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Chronic Gastritis

Diagnosis, natural history and consequences

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Cover page: Antral chronic gastritis grade III with atrophy grade III, published with the permission of F. Petersson.

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Jesus Christ, in whom are hidden all the treasures of wisdom and knowledge
Colossians 2:3

To my dear wife Anki and our children Frida, Filip, Matilda and Viktor. True love for ever!

CONTENTS

Abbreviations

CMV	Cytomegalvirus
DM	Diabetes mellitus
DU	Duodenal ulcer
ECL	Enterochromaffin-like
EGD	Esophago-gastro-duodenoscopy
ELISA	Enzyme-linked immunosorbent assay
GC	Gastric carcinoma
GU	Gastric ulcer
Hcy	Homocysteine
HCl	Hydrochloric acid
H. pylori	*Helicobacter Pylori*
IF	Intrinsic factor
IM	Intestinal Metaplasia
MALT	Mucosa-associated lymphoid tissue
MTHFR	Methylene-tetrahydrofolate reductase
NPV	Negative predictive value
NSAID	Non-Steroidal-Anti-Inflammatory-Drug
OR	Odds ratio
PA	Pernicious anaemia
PPI	Proton-pump-inhibitor
PPV	Positive predictive value
RUT	Rapid urease test
SAM	*S*-adenosylmethionine
SSRI	Selective-serotonin-reuptake-inhibitor
UBT	Urea breath test

ABSTRACT

Background & aims: The main cause of chronic gastritis is *Helicobacter pylori (H. pylori)* infection which is very common worldwide. Clinical manifestations of chronic gastritis are ulcer disease, gastric cancer and mucosa-associated lymphoma tissue (MALT) lymphoma in the stomach. It is uncertain whether gastritis can be diagnosed macroscopically at endoscopy. *H. pylori* infection may be diagnosed by several different methods, the accuracy of which needs to be explored. Some individuals with *H. pylori* related chronic gastritis will develop atrophy of the gastric mucosa. This condition is the main risk factor for cancer development and may also be associated with vitamin B12 deficiency leading to hyperhomocysteinaemia. Hyperhomocysteinaemia may be a risk factor for cardiovascular disease and dementia. The natural history of chronic gastritis in terms of development of atrophy and ulcer disease in the adult general population is largely unknown.

Material & methods: A sample of 501 volunteers from the general population in the municipality of Linköping was examined with esophago-gastro-duodenoscopy (EGD) with biopsy. Gastritis was classified according to the Sydney system. Blood samples were collected in the fasting state and the subjects answered a questionnaire about lifestyle factors, medications and disease history. The study was non-interventional as regards *H. pylori* infection without ulcer. In-hospital diagnoses and causes of death during follow-up of the population were extracted from local and national patient files. Re-examination was done in 314 subjects after a median follow-up interval of 8.4 years. Five diagnostic tests (serology UBT, RUT, culture and microscopic examination) for *H. pylori* infection were used at re-examination.

Results: The sensitivity of different macroscopic features at EGD for presence of chronic gastritis or *H. pylori* infection was unacceptably low (between 0 and 80%). The best values of sensitivity and specificity were for visible vessels in relation to microscopic presence of severe atrophy in the gastric corpus mucosa (80% and 87%, respectively).
There was a positive relation of S-homocysteine to male gender, age, S-cystatin C (renal function), methylenetetrahydrofolate reductase 677TT genotype and atrophic gastritis. During follow-up cardiovascular diseases occurred in 101/438 and dementia in 25/488 subjects, respectively. Logistic regression analysis showed an association of S-homocysteine higher than 14.5 μmol/L to cardiovascular diseases (OR 2.05), but not to dementia overall.

As observed at follow-up examination, the incidence of ulcer (duodenal or prepyloric) was 0.45 per 100 person years and was associated with weekly NSAID use, weekly alcohol consumption (OR 19.4) and smoking (OR 31.0), but not with *H. pylori* status. De novo infection with *H. pylori* was not observed, and the infection had disappeared in 11 of 113 subjects. Among subjects with chronic gastritis, the incidence of atrophy of the corpus mucosa was 1.4 per 100 person years. Atrophy development was related to age (OR 1.23) and to the severity of chronic inflammation in the corpus mucosa at baseline (OR 8.98).

Considering diagnostic test for *H. pylori* infection the accuracy was 0.86 for serology, 0.94 for UBT, 0.94 for RUT, 0.93 for culture, and 0.93 for histological examination. There was a strong correlation between the results of UBT and the histological scores of *H. pylori* colonisation as well as between the results of UBT and scores of RUT.

Conclusions: The occurrence of chronic gastritis or *H. pylori* infection is not evaluable macroscopically at gastroscopy, except for the absence of rugae or visible vessels in the

gastric corpus mucosa. Serum Hcy concentrations are dependent on gender, age, the levels of vitamin B12 and folate, renal function, the occurrence of atrophic gastritis and the MTHFR 677 TT genotype. Elevated S-Hcy is a risk factor for cardiovascular disease.
The incidence of atrophy of the corpus mucosa is 1.4 per 100 person years for chronic gastritis overall. Chronic gastritis with or without *H. pylori* infection is a variable process in which milder degrees of atrophy of the corpus mucosa may appear or disappear. In contrast, moderate-to-severe atrophy of the corpus mucosa rarely regresses. Age and the degree of chronic inflammation in the gastric corpus mucosa are major risk factors for the development of atrophy. The incidence of ulcer was 0.45 per 100 person years.
There are only minor differences in accuracy between the three invasive tests for *H. pylori* infection. The UBT is recommended for situations where endoscopy is not required. RUT may be recommended as the first non-invasive method of choice in the diagnosis of *H. pylori* infection.

List of papers

This thesis is based on the following papers, which are referred to in the text by their roman numerals:

I. Relationship of gastroscopic features of gastritis to histological findings and *Helicobacter pylori* infection in a general population sample
Redéen S, Petersson F, Jönsson K-Å, Borch K.
Endoscopy 2003 Nov;35(11):946-50.

II. Homocysteine levels in chronic gastritis and other conditions: Relations to incident cardiovascular disease and dementia
Redéen S, Ryberg A, Petersson F, Eriksson O, Nägga K, Borch K.
Dig Dis Sci 2009 Feb;55(2):351-8.

III. Natural history of chronic gastritis in a population-based cohort
Stefan Redéen, Fredrik Petersson, Stergios Kechagias, Erik Mårdh, Kurt Borch
Scand J Gastroenterol 2010 Feb 24. (Epub ahead of print)

IV. Reliability of diagnostic tests for *Helicobacter pylori* infection
Redéen S, Petersson F, Törnkvist E, Levander H, Mårdh E, Borch K.
Submitted

Introduction

H. pylori infection is very common worldwide, and is the primary cause of chronic gastritis (1-7). Chronic gastritis has a well-documented relationship to ulcer disease, gastric cancer and MALT lymphoma (7-9). The prevalence of *H. pylori* infection is higher in developing than in developed countries (10). The main risk factor for the development of gastric carcinoma is atrophy of the gastric mucosa, especially when it engages the gastric corpus (8, 11-15). In Europe, more than 100,000 people die each year in gastric cancer (5, 16) and many more worldwide (17). There is a wide geographical variation in GC incidence, high in Japan, China, South America and Eastern Europe, and low in Western Europe, including Scandinavia (5, 9, 16-19). Apart from evidence supporting the relationship between *H. pylori* and GC (6, 9, 14, 17), there are many other factors with an aetiological impact on the development of this disease such as host factors, diet, environmental and genetic factors (8, 12).

The prevalence of atrophic gastritis ranges between 10 and 37% in northern Europe (20-24). In a high-risk Chinese population, the prevalence was 26-66% (25). Severe atrophy of the gastric corpus mucosa may lead to vitamin B12 deficiency and secondary hyperhomocysteinaemia. With antrum sparing atrophy there is an association to enterochromaffin-like (ECL) cell neuroendocrine tumours, as observed in PA patients (26). The Sydney system is an international standard updated 1994 and it represents the consensus for histological assessment of chronic gastritis (27, 28), in fact there is even one part for endoscopic use (29).

Gastroscopy because of epigastric pain, suspicion of gastrointestinal bleeding or any alarm symptom is a very common everyday examination worldwide. The gastric mucosa reveals a variety of macroscopic features that may or may not be related to chronic gastritis. It is important to compare these macroscopic features to the microscopical findings. but such studies have been few (30-33).

Homocysteine (Hcy) levels in circulation are determined by several factors such as *S*-adenosylmethionine **(**SAM), vitamin B12 as a cofactor and methyltetrahydrofolate as the substrate. Deficiency of these or of other substances, as well as enzymatic defects may lead to hyperhomocysteinaemia which is reportedly associated with cardiovascular diseases and dementia. We did not find any study comparing both the relation between chronic gastritis and other conditions with possible impact on Hcy levels in circulation and their relation to incident cardiovascular diseases and dementia.

The prevalence of gastric ulcer is 1-3% among dyspeptic patients (34, 35), the equivalent number is approximately 2% in the general population (24, 36). In a 10-year follow-up study of symptomatic ulcer patients from Finland, the incidence was 7.7% and in another Finnish prospective study the ulcer incidence was 21.6% over a 32-year period (37, 38).
There are no convincing studies showing a correlation between *H. pylori* related chronic gastritis and GERD or functional dyspepsia (9). *H. pylori* infection has been linked to a number of extra gastro-duodenal diseases; further studies are needed in this field.

The natural history of chronic gastritis, has been documented in patient series (38-41), but population-based studies are quite few (40, 42, 43). The study of the natural history of chronic gastritis is a challenge because the highest incidence of atrophy is amongst the elderly and the longer the observation interval the fewer subjects are available for follow-up.

Gastritis in the corpus (corpus predominant or type A gastritis) and in the antrum (antrum predomint or type B gastritis) behave differently, that is, corpus-predominant atrophic gastritis is more related to GC, and antrum-predominant gastritis is more related to ulcer disease. Pan-gastritis is the final result from antrum-predominant chronic gastritis and it may also progress to atrophic gastritis (24).

Infection with *H. pylori* can be diagnosed by a variety of tests which are either non-invasive or invasive (44-47). *H. pylori* infection can often be successfully treated with PPI and double antibiotics (48). Treatment rarely needs to be repeated, but if so, culturing could be of value. It is important to make a definite diagnosis if aetiological treatment is to be given. Testing for the outcome of treatment is recommended.

History

After several attempts to culture bacteria from the stomach in 1982, Marshall and Warren finally succeeded at the Easter holiday (1). Because of initial scepticism, Marshall himself fulfilled the Koch's criteria with *H. pylori* (the bacteria must be present in every case of the disease, the bacteria must be isolated from the host with the disease and grown in pure culture, the specific disease must be reproduced when a pure culture of the bacteria is inoculated into a healthy susceptible host and the bacteria must be recoverable from the experimentally infected host) (49). The discovery of *H. pylori* by Marshall and Warren is one of the most important events in gastroenterology (1).

The history of chronic gastritis goes far back. Already in 1771 chronic gastritis was described by Vilardell (50). Microorganisms have been observed in the stomach of animals and humans for more than 100 years. In 1881 Rappin observed spiral-shaped bacteria in a dog stomach (51). Bizzozero confirmed Rappin's discovery in 1892 and noted that these bacteria were in the vicinity of parietal cells in the dog stomach. This was the first observation that linked the aforementioned bacteria to distinct cells in the stomach. However, it was not until 1896 that the first comprehensive study of spiral bacteria inhabiting the gastric mucosa was undertaken. In 1899 Jaworski described spiral shaped microorganisms in the sediment of gastric juice in Polish subjects, he did not receive much attention for no further research was made (52). In 1938 Doenges reported spirochetes in 40 % of human autopsy stomachs. Because of a suspicion of contamination no further research was made (53). In 1975 Steer found Pseudomonas aeruginosa when culturing biopsies from gastric endoscopic biopsies (54). The histopathologist Warren took interest in these bacteria and discovered an association with the presence of polymorphonuclear leucocytes. Soon thereafter the connection between these bacteria, chronic gastritis and ulcer disease became clear (55).

Initially the bacterium was thought to be a contamination from digested food rather than a true gastric coloniser. The conventional thinking had been that no organism could survive in the human stomach because of the low pH. A paradigm shift has occurred over the last 25 years in the etiological understanding of chronic gastritis, its consequences and its treatment.

The bacterium was initially named “Campylobacter-like organism” (CLO), “gastric Campylobacter-like organism”, “Campylobacter pyloridis” and “Campylobacter pylori”. Now we know that *H. pylori* is distinct from members of the genus Campylobacter (9).

The normal gastric mucosa

The compartments of the stomach are shown in figure1 A. The gastric corpus mucosa is thrown into prominent folds or rugae which still are present when the stomach is distended, such as during gastroscopy. There is a mucus layer for protection of the mucosa. The pH in the stomach normally is between approximately 0.9-1.5. Figure 1B shows the tubular glands and pits in a normal corpus mucosa.

Figure 1 A. The topography and gastric glands of the stomach.

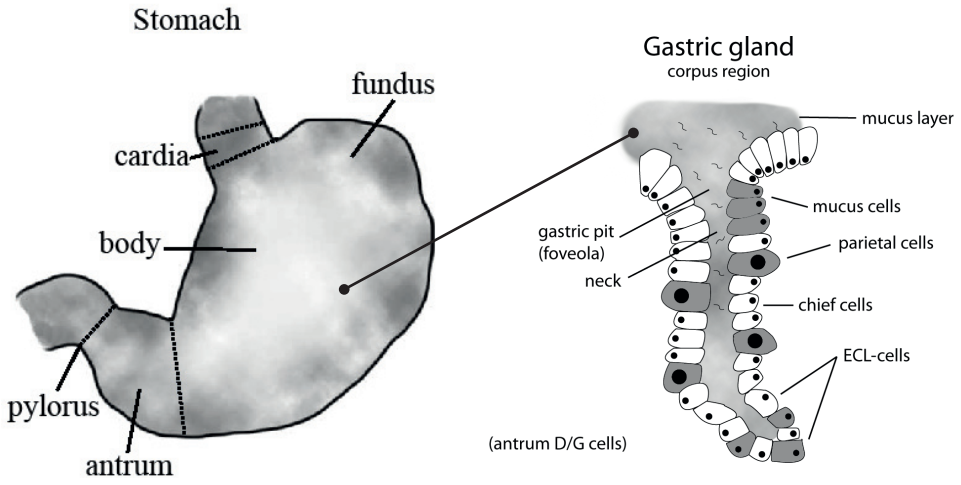
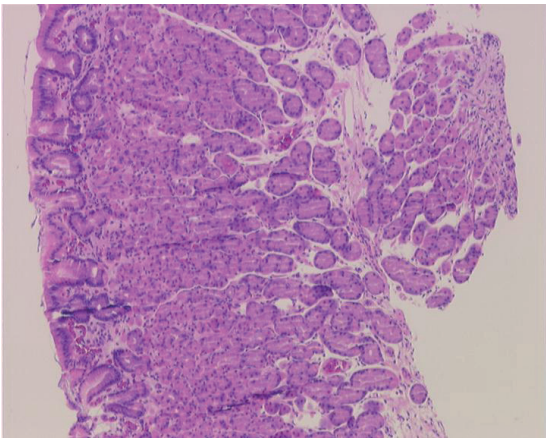


Figure 1 B. Microphotograph of gastric corpus mucosa as seen in a biopsy section. Lumen to the left. Normal foveolae (gastric pits) and tubular glands.



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In the mucosa there are several types of cells, each with a specific function (table 1).

Table 1. Gastric mucosal cell types and their main product.

Cell type	Product	Location in the stomach
Mucous neck	Mucus, bicarbonate	Corpus
Parietal (oxyntic)	HCl, intrinsic factor	Corpus
ECL	Histamin (stimulates HCl production by parietal cells)	Corpus
Chief (zymogen)	Pepsinogen I & II, gastric lipase	Corpus
Mucous glands	Mucus, Pepsinogen II	Antrum
D	Somatostatin (inhibits G cells and ECL cells)	Antrum and corpus
G	Gastrin (stimulates ECL cells and parietal cells)	Antrum

Glandular atrophy of the corpus mucosa engages both parietal cells and chief cells resulting in decreased acid secretion, decreased PG I (decreased PGI/PGII ratio) production, increased gastrin secretion and ultimately decreased IF secretion. The increased gastrin secretion is related to absence of acid (parietal cell depletion) which normally inhibits gastrin secretion from antral G cells.

Helicobacter pylori

In the year of 2005 R J Warren and B J Marshall were awarded the Nobel Prize "for their discovery of *H. pylori* and its role in gastritis and ulcer disease" (1). The common opinion today is that the *H. pylori* infection usually is acquired in childhood or youth and that the initial colonisation probably is associated with a transient acute gastritis with symptoms and even ulcer in young people (56). Transmission of *H. pylori* is considered to be via faecal-oral or oral-oral routes. It may also be transmitted by contaminated water. At present the prevalence of *H. pylori* infection seems to be decreasing "spontaneously" in the Western world (57) but not in developing countries.

H. pylori is a gram–negative spiral shaped, micro-aerophilic bacterium, highly adapted for colonisation in the stomach. Due to high acidity, limited availability of nutrients and regular gastric emptying, the environment in the stomach is unfavourable for most other known microbes. The enzyme urease of *H. pylori* increases pH through converting urea into ammonia and carbon dioxide. This results in favourable conditions for survival and attachment to the mucosa. After attachment there is a chronic inflammatory response with inflammatory cells visible upon microscopy.

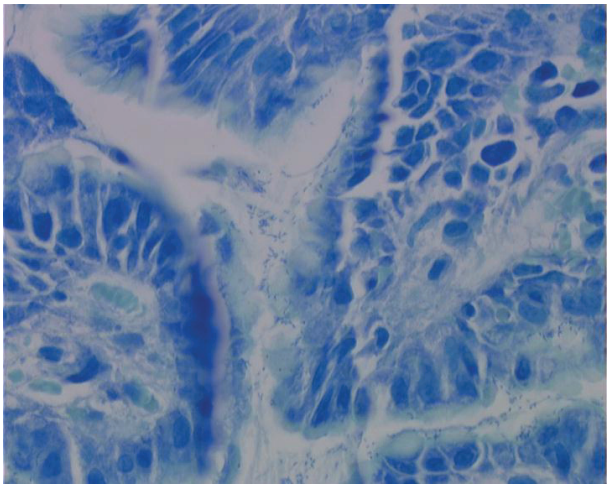
Primarily *H. pylori* colonises and causes inflammation in the antrum of the stomach. This leads to inhibition of antral D (somatostatin) cells and, thus, impaired inhibition of antral G (gastrin) cells. The ensuing increase in gastrin secretion enhances acid secretion with risk of

peptic ulcer development. If significant chronic inflammation extends to the corpus, mucosal atrophy including parietal cell atrophy may develop. This condition, with or without atrophy of the antral mucosa, is associated with the highest risk of carcinoma development (8, 9, 24).

There are numerous of variations in both pheno- and genotype of *H. pylori*. The organism constantly changes its genotype which is the reason why every infected person has his own specific *H. pylori* strain. Virulence factors can be categorised in factors required for colonisation and factors that influence of the host reaction e.g. toxins. Factors involved in the epithelial damage are vacuolising cytotoxin A (VacA), cytotoxin-associated gene A (cagA), surface lipopolysaccharide (LPS), urease, flagella, surface adhesins, oxidising radicals and cytokines produced by leukocytes in response to the infection (58).

Cag A and Vac A are virulence factors with impact on disease development. Cag A is a protein encoded by the *cag A* gene, it is highly immunogenic and present in 50-70% of strains (59). Infection with cag A+ strains is associated with more pronounced neutrophilic inflammation and a higher risk for development of ulcer or GC in Western world (60), but not in Asian populations (61). Cag A variant cag A1 is frequent in the Western world and cag A2 in Asia (62).
Vac A is an excreted protein encoded by the *VacA* gene, present in almost all *H. pylori* strains and associated with toxic activity and pathogenicity. Different toxic activities that are not found in Asian populations are found in Western populations.

Figure 2. *H. pylori* in the gastric pit in the center of the microphotograph (Giemsa stain).



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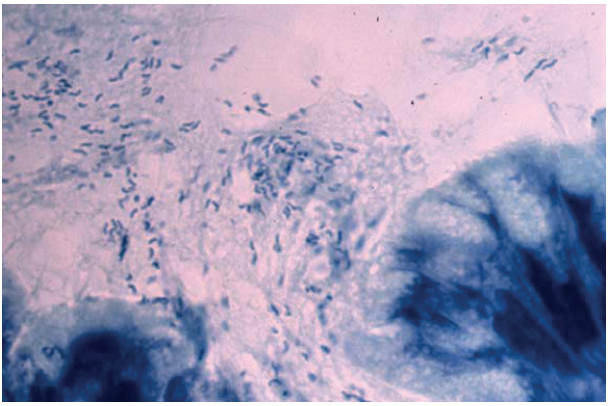

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Diagnosis of *Helicobacter pylori* infection

Recommended indications for *H. pylori* eradication therapy are: ulcer disease, MALT lymphoma, atrophic gastritis, post gastric cancer resection, chronic NSAID therapy and *H. pylori* infection in first degree relatives to gastric cancer patients (48, 63).
A correct aetiological diagnosis is mandatory in these conditions and especially in ulcer disease considering the fact that the fraction of NSAID related or idiopathic ulcers seems to have increased (36, 64, 65). This further underlines the necessity of a reliable diagnosis of *H. pylori* infection both before and after eradication therapy (44, 47, 66-68).

Non-invasive clinical tests for detection of *H. pylori* infection are serology (IgG or IgA antibodies), ^{13}C-Urea Breath Test (UBT) and faecal antigen tests (44, 47). Serology mirrors past (within years) or current infection. The reported sensitivity and specificity of serology measuring IgG antibodies is 80-100% and 69-95%, respectively (44, 47, 67, 69). Corresponding values for faecal antigen test are 90-95% and 90-95%, respectively (47). Reported sensitivities and specificities for UBT are 81-100% and 56-98%, respectively (44, 46, 47, 67, 69, 70). Differences between studies may in some instances be explained by differences in methodology and the choice of gold standard.

In patients with bleeding peptic ulcer the performance of UBT seems to be superior to biopsy-based methods (69). Invasive tests for *H. pylori* infection are the rapid urease test (RUT) on fresh biopsies, histological examination and culture of biopsies. Reportedly, the sensitivity and specificity of RUT is 80-95% and 90-100%, respectively, depending on the choice of gold standard (44, 47, 67, 69, 70). Histological examination shows a sensitivity of 88-95% and a specificity of 90-100%, respectively (47, 67, 69, 70). Regarding culture the reported sensitivity and specificity is 80-90% and 95-100%, respectively (47, 67, 69).
Considering the biopsy-based tests, the outcome probably is influenced by the location in the stomach and how many biopsies were taken for analysis (66, 69) and whether blood is present (71-73).

Host response to *H. pylori* infection

There are three main outcomes of chronic *H. pylori* infection: mild pangastritis without any significant gastric or duodenal pathology, antrum-predominant gastritis with association to DU and corpus-predominant gastritis associated with atrophy and risk of GC (9, 57).

Histologically *H. pylori* related gastritis is a chronic active inflammation with infiltration by mononuclear cells and varying degree of polymorphonuclear cell infiltration. *H. pylori* induces a Th-1 response in the host with resultant secretion of cytokines such as gamma interferon, IL-1B, IL-8, IL 10 and TNF-alfa (9). These cytokines have a number of actions including macrophage and neutrophil activation, cytotoxic T-cell recruitment and inhibition of acid secretion (e.g. IL-1B) (9). Due to antigenic mimicry *H. pylori* may induce autoimmune reactions, for instance appearance of H^+K^+-ATPase (proton pump) antibodies. In the multi-step development of GC, where *H. pylori* originated virulence factors (e.g. CagA and VacA), cytokines and free radicals are produced, angiogenesis seems to play an important role. Nitric oxide synthase (eNOS or NOS III) which is expressed in high levels is an important regulatory factor in angiogenesis (74).

Chronic gastritis

Chronic gastritis is defined as inflammation in the gastric mucosa visible upon microscopy with chronic inflammatory cells (lymphocytes and plasma cells), variable degree of activity (polymorphnuclear infiltrate) and, in some cases, glandular atrophy. Several attempts have been made to classify chronic gastritis (Whitehead, Correa). The discovery of *H. pylori* by Warren and Marshall in 1983 changed the understanding of what causes the majority, at least 80%, of cases with chronic gastritis. This led to a new conception of the process, the Sydney system (27, 28) and the Nobel prize in medicine 2005.
The Sydney system is based on the morphology, topographic distribution and aetiology of gastritis. The morphological features included are: chronic inflammation, inflammatory activity (polymorphonuclear cells), atrophy (loss of specialised glandular cells), metaplasia (transformation from one specialised set of epithelial cells to another) and bacterial colonisation, most notably *H. pylori*.
These morphological features are semi quantitatively assessed on a four-graded scale (visual analogue scale) equivalent to verbal description none, mild, moderate and severe. The recommended biopsy sites and number have varied. According to the updated Sydney system, the recommendation is to collect two biopsies from the corpus and antrum (one from the major curvature and one from the minor curvature) and one biopsy from the incisura angularis (transition zone)(28).

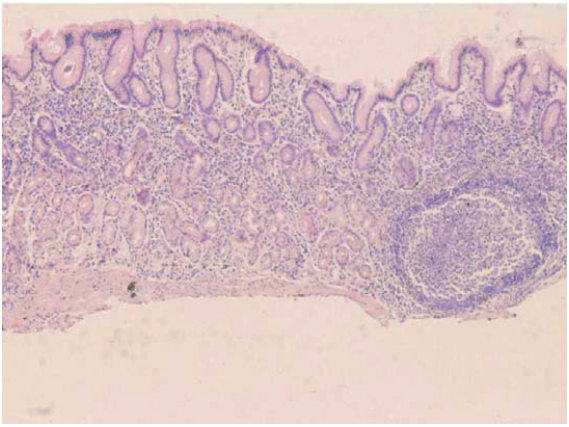
The Sydney System: Histological division

Aaetiology	Topography	Type	Morphology
Aaetiology /pathogen association	Pangastritis Antrum gastritis Corpus gastritis	Acute gastritis Chronic gastritis Special forms	Graded variables -atrophy -inflammation -activity -IM -*H. pylori* Non-graded variables Non-specific/specific
Score 0	1	2	3

It has been shown that the maximum degrees of atrophy and IM are consistently found in the region of the incisura angularis (75), which also is the most likely site to encounter dysplasia (76). Otherwise, the overall principles and grading as originally proposed in the Sydney system were retained, but grading has been aided by the provision of a visual analogue scale. It is emphasised that IM and atrophy are independent processes, but they usually coexist. The different topographical types of chronic gastritis are antrum-predominant, corpus-predominant and pan-gastritis. The antrum-predominant type is present, when the inflammatory process is strictly localised to the antrum or, when there is inflammation in both the corpus and the antrum, but the difference in the degree of chronic inflammation, is more than one grade higher in the antrum. Accordingly, corpus-predominant gastritis is when the inflammatory process is strictly localised to the corpus or, when the score for chronic inflammation is at least two grades higher in the corpus than in the antrum. Pan-gastritis is when the inflammatory process is distributed in both gastric compartments and there is not more than one grade difference in chronic inflammation. Atrophy of the gastric mucosa may be classified in a similar way (28).

The most important aetiologic agent is *H. pylori*. The density of *H. pylori* is interpreted with the visual analogue scale. Chemical gastritis e.g. from NSAID or bile are also possible aetiological factors.

Figure 3. Chronic gastritis grade II in antral mucosa. (H&E)



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Atrophic gastritis

Atrophy of the gastric mucosa is defined as loss of specialised glandular tissue. According to the Sydney system, this process could manifest itself in two ways, or a combination of these. The mucosa could maintain its thickness while the glandular compartment is replaced by cells showing a different phenotype (metaplasia). This metaplasia is mostly of the intestinal type (see below), but it may also be pancreatic or even gastric. In the latter case the corpus mucosa is replaced by an antropyloric (pseudopyloric) type of mucosa with mucus-producing cells. In the second alternative the mucosa may diminish in thickness due to an absolute reduction of the number of cells in the glandular compartment keeping the original or similar cellular composition.
There are follow-up studies showing that in some populations *H. pylori* associated chronic gastritis may lead to atrophy in up to 30% of the infected(9, 42).

Suggested risk factors for developing atrophy are certain cytokine gene polymorphisms, the genotype HLA-DQB type (77), presence of CagA (78), low acid output, low levels of ascorbic acid in the gastric juice and duodeno-gastric bile reflux (79). It should be mentioned that *H. pylori*, possibly due to its dependency of a particular acidic environment, may disappear from the stomach, when severe corpus atrophy with achlorhydria develops (80).

Figure 4 A. Antral chronic gastritis grade III with atrophy grade III (H&E)

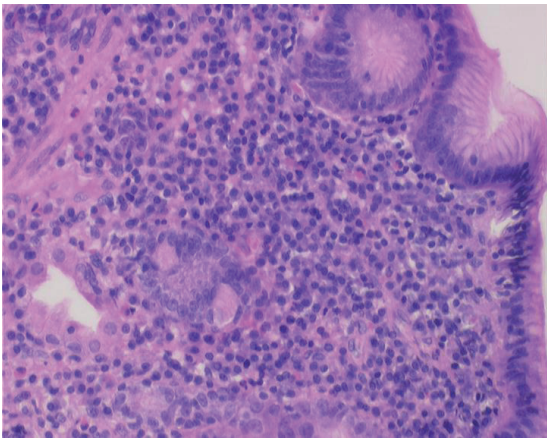

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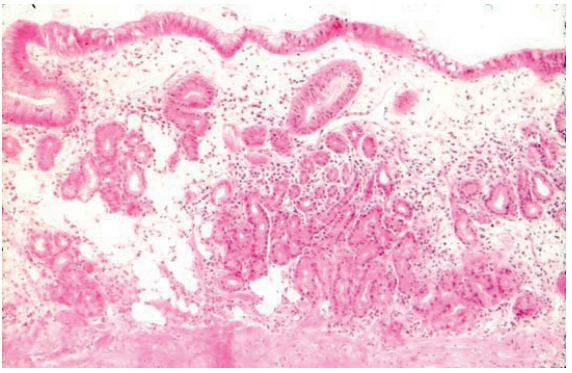
Figure 4 B. Total atrophy of the corpus mucosa (H&E).



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Intestinal metaplasia

Gastric intestinal metaplasia (IM) is an alteration of the mucosal phenotype from one specialised set of cells to another spectrum of mucosal cells with intestinal features. It has been claimed that IM rarely occurs in the gastric mucosa without any associated pathology (81). IM is also frequently seen in autoimmune atrophic corpus-predominant gastritis, as in pernicious anaemia. Epidemiological and histological studies have provided evidence of an association between IM and the intestinal type of GC which in a global perspective is the fourth most common form of cancer (8, 48).
The histomorphology of IM varies and several attempts to subclassify IM using different histological techniques have been made. Based on morphology and mucin histochemistry, three types of IM were described by Filippe and Jass. Type I: Straight crypts and trabecular

architecture. Type II: The crypts are elongated and tortuous. Type III: Distortion of glandular architecture is more pronounced.
The presence of type III has been shown to correlate with gastric epithelial dysplasia and intestinal type of gastric carcinoma in several studies (3), whereas other studies could not find such correlation (82). The prevalence of IM has been shown to be between 11 and 22 % in the Western world populations. The prevalence of IM type III in the general population of Linköping is 4% (83).

Figure 5 A. Intestinal metaplasia with goblet cells, not normally present in the gastric mucosa (Alcian blue).

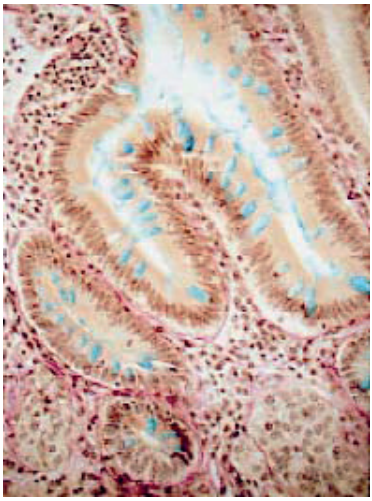
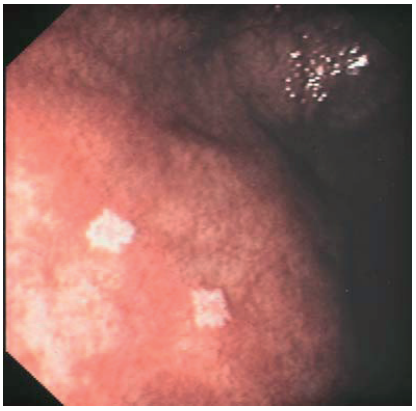


Figure 5 B. Endoscopically visible islands of intestinal metaplasia.



Gastric metaplasia

Duodenal ulcer is common and its aetiology multifactorial, although *H. pylori* infection plays a major role (84). Gastric metaplasia is characterised by the appearance of clusters of epithelial cells of gastric phenotype in non-gastric epithelium (85, 86). It may occur anywhere in the intestines, but is most commonly found in the proximal duodenum. *H. pylori* colonises these islands of gastric metaplasia (87). The proposal is that *H. pylori* in the antral mucosa causes increased gastrin secretion and thereby enhanced acid secretion which stimulates metaplastic transformation in the duodenum (87).

Other risk factors for chronic gastritis

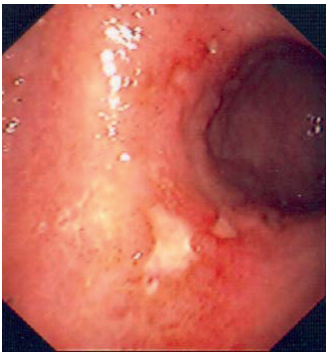
Regular intake of NSAIDs or aspirin (ASA) are known risk factors (28). Erosions or ulcer are not uncommon complications to NSAID/ASA intake. The risk of these complications is highest among the elderly.
Other suggested risk factors are smoking and alcohol consumption (9). Unusual causes of gastritis are infection with CMV, herpes simplex, parasites or fungi (27, 28).

Natural history of chronic gastritis

The prevalence of *H. pylori* infection is decreasing in the Western world (57). Most people with chronic gastritis do not develop any clinical condition at all, which is why it is important to know the natural history of the condition. In particular, it is important to know at which incidence and age atrophy occurs. Can atrophy development be predicted and how should a young person with severe atrophy be managed?

The reported prevalence of gastric ulcer is 1–3% among dyspeptic patients (34, 35) and approximately 2% in population-based cohorts (24, 36). One population-based study reported an ulcer prevalence of 8% in subjects with dyspepsia and 4% in subjects without dyspepsia (32). In a Finnish follow-up study of dyspeptic patients without ulcer at baseline examination, the incidence of symptomatic ulcer was 7.7% over a ten-year period (37). In another Finnish prospective study of dyspeptic patients, the incidence of ulcer was 21.6% over a 32-year period (38).

Figure 6. Ulcer in the antrum of the stomach.



In a study from India by Khuroo et al. 1989, it was shown that the lifetime prevalence for peptic ulcer was between 11 and 22 % (88).

The prevalence of atrophic gastritis in the general population has been assessed by screening gastric function. Screening involves analysis of S-pepsinogen I (PGI) or the S-pepsinogen I/S-pepsinogen II ratio (PGI/PGII), with subnormal levels indicating atrophy of the gastric corpus mucosa (89). According to the results of screening using these surrogate markers the overall prevalence of atrophy of the corpus mucosa among adults in parts of Europe and New Zealand ranges from 3–6% and increases with age (90-92). According to the results of histological studies the prevalence of atrophy of the corpus mucosa ranges between 10–37% in the general population in northern Europe (20-24). The corresponding prevalence of severe atrophy is 2–7% (20, 21, 23, 24). In a high-risk Chinese population, the prevalence of atrophy of the corpus mucosa (regardless of severity) was 26–66% (25).

Several studies have documented the natural history of chronic gastritis in patient series (38-41), but there are few population-based studies (40, 42, 43). The progression of *H. pylori*-associated chronic non-atrophic gastritis to atrophic gastritis is slow, and long-term observation is needed to obtain reliable quantitative data (5, 8, 93). However, the longer the observation intervals are, the fewer the number of subjects available for follow-up. This is of particular concern amongst the elderly, who have the highest incidence of atrophy.

Treatment of *H. pylori* infection

In Sweden the recommended first line treatment is of one week duration and includes a proton pump inhibitor (PPI) e.g. omeprazole 20 mg x 2 or pantoprazole 40 mg x 2 and two antibiotics e.g. amoxicillin 500 mg x 2 and clarithromycin 250 mg x 2. An alternative antibiotic is metronidazole 400 mg x 2 (48, 63). On these regimens eradication of *H. pylori* infection is achieved in approximately 90 % of the patients. Bismuth may be an alternative to PPI but is rarely used in the Western world (48, 94). Well-known side effects of eradication treatment are diarrhoea and abdominal discomfort or cramp.

Homocysteine

Hcy is an amino acid metabolite which has been under debate for several years.
The Hcy metabolism is dependent on several factors such as *S*-adenosylmethionine (SAM), vitamin B12 as a cofactor, and methyltetrahydrofolate as substrate (95, 96). Deficiency of these or other substances, as well as enzymatic defects, disturbs the metabolism and may lead to hyperhomocysteinaemia (97).

Methylenetetrahydrofolate reductase (MTHFR) catalyses the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, acting as methyl donor in the conversion of Hcy to methionine (97). The MTHFR single nucleotide polymorphism (SNP) C677T (SNPdb ID: rs1801133) is known to yield an enzyme with reduced activity, leading to mild hyperhomocysteinaemia (98).

Hyperhomocysteinaemia may cause damage to the intima of the vascular wall (99). It has been linked to cardiovascular diseases, dementia, DM, thyroid disease (hypothyreosis) and renal disease (99-106). In renal disease, the levels of Hcy in circulation are inversely related to renal function (107). Associations of Hcy to cardiovascular diseases (104, 108) and dementia (109-111) have been shown in several studies, but this issue is controversial (112-114). The cut-off value for the Hcy concentration used to define hyperhomocysteinaemia is poorly defined. Cut-off levels for P-Hcy of around 14 - 15 µmol/L have frequently been used (99, 101, 103, 108-110).
Deficiencies of vitamin B12 and folate are common causes of hyperhomocysteinaemia (115, 116). A frequent cause of vitamin B12 deficiency is atrophy of the gastric corpus mucosa with reduced IF secretion, i.e. latent or overt pernicious anaemia (117). A meta-analysis showed no association of *H. pylori* infection with coronary heart disease (118).

Aims of the study

- To determine the relations of macroscopic endoscopic findings to histological changes and presence of *H. pylori* in the gastric mucosa in a sample of the general population.

- To investigate what impact *H. pylori* infection, chronic gastritis, the MTHFR C677T SNP, renal dysfunction, and some other conditions have on variations in homocysteine levels in circulation and, furthermore, to explore the relationship of hyperhomocystaeinaemia to subsequent development of cardiovascular disease and dementia.

- To describe and explore the natural history of *H. pylori* infection and chronic gastritis in terms of the development of gastric mucosal atrophy and benign ulcer in an adult population based cohort.

- To determine the concordance between and accuracy of five different tests for *H. pylori* infection in a population based cohort examined with biopsies from both the antrum and corpus of the stomach.

MATERIALS AND METHODS

Ethics The study was performed in accordance with the updated Helsinki Declaration and was approved by the Local Ethics Committee. Informed written consent was obtained from all participants. The approval was for baseline investigation and follow-up in the studies presented in Papers I-IV. Ethical issues included personal integrity, the discomfort and risk of performing gastroscopy with biopsy, and how to handle specific findings.

The study population at baseline and follow-up

The study population was investigated twice (baseline and follow-up). Regarding *H. pylori* infection the study was non-interventional, exept for ulcer disease.
At baseline 2,000 persons (20 men and 20 women for each year of the ages 35 to 85 years) randomly selected from the population register of the municipality of Linköping, were invited to participate in the study. Exclusion criteria were ongoing warfarin treatment, severe psychiatric disease or dementia, advanced systemic disease, recent myocardial infarction or stroke and terminal disease e.g. malignancy. A total of 557 individuals were willing to participate in the study. Fifty one met one or more of the exclusion criteria. Of the remaining 506 subjects, five were unwilling to complete EGD at the first attempt, leaving 501 who underwent EGD. Ten had previously been operated with distal gastric resection for benign ulcer disease. Of the remaining 491 participants 488 subsequently had evaluable biopsy material from both the gastric corpus and antrum (paper I and II). Biopsies were also collected from the first and second part of duodenum.

Age and sex distribution of the study population at baseline. Median age was 62 (range 37-85) years in women and 60 (37-82) years in men.

Age (years)	Women N (%)	Men N (%)	Total N (%)
35-44	19 (4)	20 (4)	39 (8)
45-54	80 (16)	48 (10)	128 (26)
55-64	67 (14)	61 (12)	128 (26)
65-75	72 (15)	69 (14)	141 (29)
>75	25 (5)	27 (6)	52 (11)
Total	263 (54)	225 (46)	488 (100)

Repeat examination of the study population was planned at a minimum of eight years from baseline. Fifty participants had died, 33 had developed severe disease, 12 were on warfarin treatment, 6 had moved and 4 were lost for follow-up. For the purpose of paper III two subjects in whom histological examination was not carried out at baseline and 12 subjects who had undergone treatment for *H. pylori* infection at baseline were not included. Accordingly, 372 subjects were available for follow-up. Of these, 56 refused repeat examination, resulting in a participation rate of 314 out of 372 (84.4%) possible.
At the follow-up examination all five diagnostic tests (serology, UBT, RUT, histological examination and culture) for *H. pylori* infection were achieved in 304 participants (paper IV).

Endoscopic examination

Endoscopy was performed by three experienced endoscopists. The volunteers fasted for at least 6 hours before the examination. Blood samples were drawn and EGD was carried out after pharyngeal anaesthesia with lidocaine spray (Xylocaine, Astra, Södertälje, Sweden). Sedation with 2-3 mg intravenous flunitrazepam (Dormicum, Roche AB, Stockholm, Sweden) was given on demand. The Olympus GIF-100 gastroscope was used and biopsies were taken with the same type of forceps in all subjects at baseline and at follow-up. A clean forceps was used for each biopsy site. Three biopsy specimens for histological examination were routinely collected from the gastric body (major, anterior, and posterior aspect) and the antrum (within 3 cm of the pylorus).
One fresh biopsy was taken from the corpus and antrum and tested for occurrence of *H. pylori* with RUT (CLO®-test, Delta west Pty Ltd, Bentley, Australia), which was read after 20 min and 1, 3, and 12 h (scored 4, 3, 2, 1, respectively). Absence of *H. pylori* according to RUT was scored 0. A further biopsy specimen from each of the corpus and antrum was collected for culture of *H. pylori* (paper IV).

Ulceration was defined endoscopically as a mucosal break with unequivocal depth and a diameter of at least 3 mm. All gastric ulcers were biopsied and their benign nature verified histologically.

Histological examination

One experienced pathologist blinded to other data in the study performed all the microscopic examinations.
After orientation, fixation in neutral formaldehyde, and routine processing of the biopsies, sections cut (5 microns-thick) perpendicular to the surface were stained with haematoxylin-eosin, Alcian blue-periodic acid-Schiff, and Giemsa stain.
The density of *H pylori*, chronic inflammatory infiltrate, inflammatory activity (polymorphonuclear cells), glandular atrophy and intestinal metaplasia were scored (0: none, 1: mild, 2: moderate, or 3: severe) according to the Sydney system. When inflammation or atrophy was present in both the antrum and corpus, gastritis was classified as antrum- or corpus-predominant if there was a 2-grade or greater difference between the scores for inflammation (or atrophy), and as pan-gastritis when there was less than a 2-grade difference. Gastritis that was limited strictly to the antrum or corpus was classified accordingly as antrum- or corpus-predominant. For each location (corpus and antrum) the severity scores for gastritis is equal to the highest score for inflammation (or atrophy) in the biopsy sections.

Kappa analysis of the “blinded” repeat evaluation of the Sydney system scores of biopsy sections from the antrum and corpus in 50 participants (30 with chronic gastritis and 20 without gastritis) at baseline yielded a Cohen’s Kappa statistic of 0.782 and 0.821 for chronic inflammation, 0.882 and 0.735 for inflammatory activity, 0.640 and 1.000 for atrophy and 0.839 and 1.000 for intestinal metaplasia, respectively. Corresponding values for the density of *H. pylori* were 0.897 and 0.824, respectively.

Paper I

Endoscopic examination: At endoscopy the typical macroscopic changes evaluated were agreed upon by the endoscopists by examining at least 10 endoscopic photographs of each feature and performing numerous EGDs together.
The body and antrum were examined separately for visible blood vessels (fornix excepted), erosions (small superficial defects in the mucosa with a flat edge and white/yellow colour or small bleeding spots, petechiae) and erythema (diffuse, spotty, linear) in a standardised manner. After maximum air insufflation at the end of the examination, the corpus was examined for presence of rugae.
H. pylori infection was considered to be present when the bacteria were observed histologically and/or the urease test scored positive within 3 hours.

Paper II

Study population at baseline and follow-up: In-hospital diagnoses recorded before and after baseline including causes of death among deceased participants were extracted from local patient files (including those of the Memory Reception, Department of Geriatric Medicine) as well as from the records of Statistics Sweden (SCB) and the Swedish National Board of Health and Welfare. Nationwide reporting to the latter goes back to the year of 1964 (100% coverage by 1987). Files were screened up to 2005.

In case of incident cardiovascular disease, one diagnosis was assigned to each participant. Myocardial infarction was given the highest priority. The priority of other diagnoses in descending order was: ischaemic heart disease (angina pectoris), aortic or other arterial aneurysm, transient ischaemic attack (TIA) or stroke, and cardiac arrhythmia and/or heart failure: all without a registered diagnosis of myocardial infarction.

In the total cohort of 488 participants, 50 had a history of a cardiovascular disease at baseline. None had known dementia, since that precluded participation. Ninety-nine of 485 responders were current smokers, 78 of 476 responders used spirits and/or wine every week and 81 of 476 responders used aspirin or other NSAID every week. There were 438 participants (207 women and 231 men, age 59.4 (36.8-84.9) years) with no history of a cardiovascular disease or dementia at baseline (smokers 93 of 435 responders; use of alcohol every week 71 of 429 responders; use of aspirin or other NSAID every week 60 of 427 responders).

Histological examination and H. pylori status: Atrophy of the duodenal mucosa was histologically classified according to Alexander, and in the present study it was defined as grade III or grade IV.
H. pylori status was classified as positive if at least one of serology, histology and urease-test (read within 3 hours) on fresh biopsy specimens indicated infection.

Blood analyses: At baseline, blood samples were stored at -80°C pending analysis. Sera were analysed (Biohit Diagnostics, Helsinki, Finland) for vitamin B12 (pmol/L), folate (nmol/L) and Hcy (µmol/L) concentrations using Immulite® 1000 method (DPC, Los Angeles, CA, USA) according to the manufacturer's instructions. Samples were run at the same time to minimise assay variability. Pepsinogens in serum were measured with a sandwich enzyme

immunoassay (ELISA) utilizing a pepsinogen I (PGI) and pepsinogen II (PGII) specific capture antibody, respectively, and a horseradish peroxidase (HRP) detection antibody (from GastroPanel®, Biohit Diagnostics, Helsinki, Finland). There is no cross reactivity between the two assays. The reference interval is 30.0-120.0 µg/L for PGI, 3.0-10.0 µg/L for PGII and 3.0-20.0 for the ratio PGI/PGII. Values of PGI/PGII lower than 3.0 were considered as indicative of significant atrophy of the gastric corpus mucosa.
P-total cholesterol (mmol/L) and P-triglycerides (mmol/L) were measured with the Hitachi 717 (Boehringer Mannheim Scandinavia AB, Stockholm, Sweden) using specific reagent 1. Cystatin C in serum was analysed with Advia 1650 (Bayer AB, Gothenburg, Sweden) using reagent DakoCystatin C at the Dept. of Clinical Chemistry, University Hospital, Linköping, Sweden (reference interval for ages 1-50 years : 0.7-1.21 mg/L and for ages > 50 years: 0.84-1.55 mg/L).

MTHFR SNP 677 genotype analysis: DNA from blood samples (whole blood, plasma or serum) was extracted, amplified and quality assessed as previously described (119). Primers for MTHFR C677T (rs1801133) pyrosequencing analysis were designed based on the available MTHFR genomic sequence (GenBank accession number NT_021937, region: 6388104-6400540) using the PSQ Assay design software (Biotage, Uppsala, Sweden). The primers Biotin-MTHFR677.se (ccgaagcagggagctttga), MTHFR677.as (caagtgatgcccatgtcg), and sequencing primer Seq-MTHFR677 (cgtgatgatgaaatcg) were used. PCR products were obtained using the HotStarTaq Plus Mastermix kit (Qiagen, Hilden, Germany) following the manufacturer's instructions, using 5 pmol of each primer, and the PCR programme: 95°C 5 min; 35 cycles of 95°C 30s, 60°C 30s, 72°C 1min; final extension 72°C 10 min. The PCR products were analysed by pyrosequencing as previously described.

Paper III

EGD, histological assessment and blood analyses were performed as at baseline. The same questionnaire was distributed to the participants. The participants were also asked via the questionnaire (commercial names were listed) whether they had taken antibiotics, proton pump inhibitors or histamine-2 receptor antagonists at any time during the follow-up interval. *H. pylori* status was classified as positive when more than one of the following occurred: *H. pylori* identified by microscopic examination; a positive urease test within 3 hours; an elevated level of *H. pylori* antibodies. In all comparisons between baseline and follow-up *H. pylori* status at baseline was defined in the same way.

Paper IV

The occurrence of *H. pylori* infection was tested using five different methods (serology, UBT, RUT, culture and histology). None of the *H. pylori* infected participants had received eradication therapy.

Diagnostic tests: Serum IgG antibodies to *H. pylori* were analysed by ELISA as described above (paper II, "Blood analyses").
$^{13}CO_2$-UBT was performed as in clinical routine in a VG ISOCHROM-µG mass spectrometer (Fisons, UK). Breath samples were taken before and 15, 30, 45 and 60 min. after ingestion of

50 mg ^{13}C urea. The result used is from the cumulative curve at 30 minutes (delta over baseline 30) with an upper limit of 3.5 per mille. The participants were instructed to avoid proton pump inhibitors (PPI) two weeks before the examination. Those in need of PPI, e.g. for gastro-oesophageal reflux disease, were prescribed low dose H2-blockers during the two weeks preceding UBT.

The density of *H. pylori* in Giemsa stained biopsy sections was scored as none, mild, moderate, or severe (scored 0, 1, 2, 3), as described above ("Histology").

Frozen biopsies kept at -80° C in glycerol containing freeze medium were defrosted in room temperature, homogenised and spread onto *H. pylori* selective agar plates (developed at the Microbiology laboratory (LMC), University Hospital of Linköping, Sweden). The culture medium contains GC agar (Acumedia, UK). The bacteria were cultured under microaerophilic conditions at 37°C and read after 5-7 days. Translucent colonies typical for *H. pylori* were recultured. After another seven days urease, catalase and oxidase tests were done to confirm that the colonies were *H. pylori,* all three tests should be positive.

The result of RUT was scored as described above ("Endoscopic examination") after reading at 20 min and 1, 3, and 12 h (scored 4,3,2,1, respectively). Absence of *H. pylori* according to RUT was scored 0.

STATISTICAL ANALYSES

Continuous numerical data were summarised as median (range). The Mann-Whitney U-test was used for comparisons of non-paired continuous data between groups, while Fisher's exact test or the chi-square test (as appropriate) was used for non-paired categorical data. Wilcoxon signed rank test was used for paired continuous data and McNemar's test for paired binominal data. Sensitivity, specificity, PPV, NPV, accuracy and OR are given with 95% confidence interval. In every analysis, a two-sided P-value of less than 0.05 was regarded as significant.

Paper I

Sensitivity was defined as the percentage of subjects with histologically established gastritis (chronic inflammation/atrophy) or *H pylori* present in the stomach exhibiting an endoscopic feature, and specificity as the percentage of subjects without gastritis or *H. pylori* present. The positive predictive value (PPV) is the percentage of times that an endoscopic feature will detect an individual with microscopically verified gastritis (or presence of *H pylori)*. The negative predictive value (NPV) is the percentage of times that the absence of an endoscopic feature will detect an individual without gastritis (or presence of *H pylori)*. For each macroscopic feature and grouped macroscopic features (except visible vessels and absence of rugae in relation to atrophy), subjects with other features were excluded from the calculations. Thus, whilst evaluating erosions subjects with erythema or visible vessels (N=207 regarding the antrum and N=154 regarding the corpus) were excluded and whilst evaluating erythema subjects with erosions or visible vessels (N=70 regarding the antrum and N=77 regarding the corpus) were excluded. Whilst evaluating erosions and/or erythema subjects with visible vessles (N=43 regarding the antrum and N=71 regarding the corpus) were excluded.

Paper II

Correlations were tested with Spearman's rank correlation test. A general linear model (GLM) was used to identify factors with impact on S-Hcy concentrations. Logistic regression analysis was performed in which incident cardiovascular diseases overall or dementia overall were included as the dependent variable. Coefficients in GLM are given with (95% confidence interval).

Paper III

Logistic regression analysis was performed using incident ulcer, atrophy of the gastric mucosa and subnormal (< 3.0) PGI/PGII as the dependent variable. Gender, age at baseline, difference in BMI between baseline and follow-up, follow-up interval (months), *H. pylori* status at baseline and follow-up, weekly use of NSAIDs (no/yes) at follow-up, smoking (no/yes) at follow-up and weekly consumption of alcohol (no/yes) at follow-up were included as independent variables in all logistic regression analyses. The degree of inflammation in the corpus mucosa at baseline was an additional independent variable in the analysis that used atrophy of the corpus mucosa as the dependent variable.

Paper IV

Agreement between the results of different *H. pylori* tests was evaluated by calculating the Cohen's kappa coefficient. Dichotomised data were used to calculate sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy. Each method was tested against the other methods (except serology) taken together as Gold standard. The Kruskal-Wallis test was used to compare results of UBT between the histological scores of *H. pylori* colonisation and the scores of RUT, respectively.

RESULTS

Paper I

Among the 488 subjects, histological examination, urease test or both showed presence of *H. pylori* strictly limited to the antrum only in 4 subjects (1%), to the corpus only in 19 subjects (4%) and in both locations in 174 subjects (36%). Histological examination showed antrum-predominant gastritis in 107 (22%), pan-gastritis in 89 (18%) and corpus-predominat gastritis in 46 (9%). Histological and urease test data are given in table 2.

Macroscopic features as observed during gastroscopy are given in table 3. Sensitivities and specificities of various macroscopic features for moderate to severe inflammation according to histological examination are given in table 4. The best sensitivity was that of erosions *or* erythema *or* visible vessels which was 56% regarding the antrum and 55% regarding the corpus (specificity 55% and 70%, respectively). The corresponding PPV was 30(25-37) % and 18(12-24) %, respectively (NPV 78[72-83] % and 93[90-96] %, respectively).

As shown in table 5, the sensitivity and specificity of absence of rugae for moderate to severe atrophic gastritis in the corpus was 67% and 85%, respectively (PPV 17[10-27]%, NPV 98[96-99]%). Corresponding figures for severe atrophy were 90 (56-100) % and 84 (81-88) % (PPV 11[5-20] %, NPV 100[99-100] %). The sensitivity and specificity of visible vessels for severe atrophy in the corpus was 80 (44-97) % and 87 (83-90) % (PPV 11[5-21] %, NPV 100[98-100]%), respectively. Considering the antrum, the sensitivity and specificity of visible vessels for moderate to severe atrophy was 14 (3-35) % and 91 (89-94) % (PPV 7[1-19] %, NPV 96[93-97] %), respectively. The sensitivity and specificity of one or more of all features for presence of *H pylori* was 57% and 57% (PPV 43([37-50] %, NPV 70[64-75] %), respectively, considering the antrum and 43% and 74% (PPV 53[44-60] %, NPV 67[61-72] %), respectively, considering the corpus.

Table 2. Histological findings (severity: 0: none, 1: mild, 2: moderate, 3: severe) and results of urease test for *H pylori* in combination with histology in gastric biopsy specimens from population sample of 488 subjects. A: antrum, B: corpus.

A

Histological finding	Severity			
	0 N (%)	1 N (%)	2 N (%)	3 N (%)
Chronic inflammation	281 (58)	80 (16)	110 (23)	17 (3)
Atrophy	384 (79)	82 (17)	21 (4)	1 (0)
H. pylori (histol.)	323 (66)	48 (10)	65 (13)	52 (11)
H. pylori (urease test)	negative: 324 (66%), positive: 164 (34%)			

B

Histologic finding	Severity			
	0 N (%)	1 N (%)	2 N (%)	3 N (%)
Chronic inflammation	282 (58)	155 (32)	44 (9)	7 (1)
Atrophy	441 (90)	26 (5)	11 (2)	10 (2)
H. pylori (histol.)	328 (67)	95 (20)	45 (9)	20 (4)
H. pylori (urease test)	negative: 306 (63%), positive: 182 (37%)			

Table 3. Macroscopic features observed during gastroscopy in population sample of 488 subjects.

No of subjects: Feature		Antrum N (%)	Corpus N (%)
None		254 (52)	245 (50)
Erosions		27 (5)	6 (1)
Erythema		164 (34)	83 (17)
	spotty	96 (20)	55 (11)
	linear	41 (8)	6 (1)
	diffuse	27 (6)	22 (5)
Visible vessels		43 (9)	71 (15)
Absence of rugae		-	83 (17)

Table 4. Sensitivities and specificities of various macroscopic features for histologically diagnosed moderate to severe inflammation in the antrum (A) and corpus (B) mucosa in study population.

A

Feature	Sensitivity %(95% CI)	Specificity %(95% CI)
Erosions	10(4-20)	90(86-94)
Erythema*	49(39-58)	64(59-69)
Erythema or erosions	51(42-61))	60(54-65)
Erythema, erosions or visible vessels	56(47-65)	55 (50-60)

B

Feature	Sensitivity %(95% CI)	Specificity %(95% CI)
Erosions	0(0-15)	98(96-99)
Erythema*	39(24-57)	82(77-86)
Erythema or erosions	39(24-57)	80 (76-84)
Erythema, erosions or visible vessels	55(40-69)	70(65-74)

* Spotty, linear or diffuse.

Table 5. Sensitivities and specificities of visible vessels and absence of rugae for histological diagnosed moderate to severe atrophy in the gastric corpus mucosa in population sample of 488 subjects.

Feature	Sensitivity %(95 %CI)	Specificity %(95%CI)
Visible vessels	48(26-70)	87(84-90)
Absence of rugae	67(43-85)	85(82-88)
Visible vessels and/or absence of rugae	67(43-85)	81(77-84)

Paper II

At baseline, 246 participants had histologically normal gastric mucosa with negative *H. pylori* status, 209 had chronic gastritis with positive *H. pylori* status, and 33 had chronic gastritis with negative *H. pylori* status. Atrophy (defined as moderate to severe) of the corpus mucosa was diagnosed histologically in 21 of the 488 participants (15 with positive *H. pylori* status). Twenty of 474 participants examined had a subnormal PGI/PGII value (less than 3.0) and 28 of 475 participants had atrophy either according to histological examination and/or the PGI/PGII value. Villous atrophy of the duodenal mucosa was diagnosed in 9 participants. The distribution of the MTHFR 677 genotype among 483 successfully analyzed participants was 50.1% CC, 38.5% TC and 11.4% TT. An S-Cystatin C concentration above the reference range was present in 18 of 473 participants examined.

S-Hcy concentrations were elevated in participants with DM and in those with elevated S-cystatin C. Table 6 shows that S-Hcy concentrations were increased among participants with atrophic gastritis, but not in those with *H. pylori*-associated gastritis without atrophy.
GLM analysis (table 7) showed that S-Hcy concentrations were related to gender, age, S-cystatin C, S-vitamin B12, S-folate, MTHFR 677 TT genotype, and the occurrence of atrophic gastritis.

At baseline, 438 participants had no history of any cardiovascular disease. They were followed up for 129 (9-178) months (4796 person years), during which 101 were diagnosed with cardiovascular disease. These were myocardial infarction (28), angina pectoris (17), abdominal aortic aneurysm (6), TIA/stroke (18), and cardiac arrhythmia/heart failure (32). The median follow-up time for all 488 participants was 128 (1-180) months (5262 person years). Among all 488 participants, 25 developed dementia.
The prevalence of atrophic gastritis was 9/101 among participants with incident cardiovascular diseases and 7/337 among participants not diagnosed as having cardiovascular disease during follow-up (P=0.004). Corresponding figures for dementia were 3/25 and 18/463, respectively (P=0.09). The prevalence of positive *H. pylori* status did not differ significantly between participants with and without incident cardiovascular diseases or dementia.
S-Hcy was found to be elevated among participants developing cardiovascular diseases (14.4 (4.6-37.3) µmol/L) compared to those without such diseases (12.1 (2.9-48.3) µmol/L) (P=0.001). S-Hcy levels in sub-groups are shown in Table 8.
The median for S-Hcy was 15.7 (11.2-28.4) µmol/L among participants developing dementia and 12.7 (2.9-50.0) µmol/L among participants without dementia (P=0.004).
Table 9A shows the results of the logistic regression analyses with cardiovascular disease as the dependent variable and S-Hcy as a continuous or dichotomous independent variable with different cut-off levels. Exchanging S-Hcy in this model for gastric mucosal atrophy (as determined morphologically or with PGI/PGII), MTHFR 677 TT genotype and elevated cystatin C taken together as an independent variable, showed no significant relationship to cardiovascular diseases (OR 1.70 [0.92-3.13]). As shown in Table 9B, there was no significant association between S-Hcy and subgroups of dementia considered together.

Table 6. S-vitamin B12, S-folate and S-homocysteine concentrations in relation to histology in the gastric and duodenal mucosa at baseline.

Gastro-duodenal histomorphology	Vitamin B 12, pmol/L	Folate, nmol/L	Homocysteine, µmol/L
	median (min-max) no. examined P	median (min-max) no. examined P	median (min-max) no. examined P
Normal gastric mucosa without *H.pylori* infection	266 (118-663) 220 -	9.9 (2.1-35.2) 215 -	12.2 (2.9-33.1) 242 -
H. pylori-associated chronic gastritis without significant atrophy of the corpus mucosa	282 (98-789) 179 0.72[a]	9.0 (3.1-36.3) 175 0.21[a]	13.1 (4.8-35.7) 190 0.06[a]
Non-*H. pylori*-associated chronic gastritis without significant atrophy of the corpus mucosa	281 (161-575) 27 0.86[a]	12.1 (5.1-30.0) 24 0.08[a]	12.0 (8.7-21.2) 27 0.50[a]
Chronic gastritis with atrophy of the corpus mucosa with or without *H. pylori* infection	269 (107-497) 15 0.14[a]	9.6 (4.0-24.2) 19 0.61[a]	20.1 (10.2-50.0) 20 <0.001[a]
No significant villous atrophy of the duodenal mucosa	269 (98-789) 430 -	9.6 (3.1-36.3) 422 -	12.7 (2.9-50.0) 467 -
Villous atrophy of the duodenal mucosa (Alexander Grades III to IV)	319 (224-603) 8 0.16[b]	6.1 (2.1-12.8) 8 0.02[b]	13.9 (8.5-33.1) 9 0.47[b]

a: P-value from the Mann-Whitney U-test when compared to participants with normal gastric mucosa.
b: P-value from the Mann-Whitney U-test when compared to participants with mild or no villous atrophy of the duodenal mucosa.

Table 7. General linear model estimates of factors with putative impact on S-homocysteine concentration (dependent variable).

Independent variable	Coefficient	95% confidence interval (min ; max)	P
Male gender	1.57	0.65 ; 2.49	0.001
Age	0.08	0.0 ; 0.13	0.001
BMI	-0.03	-0.17 ; 0.10	0.62
Diabetes mellitus	2.52	-0.42 ; 5.46	0.09
Hypothyroidism	0.84	-2.92 ; 4.60	0.66
S-cystatin C	2.71	1.26 ; 4.15	<0.001
S-vitamin B12	-0.01	-0.01 ; 0.00	<0.001
S-folate	-0.16	-0.24 ; -0.08	<0.001
MTHFR 677 TT genotype	1.645	0.27 ; 3.02	0.02
Atrophy of the gastric corpus mucosa	4.62	2.00 ; 7.24	0.001
Atrophy of the duodenal mucosa (Alexander Grades III to IV)	2.50	-0.75 ; 5.76	0.13

Table 8.S-homocysteine concentrations at baseline in participants diagnosed with cardiovascular diseases during follow-up.

Diagnosis	No. of participants examined	S-homocysteine, µmol/L median (min-max)	P-value[a] when compared to participants with no known cardiovascular disease
No known cardiovascular disease (hypertension disregarded)	331	12.1 (2.9-48.3)	
Myocardial infarction	26	15.1 (8.7-37.3)	<0.001
Ischaemic heart disease (angina pectoris)	17	13.0 (7.3-25.6)	0.24
Aortic Aneurysm	5	21.0 (10.7-24.2)	0.02
TIA or stroke	18	11.7 (4.6-35.7)	0.91
Cardiac failure and/or arrhythmia	32	13.2 (6.9-28.4)	0.15

[a] Mann-Whitney U-test

Table 9. Associations of S-Hcy levels with risk for cardiovascular diseases grouped together (A) and dementia subgroups considered together (B) as the dependent variable. Results are from logistic regression analysis adjusted for gender, age at baseline (years), follow-up interval (months), body mass index (kg/m^2), current smoking (yes/no), alcohol use (weekly use yes/no), use of NSAID (weekly use yes/no), P-total cholesterol (mmol/L) and P-triglycerides (mmol/L).

A

Cut-off level for S-Hcy	Odds ratio [95% confidence interval]
14.0 µmol/L	1.81 [1.01-3.23]
14.5 µmol/L	2.05 [1.14-3.70]
15.0 µmol/L	1.74 [0.96-3.16]
None (S-Hcy continuous)	1.06 [1.00-1.12]

B

Cut-off level for S-Hcy	Odds ratio [95% confidence interval]
14.0 µmol/L	1.50 [0.50-4.46]
14.5 µmol/L	1.92 [0.64-5.79]
15.0 µmol/L	1.90 [0.63-5.68]
None (S-Hcy continuous)	1.09 [0.99-1.20]

Paper III

Median age of the cohort was 58.0 (37.0-81.0) years at baseline and 66.4 (45.3–89.8) years at follow-up examination, with no difference between the sexes. Of the 314 participants, 144 were women. Although the intention was to apply a minimum follow-up interval of 96 months (8 years), 41 participants were examined 88-95 months after baseline. The median follow-up interval was 101 (54-175) months, corresponding to 2657.5 person years.

Incidence of ulcer

At the follow-up examination, prepyloric ulcer was diagnosed in 4 and duodenal ulcer in 7 participants. In addition, one subject had received *H. pylori* eradication therapy for a symptomatic duodenal ulcer 54 months before the planned follow-up examination. Thus, a total of 12 participants were diagnosed with ulcer, yielding an incidence of 0.45 per 100 person years among the 314 participants. The corresponding figure among 141 participants with chronic gastritis was 0.58 per 100 person years (follow-up interval 1201.9 person years). Putative risk factors in participants with ulcer are listed in table 10. Of the 12 participants with ulcer, 5 were women. Out of five participants with ulcer and negative *H. pylori* status, one had only positive *H. pylori* serology and one had only histologically diagnosed *H. pylori*. UBT and biopsy cultures were negative in these five participants. Two of these used NSAIDs every week, another three consumed alcohol every week, and one was a smoker at follow-up.

Among the 11 participants with incident subclinical ulcer, there was no significant change in the weekly use of NSAIDs (2 vs. 5, P=0.250), weekly consumption of alcohol (6 vs. 8, P=0.625) or smoking (6 vs. 5, P=0.999) between the baseline and follow-up examinations.

Logistic regression analysis showed an association between incident ulcer and weekly use of NSAIDs (OR 27.8 [4.2-184.6]), weekly consumption of alcohol (OR 19.4 [3.3-114.3]) and smoking (OR 31.0 [5.3-182.2]) at follow-up. There was no significant relation to *H. pylori* status.

Course of chronic gastritis

At baseline, 173 participants had neither *H. pylori* infection nor gastritis. At the follow-up examination, none of these 173 had positive *H. pylori* status; however, 15 had chronic gastritis, 14 mild and 1 moderate (pan-gastritis without atrophy). The frequency of NSAID use, alcohol consumption and smoking did not differ between baseline and follow-up in these 15 participants.

Twenty-seven participants had chronic gastritis without *H. pylori* infection at baseline. Of these, 21 had mild gastritis, which had disappeared in 16 and was unchanged in 5 at follow-up examination. Of the remaining 6 participants, 4 had moderate-to-severe corpus-predominant atrophic gastritis, 1 had moderate antrum-predominant atrophic gastritis and 1 had moderate non-atrophic pan-gastritis at baseline. In the latter 2, gastritis had resolved at follow-up, whereas it was unchanged in those with moderate-to-severe corpus-predominant atrophic gastritis. The frequency of NSAID use, alcohol consumption and smoking did not differ between baseline and follow-up in these 27 participants, and none had acquired *H. pylori* infection.

Gastritis with positive *H. pylori* status was present in 113 participants at baseline (one participant who was treated for ulcer during the follow-up interval was excluded). The topographic types and severity of gastritis at baseline and follow-up are shown in table 11.

In 11 participants (9.7%), the *H. pylori* status had changed from positive to negative. The degree of chronic gastritis was unchanged in 4 (one with moderate atrophic corpus-predominant gastritis) of these 11 participants, and there was progress from mild atrophy (antrum-predominant in 1 and corpus-predominant in 1) to severe atrophy of the corpus mucosa in 2 participants.

Table 12 shows the development of atrophy among participants with chronic gastritis. Regarding the corpus mucosa, there was a decrease in the frequency of none-to-mild atrophy (from 93.5% to 87.7 %) and an increase in the frequency of moderate-to-severe atrophy (from 6.5% to 12.2%). Regarding the antral mucosa, the frequency of non-atrophic gastritis increased, the frequency of mild-to-moderate atrophy decreased and the frequency of severe atrophy increased. Among participants with gastritis and positive *H. pylori* status at baseline, the results were similar. There was an increase in moderate-to-severe atrophy of the corpus mucosa from 4.5% to 11.6%, ($P < 0.001$).

Thirteen of 113 participants with chronic gastritis without atrophy of the corpus mucosa at baseline (regardless of *H. pylori* status) had developed atrophy of the corpus mucosa, yielding an incidence of 1.4 per 100 person years. Thirteen of 93 participants with *H. pylori*-associated chronic gastritis without atrophy of the corpus mucosa at baseline developed atrophy of the corpus mucosa, resulting in an incidence of 1.1 per 100 person years for this group.

Logistic regression analysis was performed including all participants with chronic gastritis at baseline. With newly developed atrophy of the corpus mucosa at follow-up examination as the dependent variable, there was a positive correlation between with age at baseline (OR 1.23 [1.08-1.45]) and with more than mild inflammation in the corpus mucosa at baseline (OR 8.98 [1.41-57.23]). When using newly developed atrophy or progression of atrophy of the corpus mucosa as the dependent variable, there was also an association with age at baseline (OR 1.12 [1.04-1.28]) and with the presence of more than mild inflammation in the corpus mucosa at baseline (OR 5.49 [1.68-17.92]). None of the other independent variables at baseline or follow-up examination (sex, BMI, follow-up interval, weekly use of NSAIDs, smoking, weekly consumption of alcohol and *H. pylori* status) were related to newly developed gastritis or progression of atrophy of the corpus mucosa.

Pepsinogens at baseline and at follow-up

Table 13 shows the PGI/PGII ratios and the frequency of subnormal (< 3.0) PGI/PGII ratios. The PGI/PGII ratio decreased significantly in all groups. The frequency of subnormal PGI/PGII ratios was unchanged in participants without gastritis and in participants with gastritis without atrophy of the corpus.

Logistic regression analysis was performed that included all participants with chronic gastritis at baseline. Using a newly developed subnormal PGI/PGII ratio (< 3.0) as the dependent variable, there was a positive association with age at baseline (OR 1.13 [1.02-1.45]) and with more than mild inflammation in the corpus mucosa at baseline (OR 22.27 [4.75-104.50]). There was no other independent variable from baseline or follow-up examination that correlated with the development of subnormal PGI/PGII.

Table 10. Putative risk factors for ulcer at baseline and at follow-up among participants with and without incident ulcer at follow-up examination.

Risk factor	Ulcer[a]	No ulcer[a]	P-value Fisher's exact test
NSAIDs, weekly use at baseline	2/12	46/293	> 0.999
Alcohol, weekly use at baseline	6/12	50/297	0.011
Smoking at baseline	7/12	47/299	0.001
Positive *H. pylori* status at baseline	7/12	107/302	0.129
NSAIDs, weekly use at follow-up	5/11	21/278	0.001
Alcohol, weekly use at follow-up	8/11	65/275	0.001
Smoking at follow-up	5/11	26/278	0.003
Positive *H. pylori* status at follow-up	6/11	96/302	0.186

a: Some participants did not answer all of the lifestyle questions

Table 11. Topography and severity scores for chronic gastritis according to the Sydney system at baseline and at follow-up in 113 participants with positive *H. pylori* status at baseline.

Baseline				Follow-up													
Topography	Severity	Atrophy	No. at baseline	No gastritis	Antrum-predominant				Pan-gastritis				Corpus-predominant				
					Mild		Moderate-severe		Mild		Moderate-severe		Mild		Moderate-severe		
					Atrophy		Atrophy		Atrophy		Atrophy		Atrophy		Atrophy		
					No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	
Antrum-predominant	Mild	No	4			1			1		1			1			
		Yes	35	1[b]		10		2	9		9	2[b]		1		1	
	Moderate-severe	No	2								2						
		Yes	9	1[b]		1					3[a]	2		2			
Pan-gastritis	Mild	No	17			1			9[b]		6[a]					1	
		Yes	3									1		1		1	
	Moderate-severe	No	26	1[b]	1	3	1	2	3[b]	2	12			1			
		Yes	5							2[b]	1			1		1	
Corpus-predominant	Mild	No	1						1[b]								
		Yes	9						2	1	2			1[b]		3[b]	
	Moderate-severe	No															
		Yes	2							1						1[b]	

a: Glandular atrophy could not be evaluated in one participant. b: *H. pylori* status was negative at follow-up examination in one participant.

Table 12. Atrophy of the gastric corpus (A) and antral (B) mucosa in the study population was scored using the Sydney classification system at baseline and at follow-up in participants with chronic gastritis with or without positive *H. pylori* status at baseline.

A

Atrophy score	Baseline No. (%)	Follow-up No. (%)
None	113 (81.3)	108 (77.7)
Mild	17 (12.2)	14 (10.1)
Moderate	6 (4.3)	12 (8.6)
Severe	3 (2.2)	5 (3.6)
Total[a]	139 (100.0)	139 (100)

a: Atrophy of the corpus mucosa could not be evaluated in 1 of the 140 participants.
$P < 0.001$ (Chi-square test)

B

Atrophy score	Baseline No. (%)	Follow-up No. (%)
None	78 (56.5)	107 (77.5)
Mild	49 (35.5)	25 (18.1)
Moderate	11 (8.0)	1 (0.7)
Severe	0 (0.0)	5 (3.6)
Total[a]	138 (100.0)	138 (99.9)

a: Atrophy of the antral mucosa could not be evaluated in 2 of the 140 participants.
$P = 0.142$ (Chi-square test)

Table 13 A. The pepsinogen I/pepsinogen II ratio at baseline and at follow-up in subgroups of the study population.
B: The frequency of subnormal (< 3.0) pepsinogen I/pepsinogen II ratios at baseline and at follow-up in subgroups of the study population.

A

Group	No. of participants examined	Baseline, median (range)	Follow-up, median (range)	P-value Wilcoxon signed rank test
No gastritis	164	11.7 (3.3-23.6)	8.2 (1.2-15.6)	< 0.001
Gastritis without atrophy in corpus	109	8.1 (2-4-18.0)[a]	5.9 (1.6-14.0)[a]	< 0.001
Gastritis with atrophy in corpus (grade 1-3)	25	4.8 (0.8-9.0)[a]	2.9 (0.6-6.9)[a]	< 0.001

a: P < 0.001 as compared with no gastritis (Mann-Whitney U-test).

B

Group	No. of participants examined	Baseline No. (%)	Follow-up No. (%)	P-value McNemar's test
No gastritis	164	0 (0.0)	1 (0.6)	> 0.999
Gastritis without atrophy in corpus	109	2 (1.8)	7 (6.4)	0.180
Gastritis with atrophy in corpus (grade 1-3)	25	7 (28.0)	14 (56.0)	0.039

Paper IV

The median age of the 304 participants of whom 143 were women was 66.1 (45.3-87.9) years. The results of the different tests for *H. pylori* infection are presented in table 14. Of all 304 participants approximately 1/3 had current infection. Table 15 shows the Cohen's kappa coefficients for agreement between the diagnostic methods.
Results from comparisons between the five different diagnostic tests for *H. pylori* infection are presented in table 16. Considering the non-invasive tests, the UBT showed best accuracy at 0.94 (0.90-0.96), whereas the corresponding value for serology was 0.86 (0.82-0.90). Among the invasive tests, location in the stomach disregarded, accuracy ranged between 0.93 and 0.94. The invasive tests show slightly better accuracy in the antrum than in the corpus.

Histology and UBT were compared and the two variables were strongly correlated considering both the antrum and corpus (P<0.001, Kruskal-Wallis test). A similar strong correlation for both the antrum and corpus was present when relating results of UBT to scores of RUT (P<0.001) (Figures 7 and 8).

Table 14. Results of different methods for detection of *H. pylori* infection in population based cohort of 304 subjects.

UBT: ^{13}C-urea breath test.
RUT: rapid urease test.

a: No culture in two, b: no culture in one

Method	Positive of all tested N (%)	Corpus and/or antrum, positive of all tested N (%)	Antrum, positive of all tested N (%)	Corpus, positive of all tested, N (%)
Serology	119 (39.1)	-	-	-
UBT	91 (29.9)	-	-	-
RUT	-	95 (31.3)	88 (28.9)	89 (29.3)
Culture	-	101 (33.2)	91 (30.1) [a]	98 (32.3) [b]
Histology	-	97 (31.9)	86 (28.3)	89 (29.3)

Table 15. Agreement between the results of the tests for *H. pylori* infection as evaluated by the Cohen's kappa coefficient in the study population of 304 subjects.

RUT =Rapid Urease Test
UBT= ^{13}C-urea breath test.

a: Culture failed in two subjects, b: culture failed in one subject.

Test	Antrum and/or corpus	Antrum	Corpus
Serology-UBT	0.77	-	-
Serology-RUT	0.77	-	-
Serology-Culture	0.84	-	-
Serology-Histology	0.77	-	-
UBT-RUT	0.86	-	-
UBT-Histology	0.83	-	-
UBT-Culture	0.91	-	-
RUT-Culture	0.90	0.90[a]	0.84[b]
RUT-Histology	0.86	0.86	0.84
Culture-Histology	0.88	0.82[a]	0.85[b]

Table 16. Performance of different diagnostic tests for *H. pylori* infection. Each method was tested against all other methods (serology not included) taken together as gold standard. When the methods are taken together a positive test means that all tests are positive for *H. pylori* infection. Serology is tested against all other methods taken together as gold standard.

A: Antrum and/or corpus. N=304

B: Antrum. N=302 (culture failed in two subjects).

C: Corpus. N=303 (culture failed in one subject).

RUT= Rapid Urease Test, UBT= ^{13}C-urea breath test
c.i. = confidence interval

A

Diagnostic test	Sensitivity (95% ci.)	Specificity (95% ci.)	PPV (95% ci.)	NPV (95% ci.)	Accuracy (95% ci.)
Serology	0.99 (0.93-1.00)	0.82 (0.76-0.87)	0.66 (0.56-0.74)	0.99 (0.97-1.00)	0.86 (0.82-0.90)
UBT	0.92 (0.84-0.97)	0.94 (0.91-0.97)	0.87 (0.78-0.93)	0.97 (0.93-0.99)	0.94 (0.90-0.96)
RUT	0.96 (0.90-0.99)	0.93 (0.89-0.96)	0.83 (0.74-0.90)	0.99 (0.96-1.00)	0.94 (0.90-0.96)
Culture	0.99 (0.93-1.00)	0.90 (0.86-0.94)	0.78 (0.69-0.86)	0.99 (0.97-1.00)	0.93 (0.89-0.95)
Histology	0.95 (0.88-0.99)	0.92 (0.87-0.95)	0.81 (0.72-0.89)	0.98 (0.95-0.99)	0.93 (0.89-0.95)

B

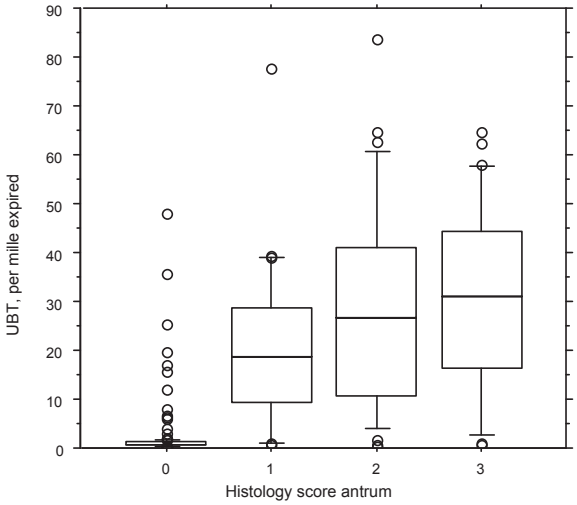
Diagnostic test	Sensitivity (95% ci.)	Specificity (95% ci.)	PPV (95% ci.)	NPV (95% ci.)	Accuracy (95% ci.)
RUT	0.97 (0.91-1.00)	0.95 (0.91-0.97)	0.86 (0.77-0.93)	0.99 (0.97-1.00)	0.95 (0.92-0.97)
Culture	0.97 (0.91-1.00)	0.93 (0.89 - 0.96)	0.82 (0.73-0.90)	0.99 (0.97 - 1.00)	0.94 (0.91-0.96)
Histology	0.90 (0.82-0.96)	0.95 (0.92-0.98)	0.88 (0.79-0.94)	0.96 (0.93-0.98)	0.94 (0.91-0.96)

C

Diagnostic test	Sensitivity (95% ci.)	Specificity (95% ci.)	PPV (95% ci.)	NPV (95% ci.)	Accuracy (95% ci.)
RUT	0.89 (0.81-0.95)	0.94 (0.90-0.96)	0.84 (0.75-0.91	0.96 (0.92-0.98)	0.92 (0.89-0.95)
Culture	0.95 (0.88-0.99)	0.90 (0.85-0.93)	0.77 (0.67-0.85)	0.98 (0.95-0.99)	0.91 (0.87-0.94)
Histology	0.90 (0.82-0.96)	0.94 (0.90-0.96)	0.84 (0.75-0.91)	0.96 (0.93-0.98)	0.93 (0.89-0.95)

Figure 7. Boxplots (showing median and interquartile range) of the relation between UBT (per mille) and histological score of *H. pylori* colonisation.

A



B

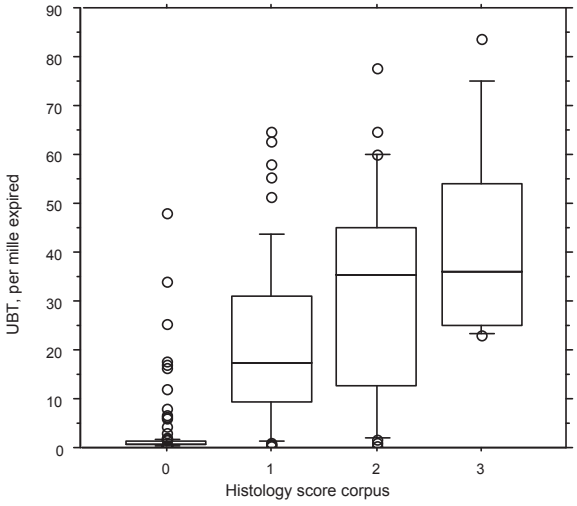
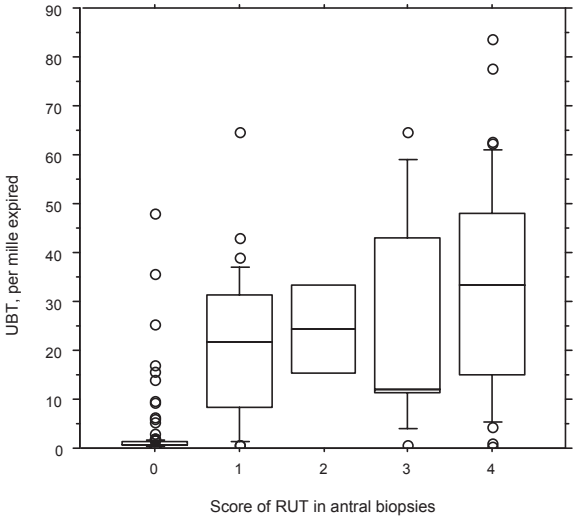
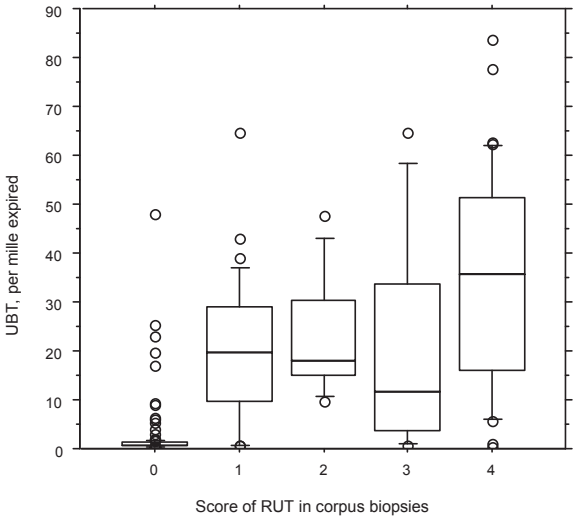


Figure 8. Boxplots (showing median and interquartile range) of relation between RUT score and histological score of *H. pylori* colonisation.

A



B



DISCUSSION

This thesis presents the results of studies on *H. pylori* infection and chronic gastritis as regards the relationship between macroscopic endoscopic changes in relation to microscopic findings. Furthermore background conditions associated with elevated homocysteine in relation to the risk of developing cardiovascular disease or dementia were explored in the same population during 10 years of follow-up. The natural history of chronic gastritis in terms of incidence of ulcer and development of atrophic gastritis was investigated and the accuracy of different diagnostic test for *H. pylori* infection was explored.

Paper I: The prevalence of erythema (any pattern) or erosions was 39% in the antrum and 18% in the corpus. Corresponding figures for visible vessels were 9% and 15%, respectively, and 17% had absence of rugae. Of all features analysed the only one, which may be of some clinical use, seems to be absence of rugae and/or visible vessels in the fully insufflated stomach indicating moderate to severe atrophic corpus gastritis. As regards the presence of *H. pylori* infection, the sensitivity of the macroscopic findings was in the range of that for moderate to severe chronic gastritis.

Lehman et al. investigated 175 healthy volunteers in one comparative study on *H. pylori* infection and erosions in the antrum with results similar to ours (120). Thirty-three subjects (19%) had macroscopic lesions on upper gastrointestinal endoscopy and of these 7 were *H. pylori* positive and 26 *H. pylori* negative. Gastric erosions occurred in 14 subjects (8%) of whom 4 were *H. pylori* positive and 10 *H. pylori* negative. Sixty-eight subjects (39%) had antral gastritis on histological examination. Bah et al. studied 66 predefined macroscopic features in 101 patients referred for elective upper gastrointestinal endoscopy (121). They found that the sensitivity and specificity for gastritis of an abnormal antral surface texture or antral erosions or a mammillated corpus surface for gastritis according to histological examination was 75 % and 63 %, respectively. The conclusion from that study is that it is not possible to diagnose *H pylori*-related gastritis on the basis of endoscopic findings. In another study by Calabrese et al. (122) on macroscopic features in 364 patients admitted for gastroscopy, a normal macroscopic picture was found in 15.1%, erythematous/exudative changes in 42.9%, chronic erosive changes in 13.7%, signs of atrophy in 8.2% and nodular changes in 20.1%. Endoscopic features correlated poorly with histology in antral biopsies, the sensitivity being 91.4% and the specificity 32.7 %. Our study gave results well in line with similar studies in this field. From ours and other studies quoted it is clear that the diagnosis of gastritis should be based on histological examination of the gastric mucosa.

If, for some reason, gastroscopy with biopsy is contraindicated and it is necessary to know whether chronic gastritis with or without atrophy is present, non-invasive determination of *H. pylori* status in combination with analysis of circulating levels of pepsinogen I, gastrin 17 and/or H^+K^+-ATPase antibodies may be of value (123, 124).

Paper II: Regarding determinants of Hcy levels, GLM analysis showed a positive relationship between S-Hcy and age, male gender, MTHFR 677 TT genotype and atrophic gastritis (both as determined morphologically and functionally with PGI/PGII). Not unexpectedly, there was a negative relationship between S-Hcy and renal function, S-vitamin B12 and S-folate. These findings are in accordance with those of other studies (90, 98, 105, 125-129).
We found an incidence of myocardial infarction and dementia in our study population at approximately the same level as reported from the Swedish National Board of Health and

Welfare (www.socialstyrelsen.se) (130). A strength of our study is the long follow-up time of 10 years (4796 person years for cardiovascular disease and 5262 person years for dementia) and that, simultaneously, putative causes of hyperhomocysteinaemia could be explored. We found no significant association between the MTHFR 677 TT genotype and Hcy levels in the univariate analysis. This is not in agreement with a previous study showing an association with mild hyperhomocysteinaemia(97). Recent studies have shown a reduced effect of the MTHFR 677 TT genotype in populations with high folate intake(131, 132). In the GLM analysis in the present study, this SNP had a significant impact on Hcy levels. The appearance of this significance may be explained by the inclusion of S-folate in the model. *H. pylori* per se had no significant impact on Hcy levels (114, 133).

There was no significant relationship between S-Hcy and occurrence of the duodenal mucosal atrophy, DM, or hypothyroidism in the GLM analysis. Others have found increased S-Hcy levels in these diseases (101, 102, 126). Since the number of cases in these groups in the present study was low we cannot draw any conclusions regarding their impact on Hcy levels.

Whilst using dichotomized S-Hcy values with a cut-off level of 14.5µmol/L we found a positive correlation between S-Hcy and the incidence of cardiovascular diseases; in fact the risk was doubled (OR 2.05 [1.14-3.70]). With S-Hcy as continuous variable the relation was weaker (OR 1.06 [1.00-1.12]). Exchanging S-Hcy for its causative variables, atrophic gastritis, MTHFR 677 TT genotype and elevated cystatin C taken together as an independent variable in the analysis, this yielded a weak if any association (OR 1.70 [0.92-3.13]). Since there are other causes of variations in Hcy levels than those studied here this lack of an association is not surprising.
Considering the association of S-Hcy with cardiovascular disease, our results are in accordance with findings in other prospective studies, although the cut-off levels for Hcy concentrations used vary between the studies (134-139). In one of these studies (134) which included 229 cases and 1126 controls (all men, mean follow-up time 8.7 years) there was a continuous dose-response relationship with a 41% (20%-65%) increased risk for death from ischemic heart disease for each 5- µmol/L increase in the S-Hcy level. In an other study by Arnesen et al. (135) of 122 cases and 478 controls, the relative risk for ischemic heart disease hospital discharges and deaths resulting from a 4 µmol/L increase in S-Hcy was 1.32 (1.05-1.65).
Whincup et al. (139), who followed a population for 12.8 years (men aged 40-59 years at, entry 386 cases and 454 controls), found that a 47 % increase in S-Hcy was associated with a slight increase in the OR for myocardial infarction of 1.15 (1.00-1.32). For values of S-Hcy higher than 16.5µmol/L, the OR for myocardial infarction was 1.77 (1.28-2.42). As shown in the comprehensive meta-analysis conducted by the Homocysteine Studies Collaboration, there are also prospective studies that show no correlation of Hcy levels to cardiovascular diseases(138). This was also the case in the study by Fallon et al. (140), which included 312 men with coronary heart disease and 1248 matched controls.

In a prospective study of 1 092 participants (667 women and 425 men) with a median follow-up time of eight years, Seshadri et al (109) found that increased P-Hcy is an independent risk factor for dementia and Alzheimer's disease. The relative risk of dementia was 1.4 (1.1-1.9) for each increase in one SD of logarithmically transformed Hcy values. The corresponding figure for Alzheimer's disease was 1.8 (1.3-2.5). In an Italian dementia-free cohort of 816 participants Ravaglia et al (106) related baseline P-Hcy to incident dementia, including Alzheimer's disease, during a mean follow-up interval of four years. They concluded that increased Hcy and low folate concentrations are independent risk factors. Hyper-

homocysteinaemia was considered to be present with a P-Hcy higher than 15.0µmol/L. With this cut-off level the hazard ratio for dementia was 2.08.

Paper III: Of 372 eligible subjects, 314 (84.4%) were re-examined after 2657.5 person years. With a longer interval, participation rate would have been lower due to co-morbidity and deaths. On the other hand, since the evolution of chronic gastritis is a slow process, a shorter interval might have been insufficient for documenting progression into atrophy (5, 8, 11, 93).

None of the participants acquired *H. pylori* infection during the follow-up interval. This is consistent with the observation that *de novo* infection generally occurs in childhood or youth (40, 141, 142). In a review of 15 population-based publications, Xia et al. found that the annual rate of seroconversion and seroreversion of *H. pylori* infection among adults was 0.2–3.8% and 0.0–2.8%, respectively (141). In a histological study by Niemala et al. 5 of 39 patients (12.8%) became *H. pylori*-negative over a 10-year period (143). Another patient-based histological follow-up study by Villako et al. (n = 139) showed that *H. pylori* infection had disappeared in the antrum and corpus of 9% and 10%, respectively, over a six-year period. The corresponding frequencies for acquiring *H. pylori* infection were 9% and 11%, respectively (144).
At follow-up examination, *H. pylori* status changed from positive to negative in 11 of 113 participants (9.7%). One of these 11 participants had unchanged moderate atrophic corpus-predominant gastritis, and two progressed from mild to severe atrophy of the corpus mucosa. In these three participants, the disappearance of the *H. pylori* infection may be explained by the fact that development of significant atrophy can be associated with the disappearance of the *H. pylori* infection (2). None of the other 8 participants developed significant atrophy. Underreporting of antibiotic use might explain some of these cases.

The incidence of ulcer was 0.45 per 100 person years. This figure must be considered an approximation, since subclinical ulcers may have occurred and resolved during the follow-up period (24). The incidence is somewhat lower than that reported in other studies from the 1990s (37, 38). In a study of 454 outpatients, Sipponen et al. found a 10-year cumulative risk for ulcer of 10.6% among patients with chronic gastritis and of 0.8% in patients without gastritis (37). In the present study, the incidence of ulcer was 0.58 per 100 person years among participants with chronic gastritis. The study by Sipponen et al. only included patients with dyspepsia, which might explain the difference.
In general, weekly use of NSAIDs and smoking decreased, whereas alcohol consumption increased. There was no significant change in the occurrence of risk factors among the 11 participants with incident subclinical ulcer. Logistic regression analysis showed that the occurrence of ulcer was dependent on these three risk factors, but not on *H. pylori* status. The latter finding could be explained by the low number of detected ulcers. However, others have recently reported a relatively high proportion of idiopathic uncomplicated ulcers in the general population (36) as well as amongst patients with bleeding ulcer (145). We found that 3 of 12 ulcers were idiopathic. In our prevalence (baseline) study published in 2000, which included 501 volunteers, prepyloric or duodenal ulcer was diagnosed in 13 (2.6%) participants, 12 of whom had positive *H. pylori* status (24).
In the Kalixanda population based prevalence study (n = 1,001) published in 2006, 4.1% had benign ulcer (20 gastric and 21 duodenal) (36). Eight (38.1%) subjects with duodenal ulcer lacked evidence of *H. pylori* infection. Five (25.0%) of the gastric ulcers and 4 (19.0%) of the duodenal ulcers were classified as idiopathic. Smoking, NSAID intake and high BMI were risk factors for gastric ulcer and smoking, NSAID use and *H. pylori* infection were risk factors for duodenal ulcer (36).

As shown in this study and in a study by Siurala et al. (42), age and the degree of inflammation in the corpus mucosa are independent determinants of atrophy development. Among participants with chronic gastritis without atrophy of the corpus mucosa, the incidence of atrophy of the corpus mucosa was 1.4 per 100 person years. The corresponding figure for participants with *H. pylori*-associated chronic gastritis was 1.1 per 100 person years. There are very few histological studies of the incidence of atrophic gastritis. Orniston et al. found that 2 of 50 (4.0%) dyspeptic patients developed atrophy of the gastric corpus mucosa in a 5-year follow-up study (39). Villako et al. published a 12-year endoscopic population-based follow-up study with biopsy data showing that the rates of appearance and disappearance of atrophy in the corpus mucosa were quite similar (40). These findings contrasted with previous findings by the same group (146).

In an 18-year population-based follow-up study by Maaroos et al., atrophy of the corpus mucosa appeared in 22 out of 64 (34.3%) subjects and atrophy of the antral mucosa in 7 (10.9%) (43). We found great variability in the occurrence and degree of antral mucosal atrophy. As illustrated by the Kappa analysis, intra-examiner error was greatest for histological evaluation of antral mucosal atrophy. Furthermore, as indicated by the results of other prospective studies, it seems that regardless of *H. pylori*, even moderate-to-severe antral mucosal atrophy can revert with time (39, 40, 43).

Orniston et al. reported in a patient-based five-year follow-up study that gastritis is a variable process (39). In the present study, chronic gastritis disappeared in 18 of 27 participants without *H. pylori* infection. Furthermore, chronic gastritis appeared in 15 of 173 participants without gastritis or *H. pylori* infection. It seems that chronic gastritis without *H. pylori* infection is frequently a reversible and temporary condition. This could be explained by variations in the occurrence of risk factors, such as use of NSAIDs, alcohol consumption and smoking. However, as illustrated in this study, some subjects (4/27) presented with significant corpus-predominant atrophy and negative *H. pylori* status upon initial screening (baseline). The degree of atrophy in these subjects was unchanged at follow-up examination. Among 139 participants with gastritis at baseline, mild atrophy of the corpus mucosa had disappeared in 7 and moderate atrophy in 1. There was good agreement between the histological findings and the results of the pepsinogen analyses. The PGI/PGII ratio decreased with age even in the absence of chronic gastritis. Although it is somewhat controversial, other studies have shown similar results (89). An increase in the frequency of subnormal PGI/PGII was observed only in participants with atrophy of the corpus mucosa.

Paper IV: Primary diagnosis and check of treatment success of *H. pylori* infection is crucial for patients with uncomplicated or complicated ulcer disease, MALT lymphoma, atrophic gastritis, previous partial gastric resection for gastric cancer and probably also for *H. pylori* infected patients starting long term medication with NSAID or low dose ASA (48, 147).

The methods studied included serology, UBT, RUT, culture and histological examination. Concordance between the tests according to Cohen's kappa analysis was calculated and we used sensitivity, specificity, PPV (precision), NPV and accuracy to evaluate which test or combination of tests may be recommended.
Potential sources of error with invasive tests are that the number of gastric biopsies are too few and that the main compartments of the stomach are not represented (66, 68). In the present study, three biopsies from each location were analyzed histologically. According to Cohen's kappa analysis the intra-examinator error for histological diagnosis of *H. pylori* colonisation was low. RUT and culture, respectively, were performed on one biopsy from

each location. Collection of more than one biopsy from each location for these tests could potentially have influenced the results. A potential error in the UBT is use of PPI prior to testing. The participants in the study were instructed to avoid PPI two weeks before the examination. Participants on PPI medication for gastro-esophageal reflux disease, for example were prescribed low dose H2 blockers during the two weeks preceding UBT.
Agreement between the tests according to Cohen's kappa analysis was best (0.91) for culture (location in the stomach disregarded) and UBT. A similar result between RUT and culture (0.90) was found. Agreement between the latter two was better in the antrum (0.90) than in the corpus (0.84).
We chose to use accuracy as a measure of the performance of the tests. Of the two non-invasive tests, UBT showed the highest accuracy (0.94 vs. 0.86 for serology). Considering the invasive methods, results were quite similar; RUT (0.94), culture (0.93) and histology (0.93). The accuracy of the invasive tests was slightly lower (0.91-0.93) in the corpus than in the antrum (0.94-0.95). We found no studies reporting accuracy of the different diagnostic tests.

In this study the lowest sensitivity was for UBT (0.92) and the highest for culture (0.99). Considering the specificities the lowest was for serology (0.82) and the highest for UBT (0.94). PPV was lowest for serology (0.66) and highest for histology (0.81), whereas NPV only differed slightly between the tests (0.97-0.99).

In a study from 2009 by Calvet et al. on 118 patients using a pre-defined gold standard (more than one positive test result), the performance of ^{13}C UBT, RUT, microscopic examination and fecal tests was evaluated (70). The PPVs found in that study were somewhat higher than those in the present study (RUT 1.0 vs. 0.83, histology 0.99 vs. 0.81, UBT 0.92 vs. 0.87). These differences may partly be explained by the fact that only antral biopsies were examined in that study, whereas both compartments of the stomach were examined in the present one. We found a lower test accuracy in the corpus than in the antrum and furthermore Calvert's study involved two pathologists as opposed to one in the present.

In a study by Cutler et al. (44) using several tests taken together as gold standard, sensitivity, specificity, PPV and NPV were calculated for ^{13}C UBT, serology, RUT, microscopic occurrence of *H. pylori,* and chronic and acute gastritis. Considering the first four tests differences in results between that study and the present were minor regarding sensitivity and specificity, whereas differences were greater for predictive values, that is, PPVs were lower (UBT 0.87 vs. 0.98, RUT 0.83 vs. 1.0, serology 0.66 vs. 0.97 and histology 0.81 vs. 0.99) and NPVs higher in the present study. This finding may be related to differences between the studies with regard to the number and location of biopsies collected and that chronic gastritis was included among the diagnostic methods in the referred study (44). Furthermore, there was a difference in the administered dose (150 mg vs. 50 mg) of urea and the time interval (60 min. vs. 30 min) until the reading of the UBT.

Average values of sensitivity and specificity of invasive and non-invasive tests were calculated in an overview of epidemiology and diagnosis of *H. pylori* infection by Logan et al (47). Our results were were within their range. The sensitivity and specificity of microscopical examination was 88-95% and 90-95%, respectively. Corresponding values for culture were 80-90% and 95-100% and 90-95% and 90-95% for the urease test. For serology the sensitivity was 80-95% and the specificity 80-95%. Sensitivity and specificity for ^{13}C UBT was 90-95% and 90-95%, respectively. Culture was mentioned as the theoretical gold standard.

In a review by Chey et al(63), in the guidelines from the American College of Gastroenterolgy the urease test showed a sensitivity of more than 90% and specificity of more than 95%. Corresponding values for histology were more than 95% and more than 95%. The sensitivity of serology ranged between 76% and 84%, and the specificity between 79% and 90%, respectively. For UBT the sensitivity and specificity were both higher than 95%. Histological examination was used as gold standard.

Conclusions

- Macroscopic features observed during gastroscopy are of very limited value in the evaluation of whether or not gastritis or *H. pylori* infection is present. We emphasise that gastritis is a histological diagnosis.
- Hcy concentrations in circulation are dependent on gender, age, the levels of vitamin B12 and folate, renal function, the occurrence of atrophic gastritis and the MTHFR 677 TT genotype.
- There is a positive relation between S-Hcy levels and the occurrence of cardiovascular disease.
- The incidence of ulcer in this cohort was 0.45 per 100 person years overall and 0.58 per 100 person years among subjects with chronic gastritis.
- NSAID, alcohol and smoking were found to be risk factors for ulcer disease.
- The incidence of atrophy of the corpus mucosa in this cohort was 1.4 per 100 person years for gastritis overall, and 1.1 per 100 person years for *H. pylori* associated gastritis.
- Chronic gastritis with or without *H. pylori* infection is a variable process in which milder degrees of atrophy of the corpus mucosa could appear or disappear. In contrast, it seems that moderate-to-severe atrophy rarely goes into regress.
- Age and the degree of chronic inflammation in the gastric corpus mucosa were risk factors for the development of atrophy.
- There are only minor differences in accuracy between the three invasive tests for *H. pylori* infection. The accuracy of UBT was comparable to that of the invasive tests and it is recommended for situations where endoscopy is not necessary.
- RUT may be recommended as the first invasive choice of diagnosing *H. pylori* infection.

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Summary in Swedish/Populärvetenskaplig sammanfattning på svenska

Magsår och magsäckscancer är sedan länge vanliga sjukdomar i stora delar av världen, så även i Europa. Livshotande komplikationer med blödning och hål på magsäcken förekommer regelbundet. Främsta orsaken till magsår och magsäckscancer är magsäcksinflammation (kronisk gastrit) som till största delen beror på infektion med bakterien *Helikobakter pylori (H. pylori)*. Hos en del personer med *H. pylori* infektion utvecklas under åren förtvining (atrofi) av körtlarna i magsäcken. Atrofisk gastrit anses vara den viktigaste riskfaktorn för cancer i magsäcken.

År 2005 fick Robin J Warren och Barry J Marshall från Australien Nobelpriset för upptäckten av *H. pylori*. I västvärlden syns en spontant sjunkande förekomst av *H. pylori* medan den i utvecklingsländer fortfarande är mycket vanlig. En del läkemedel samt ännu okända faktorer kan också bidra till utvecklingen av kronisk gastrit och dess följdtillstånd.
Vi har undersökt hur magsäcksslemhinnan ser ut i ett gastroskop jämfört med den mikroskopiska bilden i slemhinneprover från magsäcken, relationen mellan kronisk gastrit och homocystein samt utvecklingen av hjärtkärlsjukdom i samma grupp och naturligt förlopp vid kronisk gastrit samt avslutningsvis hur man säkrast påvisar *H. pylori* infektion.

Den här avhandlingen grundar sig på undersökningar av 501 frivilliga Linköpingsbor.
Först genomfördes gastroskopi för att jämföra den makroskopiska bilden av magsäcksslemhinnan med mikroskopiska fynd i slemhinneprover. Jämförelser gjordes med rodnader av olika slag, vita/gula små ytliga jämna slemhinnedefekter eller små punktformiga blödningar samt frånvaro av slemhinneveck i mellersta delen av magsäcken eller närvaro av synliga blodkärl i magsäcksslemhinnan och mikroskopisk inflammation.
Vi kunde inte se några samband mellan de makroskopiska och de mikroskopiska fynden förutom frånvaro av slemhinneveck och synliga blodkärl vid atrofisk gastrit. Slutsatsen blir att diagnosen gastrit ställs med mikroskopisk undersökning av magsäcksslemhinnan.

Vi studerade även relationen mellan kronisk gastrit m.fl. tillstånd och homocysteinnivåerna (en aminosyra) i blodet samt relationen till hjärtkärlsjukdom och demens under 10 års uppföljning. Samma personer var undersökta för kronisk gastrit samt hade besvarat ett frågeformulär om sjukdomshistoria och livsstilsfaktorer. Blodprov togs precis innan gastroskopierna genomfördes, och analyserades för pepsinogen I & II (magsäckens funktion), vitamin B12, folat, homocystein och cystatin C (njurfunktionen) samt genetiska faktorer. Sjukdomsutvecklingen följdes 8-10 år efter första undersökningarna. Analyser utfördes för att undersöka bakgrundsfaktorer såsom kön, ålder vid start av studien, uppföljningstid, BMI, rökning, alkohol samt NSAID användning (läkemedel mot inflammation och smärta).

Vi fann en positiv relation mellan kön, ålder, vitamin B12, folat, njurfunktion, atrofisk gastrit, genotyp MTHFR 677TT och förhöjt homocystein vilket i sin tur var positivt relaterat till utvecklingen av hjärtkärlsjukdom. Slutsatsen blir här att atrofisk gastrit m.fl. tillstånd kan ge högt homocystein vilket i sig ser ut att vara en riskfaktor för hjärtkärlsjukdom.

Det tredje arbetet fokuserade på den naturliga utvecklingen av *H. pylori* infektion, kronisk gastrit och magsår efter 8,4 års uppföljning i samma undersökta befolkningsgrupp. Av alla tidigare deltagare kunde 314 undersökas. Gastroskopi med vävnadsprov och bedömning av *H. pylori* förekomst utfördes liksom analys av pepsinogen och livsstilsfaktorer.
I denna studie var nyinsjuknandet i magsår 0.45 per 100 personer och år. Riskfaktorer för magsår var rökning, alkohol och NSAID, *H. pylori* fann vi inget samband med just här.

Utvecklingen av atrofisk gastrit var 1.4 per 100 personer och år med en tydlig relation till ålder och grad av kronisk inflammation vid start. Atrofin var oförändrad i avancerade fall.

I det sista arbetet undersöktes känsligheten i diagnostik av *H. pylori* hos fem olika diagnostiska metoder med eller utan gastroskopiundersökning. De diagnostiska metoder som jämfördes var utandningstest, blodprov, RUT (slemhinneprov, enzymreaktion), odling och mikroskopisk diagnostik av magsäcksslemhinnan. Utandningsprov visade störst känslighet av prover utan gastroskopi. De gånger man gör en gastroskopi så kan man ta ett s.k. RUT test för säker och enkel diagnos, om man tar minst två slemhinneprover utan samtidig närvaro av blod i magsäcken. Odling och mikroskopisk undersökning är nästan lika säkra men kräver extra procedurer, de kan användas vid speciella behov. Blodprover uppvisar sämre träffsäkerhet.

Sammanfattningsvis framkommer i denna avhandling att gastrit är en diagnos som ställs med mikroskopisk undersökning samt att kronisk gastrit är en orsak till förhöjt homocystein vilket i sig kan vara en riskfaktor för hjärtkärlsjukdom. Naturlig utveckling av kronisk gastrit går långsamt, nyinsjuknandet i magsår är 0.45 per 100 personer och år. Atrofi av magsäcksslemhinnan utvecklas hos 1.4 per 100 personer och år. Om man ska diagnostisera *H. pylori* utan gastroskopi så är utandningstest säkrast medan RUT (enzym) test verkar säkrast om man gör en gastroskopi.

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