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HELICOBACTER PYLORI INFECTION AND ASSOCIATED STOMACH PATHOLOGY IN THE ADULT GENERAL POPULATION

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At last !

To Åsa and our boys

ABSTRACT

The discovery of *H. pylori* infection has had a tremendous impact on the understanding and treatment of upper gastrointestinal diseases. It causes gastritis, sometimes developing to atrophy and intestinal metaplasia (IM), both considered as premalignant conditions and it is an important risk factor for peptic ulcer and non-cardia gastric cancer. The purpose of this thesis was to explore aspects of the infection in the general adult population. A random sample of 3,000 adults aged 20-80 years (mean age 50.4), from two Swedish municipalities (n=21,610) was surveyed using a validated postal abdominal symptom questionnaire with a response rate of 74%. A representative sub-sample of the responders (n=1,000) was investigated, in random order, by upper endoscopy and histology. Blood samples were drawn for *H. pylori* serology and assessment of other biomarkers. Medical history was taken and repeated recording of symptoms were made. .

Diagnosis of current infection can be made by histology and/or culture, while serology also detects past infection. The diagnosis of atrophic gastritis and IM is based on histology. We found that 43.0% of all individuals were positive on *H. pylori* serology, 33.9% had signs of current infection on either histology or culture, and 9.3% were seropositive but negative on histology and culture. Corpus atrophy and IM was found in 10% and 13%, respectively, in those with current infection, and in a significantly higher proportion, 17% and 21%, respectively, in those with only serological evidence of past infection. Among those who were sero-negative, the corrsponding proportion of individuals were 1% and 2%, respectively.

Gastric precancerous lesions (atrophy and IM) are often found in the junctional (incisura and gastro-esophageal junction) mucosa. Non-cardia gastric cancer is most common in the antral mucosa, and thus a proximal *antralization* might imply increased cancer risk.We found *antralization* at the incisura in 45% of all 1000 subjects and it was significantly more common in *H. pylori* infected and in smokers. Atrophy and/or IM were present in 9.8% of those with antralization. In the cardia, 7.3% had corpus mucosa and 92.7 % had cardia mucosa. Among the latter subjects, 10.3 % had atrophy/IM compared to 2.8 % among the former, p=0.04.

Diagnosing *H. pylori* infection and atrophy by means of serological biomarkers has some advantages. The overall agreement between histology and serological biomarkers for diagnosing corpus atrophy in this study was 96%. The sensitivity and specificity of markers for atrophic gastritis were 71% and 98%, respectively, and the Positive Likelihood ratio was 35.5. Only 0.8% of individuals with no histological evidence of corpus atrophy were positive for atrophy based on biomarker assessment. Biomarkers detected corpus atrophy in 6.6% of all study subjects.

Triple therapy for *H. pylori* eradication includes two antimicrobials and a proton pump inhibitor. Knowledge of antimicrobial resistance is crucial. We found that 16.2 % of the *H. pylori* strains were resistant to metroniazole, 1.5 % to clarithromycin, 0 % to amoxicillin and 0.3 % to tetracycline.

Conclusions: The prevalence of current *H. pylori* infection in a random sample of an adult population in two municipalioties in Sweden, was 33.9% and a further 9.3% had serological evidence of past *H pylori* infection. The prevalence of corpus atrophy and/or IM was higher among the latter. We found that *Antralization* is related to *H. pylori* infection and smoking. Serological biomarkers for current and past *H pylori*-infection are useful for corpus atrophy detection. Antibiotic resistance to *H. pylori* was low in the study population.

Key-words: *H. pylori* infection, gastritis, gastric mucosal atrophy, antibiotic resistance, serological biomarkers, population based, epidemiology

LIST OF PUBLICATIONS

I **Storskrubb T**, Aro P, Ronkainen J, Vieth M, Stolte M, Wreiber K, Engstrand L, Nyhlin H, Bolling-Sternevald E, Talley NJ, Agréus L. **A negative Helicobacter pylori serology test is more reliable for exclusion of premalignant gastric conditions than a negative test for current H. pylori infection: a report on histology and H. pylori detection in the general adult population**. Scand J Gastroenterol. 2005 Mar;40(3):302-11.

II **Storskrubb T,** Talley NJ, Walker MM, Aro P, Ronkainen J, Sipponen P, Vieth M, Stolte M**,** Agréus L. **Cancer risk factors in gastric incisura and cardia: An adult endoscopic population based study (Kalixanda).** Submitted

III **Storskrubb T**, Aro P, Ronkainen J, Sipponen P, Nyhlin H, Talley NJ, Engstrand L, Stolte M, Vieth M, Walker MM ,Agréus L **Serum biomarkers provide an accurate and non-invasive screening method for diagnosis of atrophic gastritis compared with "gold standard" histology in a large population sample. The Kalixanda study.** Submitted

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

CONTENTS

LIST OF ABBREVIATIONS

ASQ	Abdominal symptom questionnaire
CDG	Corpus dominant gastritis
DDD	Defined daily dosage
EGD	Esophagogastroduodenoscopy
ELISA	Enzyme-linked immuno sorbent assay
GERD	Gastroesopgageal reflux disease
GERS	Gastroesophageal reflux symptoms
H.pylori	*Helicobacter pylori*
H_2RA	Histamine$_2$ receptor antagonist
IM	Intestinal metaplasia
MIC	Minimal inhibitory concentration
NPV	Negative predictive value
PPI	Proton pump inhibitor
PPV	Positive predictive value
UBT	Urea breath test

1 INTRODUCTON

1.1 ABOUT *HELICOBACTER PYLORI*

1.1.1 History

When Robin Warren and Barry Marshall in 1982 described "unidentified curved bacilli on gastric epithelium in active chronic gastritis"[1]it was the start of an intensive development in the understanding of gastric and duodenal bulb pathology such as gastritis, peptic ulcer disease and gastric cancer.

The main research focus before this landmark discovery, was on the role of gastric acid and the hypothesis that these upper GI diseases could have an infectious course was only proposed by a few researchers [2]. There had, however, been reports of curved bacilli in the stomach of both animals and man for more than a 100 years as the "bacterial hypothesis" was articulated already in 1875. Bizzozero reported curves bacilli in the stomach of dogs in 1893 [3]. In 1940 Freedberg and Barron described spirochetes, in vivo, in human stomach samples. Already in the 50´s, the Greek physician John Lykoudis was convinced that peptic ulcer disease was caused by a microbial agent and in one tablet, he combined a mixture of several antimicrobial agents and vitamin A for treatment of gastritis and peptic ulcer disease. Lykoudis tried to convince fellow physicians, authorities and pharmaceutical companies about his treatment method but without success and even with legal consequences [2].

The closest call to a breakthrough was in 1975 when Steer and Colin-Jones described bacteria in the inflamed mucosa of gastric ulcer patients and the absence of these bacteria in normal mucosa [4]. They classified the bacteria found as pseudomonas-like but what they saw was most likely *H. pylori* and this was almost 10 years before Warren and Marshall described and classified this bacterium, first named *Campylobacter pylori* but later renamed to its present name in 1989 [5]. When Warren and Marshall proved the presence of numerous spiral bacteria in the human stomach and its association with chronic active gastritis, they fulfilled Koch's first postulate and with the unintentional but successful culture of *H pylori* over Easter in 1982, they also fulfilled Koch's second postulate. Following ingestion of a culture of *H pylori* Barry Marshall developed histological gastritis within10 days after ingestion and Koch's third postulate was fulfilled [6]. Morris in New Zealand did the same thing shortly thereafter and his infection was cleared with a "triple therapy" regimen as shown by negative cultures and only minimal residual chronic gastritis in his biopsies [7], this fulfilling Koch's fourth postulate.

Table 1. Koch`s postulates [8]

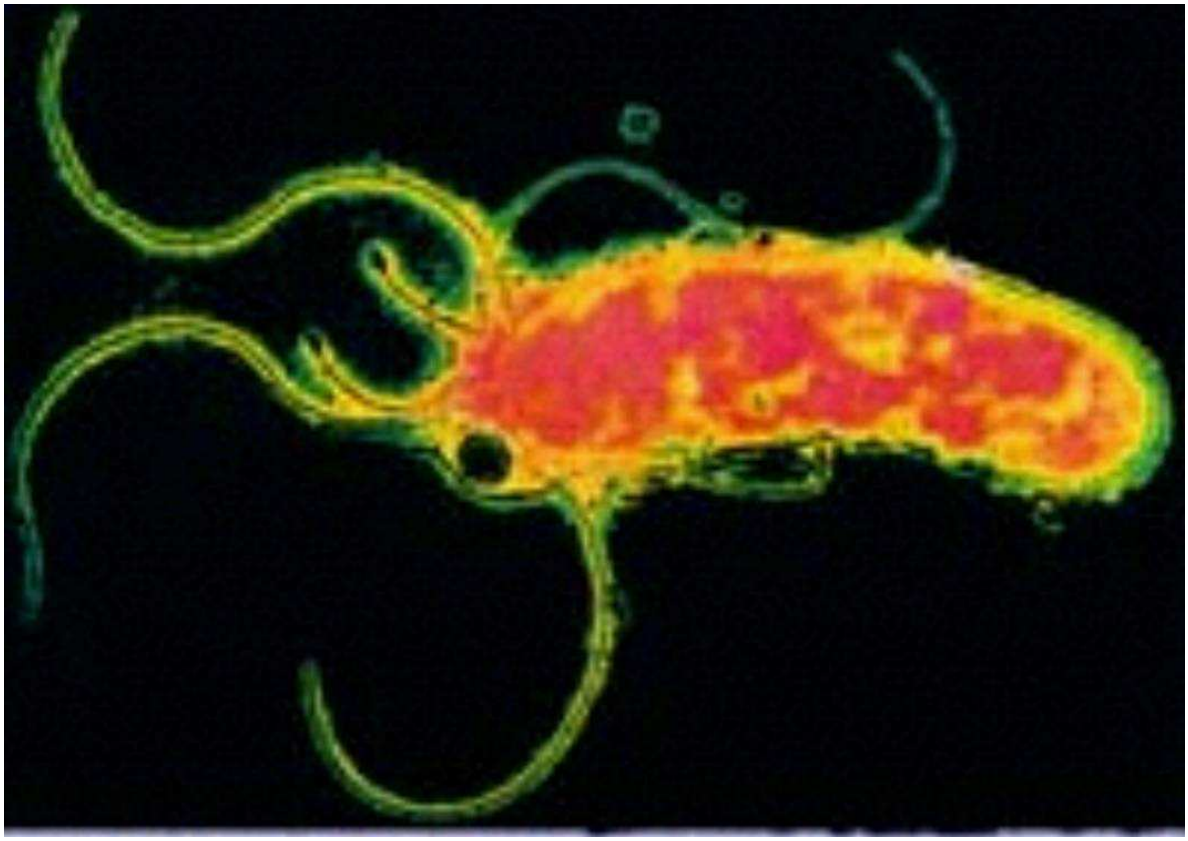
I	"The organism must always be found in the diseased animal but not in healthy ones"
II	"The organism must be isolated from diseased animals and grown in pure cultures away from the animal"
III	"The organism isolated in pure culture must initiate and reproduce the disease when re-inoculated into susceptible animals"
IV	"The organism should be re-isolated from from the experimentally infected animals"

1.1.2 Microbiology

The *Helicobacter* genus belongs to the Spirillaceae family. It is a spiral shaped organism (2-4µm long) with several flagella located at one of short ends of the bacterium.as shown in picture 1. The flagellae makes *H. pylori* highly mobile. There are several different *Helicobacter* species, mainly found in different animals species [9]. The possible pathological role of these other *Helicobacter* species in animals is unclear.

H. pylori is found in the human stomach mucosa, mainly in the mucus layer but also adherent to the gastric mucosal cells and it is highly interactive with the host immune system. *H. pylori* prefers a microaerophilic environment and it grows slowly on supplemented blood agar plates, taking seven days to show visible colonies. *H. pylori* is a gram-negative, urease-, catalase- and oxidase positive rod [10].

Picture 1. H.pylori caught by electron microscopy



1.1.3 Colonisation and virulence factors

H. pylori has developed several mechanisms enabling it to colonise the hostile environment in the stomach [11]. The capability *H. pylori* has to produce large amounts of urease and convert urea to ammonia and carbon dioxide. This creates a microenvironment with elevated pH which seems to be important for survival of the bacterium. The flagellae gives *H. pylori* motility in the mucus layer, which is essential when it colonizes the gastric mucosa [12].

H. pylori expresses several adhesion molecules on its surface in order to make adhesion to the gastric epithelium possible. Binding is to Lewis^{b} antigen, where laminin and hemagglutinins are receptors[13].

The Cag pathogenicity island is a major virulence factor containing 31 putative genes including CagA. About 60% of *H. pylori* strains isolated in Western countries are CagA positive while the corresponding figure in East Asian strains is almost 100% [14]. There are indications that CagA positive strains are associated with more intense gastric inflammation and subsequent atrophic gastritis and possibly carry a greater risk for gastric cancer. However, it is still not possible to identify which strains are associated with gastric morbidity, in order to guide treatment. .

1.2 EPIDEMIOLOGY

1.2.1 Prevalence and geographical distribution

H. pylori is prevalent worldwide and it is estimated that it colonises the gastric mucosa of 50% of the global population. The distribution is not even though. In areas with high socio-economic conditions such as Europe and North America, the prevalence among adults is 20-50 %, whereas the corresponding figures in developing countries is 80-90% [15]. In children, the corresponding figures vary from less than 10% to more than 80%, respectively [16]. The prevalence rises with age which is considered to be a cohort phenomenon, i.e. more people were infected as a child in the earlier days when the socio-economy was less developed [17].

In the developing parts of the world the annual incidence in childhood is as high as 20% compared to 1-10% in high-income areas [18-21]. The overall prevalence in Sweden was estimated to 38 % based on serology among adults in a study from 1995 [22]. Bergenzaun et al found corresponding results in Sweden and Iceland [23] as did the EUROGAST Study Group in comparable Northern European countries [24].

1.2.2 Transmission

Humans are the only known host of *H.pylori*. The route of transmission is not yet clearly understood [17]. It is either gastro-oral (in developed countries water could be a vehicle) or faecal-oral (in developing countries) [25]. The bacteria has been cultured from saliva, gastric content and faeces [16, 26, 27]. The most common transmission is between members of the same family, especially between mother and child [16, 28]. Family size and crowding also seem to be risk factors for infection [29].

1.3 DIAGNOSTIC METHODS FOR *H. PYLORI* INFECTION

1.3.1 General considerations

There are several different methods for diagnosing *H. pylori* infection. Some methods requires upper GI-endoscopy and mucosal biopsies and are thus named invasive, while others rely on blood-, breath- or faecal samples and are referred to as non-invasive. All tests that are based on identifying living bacteria on the mucosa are affected by concomitant acid suppression therapy, mainly proton pump inhibitors (PPI). The intragastric pH elevation caused by PPI leads to redistribution of *H. pylori* and alteration of the histological picture; the amount of bacteria, as well as the inflammation, is reduced in the antrum but increased in the corpus and fundus. Also the total amount of *H.pylori* bacteria seems to decrease. Due

to this, invasive methods can be false negative during PPI therapy in as many as 30-40% of the cases [30].

1.3.2 Invasive methods

1.3.2.1 Histology

H. pylori has a characteristic spiral appearance making it relatively easy to identify during histological evaluation. It can be seen on Hematoxylin-Eosin, Warthin-Starry or modified Giemsa staining. Histology also gives additional information about the grade of inflammation in the gastric mucosa by evaluating the infiltration of granulocytes and lymphocytes as well as presence of gatric atrophy and intestinal metaplasia. Biopsies needs to be taken both from the antrum and the corpus and at least two biopsies are needed from each location [31]. Specificity is high but sensitivity can vary due to the patchy distribution of the bacteria [32, 33].

1.3.2.2 Culture

Biopsies from the gastric mucosa are cultivated in a microaerophil environment for up to seven days. Colonies of bacteria can typically be seen after 4-5 days. The colonies are identified as *H. pylori* by typical appearance of the colonies and by urease-, catalase- and oxidase tests and Gram-staining. The accuracy of *H.pylori* culture is considered to be high and it is dependent on the laboratory settings and low-temperature transportation of the samples.

1.3.2.3 Rapid urease test

Biopsies from the gastric mucosa are placed in a gel containing urease and a pH-dependent colour indicator. If the sample contains active *H. pylori*, its urease will convert urea to ammonia and bicarbonate which will raise in pH and lead to a colour change in the gel from yellow to red. The sensitivity and specificity are both about 95 % respectively. If the patient is infected with *H.pylori*, the test will be positive in 3 hours in 90% of the cases and the final evaluation is possible after 24 hours [34].

1.3.3 Non-invasive tests

1.3.3.1 In-office tests

In-office, enzyme-linked immuno sorbent assay (ELISA) -based tests detecting *H. pylori* antibodies, are easy to use but due to lack of accuracy they are not in wide-spread use [35].

1.3.3.2 Serology

H. pylori infection induces an immune response from the host, resulting in specific antibodies against the bacteria, mainly IgG but also IgA. These antibodies are best detected by the ELISA methods. Serology is a suitable method to diagnose *H. pylori* infection in large scale epidemiological settings and in screening protocols because it is easy to perform and non-invasive. The serology test used has to be customized (or calibrated) to the population investigated [36, 37]. There is a risk of false positive results since circulating antibodies against *H.pylori* can persist for years after successful eradication [38-40]. Due to this, serology is not so suitable for monitoring eradication success. The number of false positive results on serology increases with rising patient age decreasing the specificity to 75 % in patients over 65 years of age [41]. When excluding patients with false positive serology and atrophic gastritis, the specificity in the older age groups rises to 93-97 % [41]. Serology may thus be used to diagnose *H. pylori* in situations with patchy distribution of

the infection or when minimal colonization of bacteria is at hand due to mucosal atrophy [33] or in an episode of upper gastro-intestinal bleeding. The detection of cagA antibodies with immunoblot can detect past infection when circulating IgG-antibodies no longer can be found with ELISA [42].

1.3.3.3 Urea Breath Test (UBT)

^{13}C or ^{14}C breath-test are based on the same principle as the rapid urease test. Urea labelled with a carbon isotope, usually ^{13}C, is ingested and if *H. pylori* is present in the stomach, the isotopecan be detected, as radiolabelled CO2, in the exhaled air. The C^{14} method is not so widely used because of its higher radioactivity. The C^{13}-method requires a mass spectrometer for the analysis, and the samples can be sent by ordinary mail without loss of accuracy. The results of the UBT is influenced by concomitant use of acid suppressive agents and such treatment should be stopped one week before the test is performed, although some results indicate that the test is also accurate under acid suppression, when the test includes a meal including citric acid [32, 43]. UBT is suitable for monitoring outcome of treatment [43, 44].

1.3.3.4 Stool Antigen Test

The stool antigen test detects presence *H. pylori* antigen in the stool, either by poly- or monoclonal antibodies where the latter seems to be the more accurate. It is suitable both for primary diagnosis and monitoring treatment outcome. The best accuracy of this test is achieved in previously untreated individuals where it has a sensitivity of 91% and a specificity of 94%. However, slightly conflicting test results have been seen in studies assessing *H. pylori* eradication, 4-8 weeks post treatment. In-office stool antigen tests are now available and encouraging results have been reported with a mean sensitivity and specificity of 95 and 87 % respectively, and test results are available in about 10 minutes. These tests are also used in hospital laboratories. Concomitantuse of proton pump inhibitors affects the test accuracy negatively, but this effect disappears1-2 weeks after withdrawal of the medication [45].

1.4 *H.PYLORI*, GASTRITIS AND POTENTIALLY PREMALIGNANT FINDINGS

1.4.1 Normal stomach histology and secretion

The gastric mucosal surface is lined primarily with a simple layer of columnar epithelial cells. They secrete mucus into the gastric lumen via exocytosis and the primary role of the neutral mucus and the secreted bicarbonate is luminal cytoprotection from acid, pepsin and ingested substances including pathogens. The surface epithelial lining is invaginated by gastric pits or foveolae, which provide access to the gastric lumen for the gastric glands. The gastric glands in the different anatomic regions of the stomach are lined with different types of specialized epithelial cells allowing differentiation of these regions by type of glands.as shown in.Picture 2.

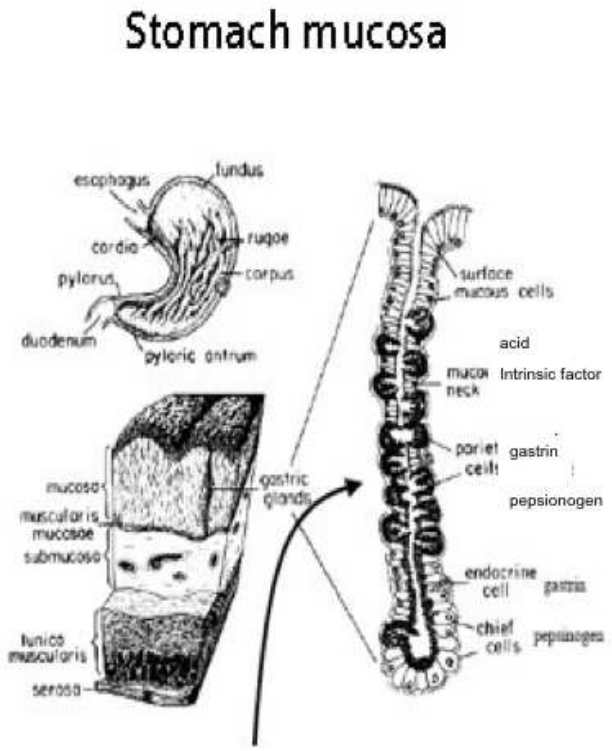
In the *cardia*, a 1,5-3 cm long transition zone from esophageal squamous epithelium to gastric columnar epithelium, the glands are populated by mucous, endocrine and undifferentiated cells.

In the acid-secreting corpus and fundus, the oxyntic (or parietal) glands are found and they are composed of parietal (oxyntic), chief, endocrine, mucous and undifferentiated cells. These glands are responsible for the secretion of acid and intrinsic factor (parietal cells),

pepsinogen I and II (chief cells), somatostatin and histamine (endocrine cells) and mucous (mucous cells).

In the more distal part of the stomach, i.e antrum and pylorus, glands are composed of endocrine G-cells producing gastrin and D-cells which produce somatostatin, a potent inhibitor of gastrin secretion. The secreted gastrin is a mixture of different acid stimulatory gastrins, of which amidated gastrin-34 and-17 are the dominant forms in plasma/serum in healthy humans and gastrin-17 is the predominant and potent tissue form[46]. Also, parietal cells producing pepinogen II and mucous cells are found in the antral glands.
Under the epithelial layer the basement membrane is found and immediately below that membrane, the lamina propria is located containing different leucocytes, fibroblasts and endocrine-like cells. A few lymphatic channels and the mucosal capillary plexus are also found in the lamina propria.

Picture 2. The stomach mucosa.



1.4.2 About Gastritis

The diagnosis of gastritis is histological and should be evaluated according to the updated Sydney system [31]. Histological gastritis is not considered to trigger symptoms and the term gastritis is often used inadequately as a synonym for dyspepsia. For histological evaluation at least two biopsies should be taken from both the antrum and corpus. The start of the gastritis research reaches far back into the 19th century, and into early decades of the 20th century [47].

Modern aspects in classification of gastritis and knowledge of the biological course of gastric inflammation as well as its links to chronic gastritis and several important gastric disorders were well known even before the discovery of *H. pylori* in 1982. This discovery, however, significantly changed the field and raised the interest in gastritis among

gastroenterologists. With the *H.pylori* discovery, chronic gastritis became a curable disease with known etiology and with its well established links to main gastric diseases, including gastric cancer.

1.4.3 Acute Gastritis

Infection with *H. pylori* leads to an inflammatory response in the gastric mucosa, i.e. gastritis. In the acute phase, the infection leads to dense infiltration of polymorphonuclear leucocytes into the gastric mucosa with formation of microabscesses and exudation of inflammatory cells to the mucosal surface. The whole stomach is affected in the initial phaseleading to an almost total loss of acid secretion for up to 40 days followed by normalisation within 2-3 months. This acute infection may lead to unspecific symptoms such as dyspepsia, nausea and diarrhoea for 1-2 weeks [6]. After this acute phase, the inflammation develops into chronic active gastritis with peak activity in the antrum.

1.4.4 Chronic Gastritis

1.4.4.1 *H. pylori* and Chronic Gastritis

The most common cause for chronic gastritis is *H. pylori* infection. After the acute phase, the amount of lymphocytes in the mucosa increases as a sign of chronic inflammation. The presence of both polymorphonuclear cells and lymphocytes in the mucosa characterizes chronic active gastritis where the former indicates the activity and the latter the chronic part of the inflammatory response. Lymphoid follicles may be present. The interaction between the hosts immune system and *H. pylori* leads to high levels of cytokines, mainly TNF-alfa, interleukins (IL-6, IL-8, IL-10) and leucotrienes (B4) in the gastric mucosa. In the majority of the cases, *H. pylori* infection leads to mild, chronic active gastritis with highest activity in the antrum and no further morbidity develops. This is most often the case in young people.

In some of the infected individuals, a more intense antral inflammation is seen leading to increased gastrin release and subsequent increased acid secretion. This leads to increased acid load and gastric metaplasia in the proximal duodenum, which can also be colonized by *H. pylori*, and in some this can case duodenal ulceration [48]. This phenotype has a very low risk for developing gastric cancer [33].

In some cases and with rising age, the infection can also affects the more proximal corpus mucosa, where the inflammatory activity can increase and lead to corpus-dominant gastritis (CDG) [49].

The inflammatory response to *H.pylori* infection may lead to destruction of normal glands in the gastric mucosa resulting in gastric mucosal atrophy and these glands might be replaced by an epithelium of intestinal type, so called intestinal metaplasia (IM). This process often begins at the junction between antrum-like and corpus-like mucosa, located in the area of incisura angularis [50]. The acid output is often reduced when the corpus is substantially affected in this way, due to loss of parietal cells and acid production capacity. This pattern of corpus -dominant gastritis with resulting atrophy and IM is associated with a greater risk of gastric ulcer and cancer in the stomach [49]. These tumours are typically located in the mid- or distal parts of the stomach[51-53].

Corpus- dominant gastritis, atrophy and IM can thus be considered potentially premalignant lesions [48, 49]. In some cases, atrophic pangastritis is present where loss of glandular epithelium and IM is seen, both in antrum and corpus, and this is the pattern considered to

pose the greatest risk for gastric adenocarcinoma [33]. Overall, it has been estimated that 70-90% of the gastric adenocarcinomas are attributable to *H.pylori* infection [42].

1.4.4.2 Autoimmune Gastritis

In autoimmune gastritis, chronic active gastritis is seen in the corpus and fundus of the stomach while the antrum is unaffected by this inflammation. The patients are mostly *H. pylori* negative and antibodies against parietal cells, pepsinogen and intrinsic factor (IF) are found.

In these patients, progressive atrophy of glandular structures is present and reduced output of gastric acid, pepsinogen and IF occurs. This increases the risk for vitamin B12-deficiency and pernicious anaemia (PA) [54].

There are data however, indicating that autoimmune gastritis can be associated to *H. pylori* infection as well [55]. Studies have shown that infection with *H. pylori* can lead to antigastric autoimmunity and gastric atrophy [55-57]. In 30 % of infected patients, antigastric antibodies can be found and it has been shown that classic autoimmune gastritis, with antibodies against parietal cells, and *H. pylori* -induced autoimmune gastritis have several common features [56, 57]. This antigastric autoimmunity represents a host factor which affects the course of gastritis development.

1.5 TREATMENT FOR *H.PYLORI* AND HISTOLOGICAL RESPONSE

1.5.1 Eradication and Histology

After successful eradication of *H. pylori*, the polymorphonuclear infiltration diminishes rapidly within weeks as well as the lymphocytic infiltration within a few months [31]. The potential effect of eradication on regression of gastric mucosal atrophy and IM, is controversial, but it seems that further progression of such atrophy and IM halts and that regression of these potentially premalignant changes may occur in some cases[58]. Most data support the notion that only a partial regression of gastric mucosal atrophy can occur in some patients. Many studies have significant limitations in their design, including inability to blind pathologists to the presence of *H. pylori* to allow for generalized conclusions [59]. In a recent randomized prospective study by Wong et al in 2004, the authors conclude that eradication of *H. pylori* reduces the risk for gastric cancer only in patients without pre-existent gastric mucosal atrophy or IM [60].

Even though we can see reduction of mucosal atrophy and IM in some patients, we have no markers that tells us in whom this will occur. As a consequence of this, eradication of *H. pylori* in the earliest stage of the infection or at the latest before “the point of no return”, i.e. before the development of gastric atrophy and IM, seems reasonable, as this would be the time when the probability of progression to these potentially premalignant lesions is likely to be at its lowest [58, 61, 62].

1.5.2 Antibiotics and Resistance

Triple therapies, including two antimicrobials (*clarithromycin* and *amoxicillin* or *metronidazole*) and a proton pump inhibitor (PPI) , have become the undisputed first option to treat *H.pylori* positive patients with peptic ulcer and other associated clinical conditions [63].

The increasing prevalence of antibiotic resistance in *H. pylori* strains has serious implications as, apart from patient compliance, antimicrobial resistance is the most

important factor in determining the outcome of antibiotic treatment. The prevalence of antimicrobial resistance varies geographically and ranges from 10 to 90% for *metronidazole* and from 0 to 15% for *clarithromycin* [64]. The overall *clarthromycin* resistance in Europe was measured in an European study and was found to be on average 10%, with 4% in Northern and 18.5% in Southern European countries [65]. In a recent Italian study by Zullo et al [66], 255 *H. pylori* strains were evaluated for antibicrobial resistance and primary resistance to *clarithromycin* was found to be 16.9%, the corresponding figures for *metronidazole* and *levofloxacin* were 29.4 and 19.1 % respectively.

The threshold for *clarithromycin* resistance, at which this antibiotic should not be used or susceptibility testing needs to be preformed, is 15-20% [67]. Moreover, it has also been shown that increased consumption of macrolides, other than *clarithromycin*, such as *erythromycin*, also leads to increased clarithromycin cross resistance [68]. Recent studies have shown that *clarithromycin* resistance among *H. pylori* strains is a predominant cause of therapy failure [69].

Testing for *metronidazole* resistance is not routinely done, since resistance in vitro does not necessarily mean resistance in vivo and this testing needs further standardisation. The E-test is the method of choice, when testing for metronidazole resistance. [70] Cross resistance against clarithromycin and *metronidazole* is also frequently seen and this is of particular clinical importance, as these drugs are used together in almost all standard *H. pylori* eradication regimens [71].

Resistance against *amoxicillin* is rare.

The prevalence of resistance to antibiotics in current use, has increased during the *H. pylori*-treatment era, most probably because of liberal use of these in other infections [64]. At a consensus meeting in 2005, it was concluded that the use of antibiotics constitutes a risk for antimicrobial resistance. In the case of *H. pylori*, due to the fact that the resistance mechanism is a mutation leading to vertical transmission of resistance, the spread of resistant *H.pylori* bacteria in Western societies is limited. Furthermore, as the eradication therapy typically consists of more than one antibiotic, the risk for development of resistance is moderate [58]. Antimicrobial resistance might increase though for other bacteria [72], but the magnitude of this risk is difficult to estimate at present because of lack of data.

1.6 CARCINOGENESIS OF *H. PYLORI*-ROLE OF *ANTRALIZATION*

A model of gastric carcinogenesis presented by Correa has been generally accepted and consists of the following cascade of events; chronic active gastritis, multifocal atrophy, intestinal metaplasia, dysplasia and invasive carcinoma [73]. The initial chronic gastritis is characterised by infiltration of leucocytes as described elsewhere in this introduction (1.4.4.1). In some individuals, the cascade progresses slowly but steadily with focal loss of glands (atrophy) and initially it often takes place in the incisura angularis. More virulent bacterial strains and a permissive host immune response are strongly associated with development of atrophy and progression to severe disease [50].

At this stage, the original foveolar glands are replaced by cells of intestinal phenotype, initially small intestine-like (complete) and later large intestine-like (incomplete) cells, a process named intestinal metaplasia (IM). The former dominates in younger patients and the latter in older. Up to this point in the cascade, the epithelium of the atrophic and metaplastic lesions remains well differentiated [50].

The presence of gastric glands with antral phenotype in the oxyntic mucosa, has long been recognized and described as "*antralization*" of the corpus or "pseudopyloric metaplasia" [74] and this is the predominantly precancerous lesion in several animal models [75, 76].

Intestinal metaplasia and intestinal-type adenocarcinoma are usually found in the gastric antrum and incisura. The incisura is a junctional mucosa, normally comprised of oxyntic (acid secreting) type cells. It has previously been reported that antral -type mucosa in the gastric incisura (*antralization* or pseudopyloric metaplasia) is more prevalent in *H. pylori*-infected patients and is associated with an increased risk of atrophic gastritis and intestinal metaplasia [77]. This has been confirmed in a subsequent study of patients in Europe [78]. *H. pylori* infection may induce the expansion of antral mucosa by initiating a cycle of damage and regeneration of the gastric epithelial cells, precipitating intestinal metaplasia by increasing gastric epithelial cell proliferation.

The next step in the cascade is dysplasia which is characterized by atypical changes in nuclear morphology and tissue architecture. Dysplasia is classified as either low-or high-grade. Dysplasia within the epithelium is called intraepithelial neoplasia but if it penetrates the basal membrane, it it is referred to as invasive carcinoma.

The supposed role of *H. pylori* in this process is that the infection and resulting inflammation leads to ongoing tissue injury and peripheral stem cell failure with atrophic changes and loss of glandular cells and over time this is replaced by fibrous tissue. This loss of peripheral stem cells and of cell-cell crosstalk as well as the introduction of fibrous stromal tissue, may lead to the recruitment of bone-marrow derived cells (BMDCs) into the peripheral tissue stem cell niche taking over that function [50]. The BMDCs are then exposed to an abnormal tissue environment characterized by elevated cytokine and growth factor levels and lacking parietal and chief cells and their secretions. It is likely that the BMDCs fail to regulate cell growth appropriately in this environment and instead progress through metaplasia and dysplasia to carcinoma [50].

The primary sites for distal gastric cancer are the antrum and incisura, along the lesser curvature of the stomach, at the sites of intestinal metaplasia and atrophy [51-53]. *H. pylori* is an established risk factor for gastric cancer (Class I carcinogen according to WHO) [79]. A high prevalence rate of *H.pylori* infection mirrors the gastric cancer rate, but for cardia cancer there is increasing evidence that there is an inverse relationship to *H.pylori* infection [80-82], at least in Western populations. Studies of Eastern populations have showed an increased risk of cardia cancer in *H.pylori*- infected individuals [83]and gastric atrophy has thus been postulated to be associated with an increased risk for gastric cardia adenocarcinoma as well [81].

1.7 CARDIA CANCER

Adenocarcinoma at the gastroesophageal junction may arise either from the proximal stomach (cardia) or within columnar metaplasia of the distal esophagus (Barrett's esophagus).The mucosa of the cardia is in some aspects comparable to antral mucosa, although it has admixed corpus glands [84]. Cardia cancer may arise here in foci of intestinal metaplasia. In the gastric cardia, including its junctional epithelial region, gastroesophageal reflux disease (GERD) might trigger development of intestinal metaplasia, although there is considerable debate regarding the putative role of GERD versus *H. pylori* in cancer induction at this site. Other known risk factors associated with adenocarcinoma of the gastric cardia cited in literature include obesity and Barrett's esophagus [85-87].

1.8 IMPORTANT CLINICAL CONSEQUENCES OF *H. PYLORI* INFECTION

Infection with *H. pylori* leads to a chronic active gastritis and in some cases to atrophic gastritis and intestinal metaplasia as discussed above. The consequences of the infection in clinically significant morbidity is well described. The life-time risk of peptic ulcer disease is 10-20% among *H. pylori*-infected individuals [88]. It is considered that 95 % of duodenal ulcers and 70 % of all gastric ulcers are caused by *H.pylori* infection [88] and the corresponding figure for non-cardia gastric cancer is 70% [42]. It has been estimated that the life-time incidence of gastric cancer in *H.pylori*-infected subjects is 1-3 % [88, 89]. There are though studies indicating that *H. pylori* infection might have protective effects against cardia cancer [82].

A “protective” effect of *H. pylori* infection, in some individuals, against reflux esophagitis has also been debated. This putative association could be related to reduced acid output in individuals with H.pylori-induced corpus mucosal inflammation and atrophy [90-93].
H. pylori infection can contribute to iron deficiency anemia and idiopathic thrombocytopenic purpura (ITP) [94, 95]. Vitamin B12 deficiency as a consequence of atrophic gastritis and destruction of parietal cells, resulting in lack of intrinsic factor (IF) production [54].
These diseases are probably subclinical in the majority of cases.

2 AIMS OF THE STUDY

The aims were

To explore the epidemiology of gastric histology in the general adult population and to investigate whether signs of not only current, but also a past H. pylori infection are needed for accurate screening of risk factors associated with gastric malignancy**. (Study I)**

To determine the prevalence and distribution of mucosal types at the incisura and cardia in *H. pylori*-infected and uninfected subjects and associated risk factors for development of *antralization* at the incisura, and atrophy and/or intestinal metaplasia at the cardia and whether junctional mucosal changes reflects an increased cancer risk, as suggested by an increased association with gastric atrophy and intestinal metaplasia elsewhere in the stomach. **(Study II)**

To examine the value of biomarker assays (*H. pylori* antibodies, pepsinogen I (PGI) and pepsinogen II (PGII), the ratio (PGI/PGII) and gastrin-17 (G-17)) to diagnose atrophic gastritis in a large sample of subjects who represent randomly selected adults in a population sample where the histopathological status of gastric mucosa was also evaluated independently. **(Study III)**

To evaluate the distribution of minimum inhibitory concentrations (MICs) of commonly used antimicrobial agents against *H. pylori* strains, the prevalence of antimicrobial resistance to these antimicrobial agents for treatment of *H. pylori* in a random adult Swedish population, the difference in antimicrobial resistance between *H. pylori* isolates from subjects with symptomatic and asymptomatic infection and the antimicrobial use in the same geographic area. **(Study IV).**

3 MATERIALS AND METHODS

3.1 SETTING

Kalix and Haparanda with a total of 28, 988 inhabitants (as of December 1998) are two adjoining municipalities in the Northern part of Sweden and they were the setting of the "Kalixanda Study". The age and gender distribution in this area is similar to the national average in Sweden [96] while some socio-economic variables (employment status, income, higher education) was slightly lower in Kalix and more markedly in Haparanda compared to the Swedish national average. Seventy-eight percent lived in urban areas compared to 84 % in the national average [97, 98]. Of the inhabitants in Haparanda, approximately one third was born outside Sweden, mainly in Finland. The corresponding figure for Kalix was 11.3%.

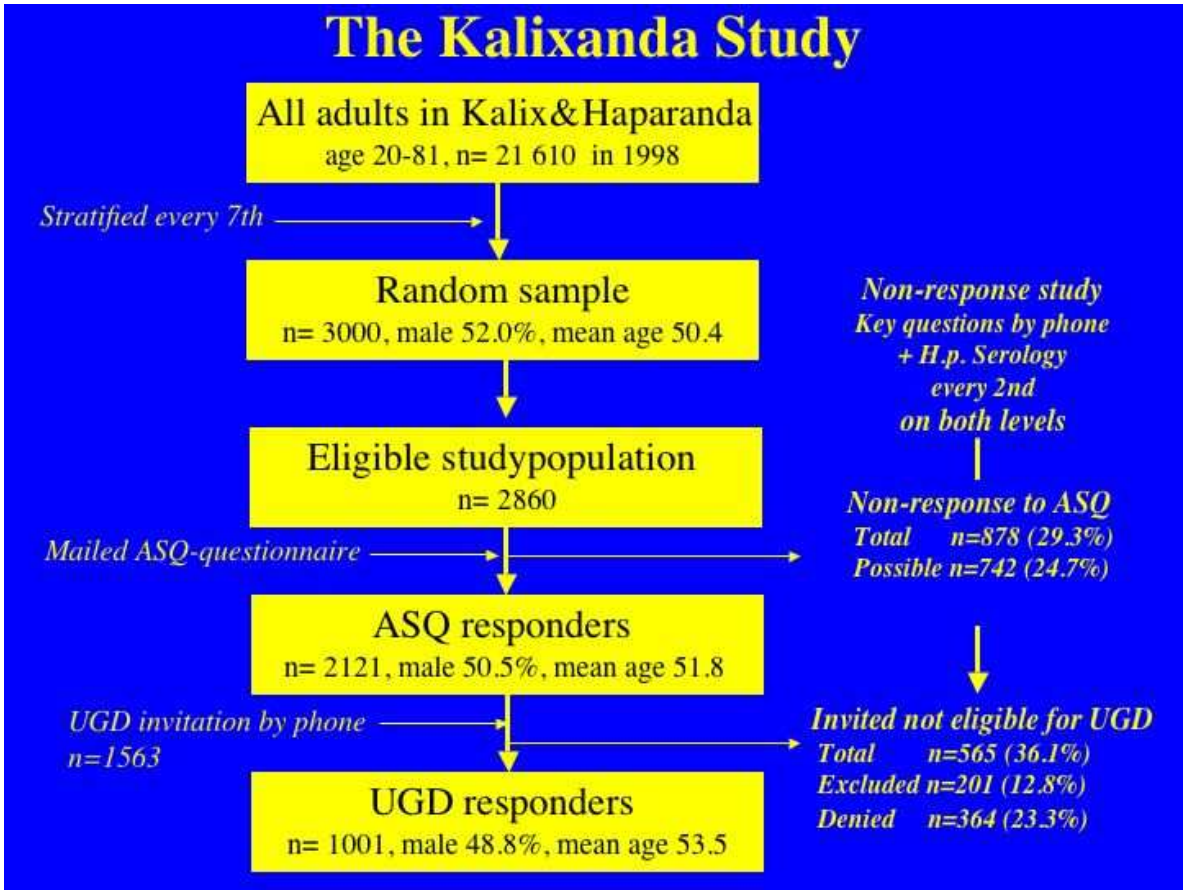
3.2 SAMPLING AND STUDY DESIGN

The official population register contains all Swedish residents and they can be identified by their unique national registration number [99].

With the aid of this register, the adult population (18-80 years) living in Kalix and Haparanda was identified. This was defined as the target population of the study(n=21,610 in September 1998 (Figure 1). Every seventh individual of this population sample was enrolled as the study population (n=3000) in a procedure equivalent to random sampling.The subjects selected were then given a randomized identification number (ID) [96].

The aim of the study was to perform an EGD (esophago-gastro-duodenoscopy) in one third of the study population (n=1000) in a random order (the EGD study sample, 4.6% of the target population).The EGD study sample size was calculated to allow appropriate precisions around the 95% confidence intervals.

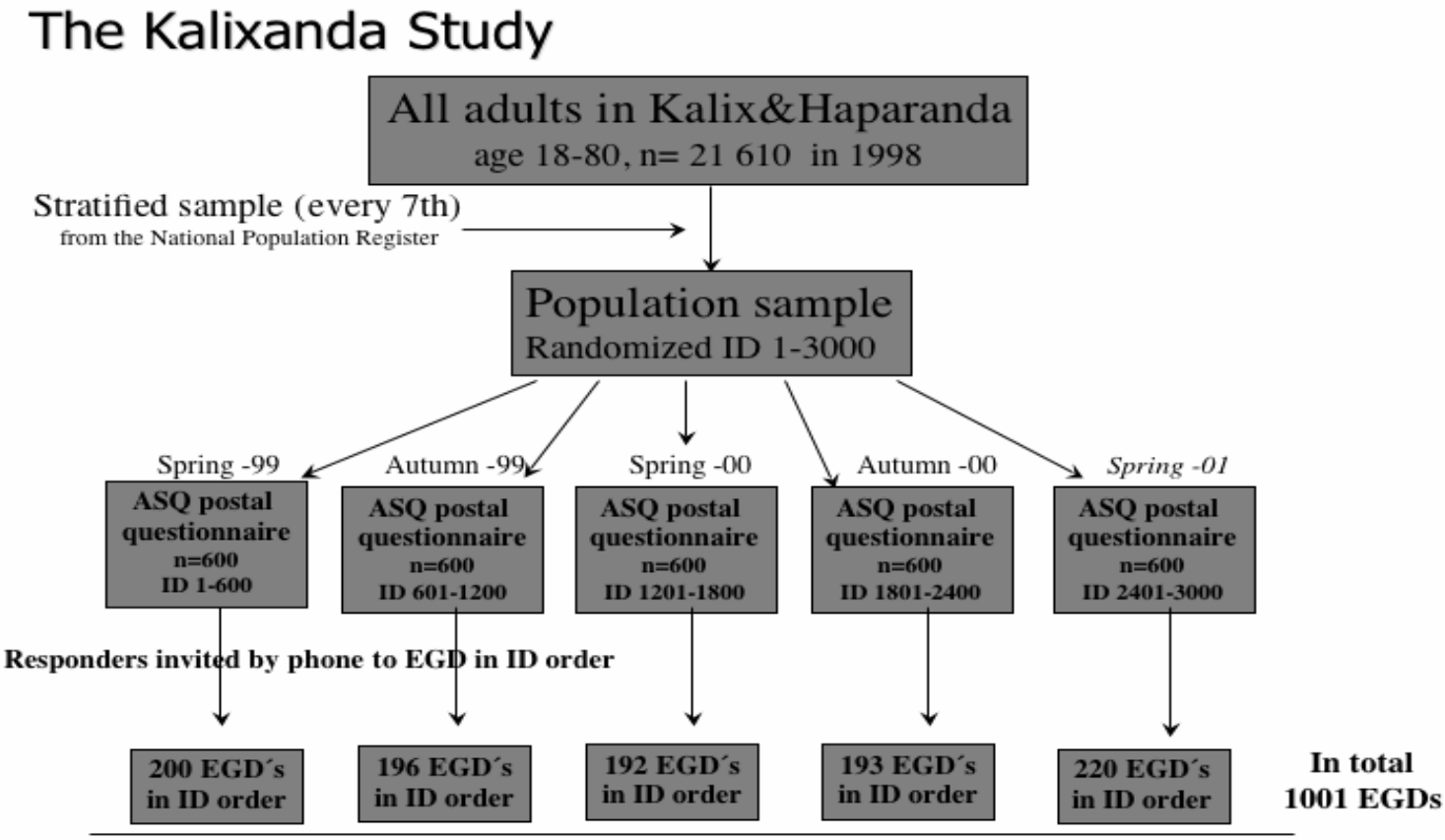
Figure 1. Study design



The study population was first approached by mail with a validated questionnaire (the Abdominal Symptom Questionnaire, ASQ [96, 100] sent by mail, that included a question regarding level of education (elementary, comprehensive, secondary, upper secondary, university). Up to two reminders were sent if needed. Of the 3000 subjects in the original study population, 2860 where still eligible at the time of the mailing [96]. The 140 non-eligible individuals had a mean age of 49.7 years and 55 % of them were men. Of these, 21 had deceased, 17 had dementia or mental retardation, 87 had moved or had an unknown address and 15 additional individuals had other reasons for not being included in the first outmailing.

For logistic reasons, the study population was divided into five groups in ascending order, ID 1-600, 601-1200 etc as shown in figure 2. The first subset of subjects was included in the study in November 1998 and the EGD:s were completed in 2.5 years. Altogether, 1001 subjects had an EGD, of which one refused biopsies.

Figure 2. Study logistics



Non-response
Every fourth non-responder to the initially mailed questionnaire (185 out of 738) was approached by telephone or mail and asked, besides completing a short key question interview, to give a blood sample for *H. pylori* serology. In total, 143 (77.3%) were reached and willing to answer, but only 19 agreed to give a blood sample. Of these, eight subjects (42%) had a positive test. In the endoscoped group 430 (43%) were seropositive.

This study was approved by the Umeå University Ethics Committee on May 29, 1998 (Um dnr 98-99) and was conducted in accordance with the revised Declaration of Helsinki. All participants gave their informed consent to participate in the study and no economical reimbursement was given for the participation in the study.

3.3 ESOPHAGOGASTRODUODENOSCOPY (EGD)

The EGD procedures in the survey were performed by two endoscopists in primary care and one in secondary care; the two endoscopy units covered the whole catchment area. Prior to endoscopy, the three endoscopists were blinded to the medical history and symptoms of the study individuals. In Haparanda, an Olympus XQ-20 fibre endoscope was used and in Kalix an Olympus GIF-100 video endoscope. In order to standardize the

endoscopic assessment, the endoscopists participated in training sessions focusing on assessment of Barrett's esophagus and relfux esophagitis lead by a professor of gastrointestinal surgery. Moreover, a test session with a professor of gastroenterology, with review of video sessions, were performed and only one of 18 esophageal diagnoses had a mismatch. During the study, there were no difference in endoscopically suspected columnar-lined esophagus between the two endoscopy units (p=0.9) [101]. A predefined endoscopy protocol was applied. All three endoscopists were experienced and had performed between 2500 and 6000 EGDs.each. All three had been participating in regular quality assessment programs over several years.

According to the protocol, at least two biopsies were taken from the following locations in the esophagus: 2 cm above the Z-line, at the Z-line and with an inverted endoscope at the cardia adjacent to the Z-line, with the aim of obtaining both squamous and columnar epithelium in the biopsies from the last two locations. In addition, two or more biopsies were taken from any suspected abnormal areas. In the stomach, biopsies were obtained according to the recommendations of the updated Sydney System [31] as well as for culture of *H. pylori*. Biopsies from the angulus were added to the protocol after 247 cases.

3.4 HISTOLOGICAL EVALUATION

The biopsies were first fixed in 10% buffered formalin, dehydrated in an increasing series of alcohols and xylol, and then embedded in paraffin. Prior to embedding, the specimen was orientated in such a manner that histological sectioning was carried out perpendicular to the plane of the mucosal surface. After deparaffinization, the 4-micrometer thick sections (at least 8 sections per paraffin block) were stained with haematoxylin and eosin (H&E). For detection of *H. pylori*, a Warthin-Starry-Silver-Stain was also performed on each specimen. Histological features of the gastric mucosa, i.e. inflammation, atrophy, (complete) intestinal metaplasia (IM), and lymphoid follicles and lymphatic aggregates were recorded according to the updated Sydney System definitions [31] and scored as 0=none, 1= slight, 2= moderate and 3= marked when applicable.

Gastritis, including features of former *H. pylori* gastritis (Minimal chronic inactive or ex-*H. pylori*) was diagnosed whenever basal lymphoid aggregates in antrum or corpus were found besides slight chronic, but not active, gastritis [102].

Corpus- dominant, chronic active *H. pylori* gastritis (CDG), was diagnosed whenever the activity of the corpus gastritis scored one score or more higher than the antrum activity, and the antrum activity was scored at least as slight [103].

Atrophic *autoimmune gastritis* was diagnosed whenever there was a total loss of parietal and chief cells, often in combination with focal intestinal metaplasia and hyperplasia of the gastric ECL cells [31].

Pre- autoimmune gastritis (not yet atrophic) of the corpus mucosa was diagnosed when diffuse lymphocytic infiltration of the entire (width of the) lamina propria in the corpus mucosa was present, with focal lymphocytic destruction of the corpus glands and reactive hypertrophy of the parietal cells with or without morphological detection of *H. pylori* [55, 104].

Antralization was recorded if antral type mucosa was present at the incisura. Transitional- type mucosa was defined as a mix of antral and corpus- type mucosa.

Cardia mucosa was recorded if the mucosa consisted of surface mucous, columnar- type epithelium and either pure mucous glands or a mixture of mucous or parietal cells of the corpus [84].

3.5 SEROLOGY

Gastrin-17, pepsinogen I(PGI), pepsionogen II(PGII) and IgG class antibodies to *H. pylori* were determined using specific EIA (Enzyme Immuno Assay) tests according to the instructions of the manufacturer (Biohit Plc, Helsinki, Finland). For determination of PGI, PGII and G-17 values, 2nd order fit on standard concentrations are used to interpolate/extrapolate unknown sample concentration automatically, with the help of the BP800 in-built software (Biohit Plc, Helsinki, Finland). The *H. pylori* antibodies are expressed as enzyme immuno units (EIU) according to the formula included in the test kit (sample EIU= $(X(A_{Sample})-X(A_{Blank})) / (X(A_{Calibrator})-X(A_{Blank}))$. EIU levels ≥ 38 are considered to be *H. pylori* positive. For G-17 <5 pmol/l was considered as normal, and the cut off for PG I was < 25 microgram/l, and for the PGI/PGII ratio the cut off was < 3. The monoclonal antibodies of G-17, PGI and PGII used in the EIA tests are highly specific according to the information from the manufacturer. The G-17 antibody detects only amidated gastrin-17 but no other gastrin molecules or fragments [105]. In immunohistochemistry, for dilutions up to 1/10.000, the G-17 antibodies stain only antral G cells, but not other cells or tissues in the stomach, duodenum, small or large bowel, or pancreas. This specificity also applies to the PGI and PGII antibodies, which only stain chief and neck cells of the gastric corpus (oxyntic gland mucosa).

3.6 *H.PYLORI* CULTURE AND RESISTANCE TESTING

Biopsies were placed in freezing medium with 10 % glycerol and the tubes were frozen at -20 °C immediately after endoscopy and moved to -70 °C within two weeks. After thawing, samples from antrum and corpus of each subject were homogenized and analysed separately under standard conditions. Primary cultures were done on blood agar with Skirrow supplement, and subsequent passages on Columbia agar with 8,5 % horse blood and 10 % horse serum. All cultures were performed at 37 °C in 5 % oxygen.Colonies of *H. pylori* were tested by Gram´s stain and biochemically(urease, catalase and oxidase positive) before harvest [106].

Minimum inhibitory concentrations (MICs) of clarithromycin, amoxicillin, metronidazole and tetracycline, for *H. pylori* isolates, were determined by the agar dilution method as described by National Committee for Clinical Laboratory Standards [107]. The MIC was defined as the lowest concentration of the antibiotic at which the growth of the inoculum was completely inhibited. The MIC breakpoint used for amoxicillin and clarithromycin was >0,5 μg/ml, for tetracycline MIC > 2μg/ml, and for metronidazole MIC > 8 μg/ml [108, 109].

Determinations of resistance were performed on one isolate per individual, normally from the antrum biopsy. For some subjects it was only possible to isolate *H. pylori* from the corpus biopsy and in those cases the corpus isolate was used. *H. pylori* strain 26695 was used as a control in all resistance determinations.

3.7 DEFINED DAILY DOSAGES OF ANTIBIOTIC CONSUMPTION

The consumption of antibiotics is reported as defined daily dosages (DDD)/

1000 inhabitants/day, as defined by WHO [110]. The data from Sweden were obtained from the National Corporation of Swedish Pharmacies [111], which is the only retail pharmaceutical company in Sweden, and the data concerning the rest of Europe were obtained from STRAMA (Swedish Strategic Programme for the Rational use of Antibiotics) [112].

3.8 QUESTIONNAIRES

The Abdominal Symptom Questionnaire has been used previously and been found to be reliable and valid [96, 113, 114]. In the questionnaire, participants indicated whether they had been troubled (yes/no) by any of the listed gastrointestinal symptoms over the prior three months. They were also asked about their previous antibiotic treatments, including eradication therapy for *H. pylori* , their consumption of antisecretory drugs and their level of education (Lower: elementary, comprehensive or secondary school. Higher: upper secondary school or university).

3.9 DYSPEPSIA DEFINITION

Dyspepsia was defined as troublesome pain or discomfort expressed as one or more of the 11 listed pain or discomfort modalities indicated in the upper (epigastric) part of the abdomen, or reporting of one or more of the symptoms “uncomfortable feeling of fullness“, “early satiety“ or “nausea“ (“upper abdominal bloating“ was not recorded) in accordance with the Rome II definition of dyspepsia [114].

3.10 STATISTICAL EVALUATION

Pearson Chi-2, Wilcoxon-Mann-Whitney test and Fischers exact test were used for nominal and ordinal data, and Student’s t-test for continuous data. All tests were two- tailed and accepted a p -value of less than 0.05 as statistically significant. The estimates are shown with 95% confidence intervals (CI) when applicable. Kappa values was used to estimate agreement when applicable on dichotomized data (corpus atrophy and intestinal metaplasia none= 0, ≥ slight =1) for the second opinion on corpus histology. Likelihood ratio and ROC curves were calculated with standard procedures.

Multivariate analyses were performed using the logistic regression analysis. The results are shown as odds ratios (OR) with 95% confidence intervals (CI). In multivariate analyses, where level of education, age and gender were introduced into models with *H. pylori*-positivity, education was dichotomized: elementary, comprehensive and secondary school constituted the lower educated group and upper secondary school and university the higher educated group.

The risk for antralization (antral mucosa on histology) at the incisura and for corpus mucosa in the cardia biopsies were first analysed for each item in a logistic regression model where age and gender were taken into account. The following factors were dichotomized into a yes/no variable: Reporting troublesome gastroesophageal reflux symptoms daily or anytime the past three months, reporting troublesome dyspepsia the past three month, ongoing *H. pylori* infection, CagA status of *H. pylori*, smoking (those who also used moist snuff were excluded), and moist snuff (only, not combined smokers), alcohol (>100 g per week), overweight (BMI ≥25 - <30), obesity (BMI ≥ 30), any use of antacid/alginates, H_2-blockers or PPI the past week or the past three months, or any combination, any use of NSAID and/or aspirin, low or high level of education as defined above, any esophagitis, Barrett’s esophagus, gastric, duodenal or any peptic ulcer, any

mucosal atrophy as defined above, any intestinal metaplasia, G-17, PGI, PGII and the PGI/II ratio as defined above. When the risks for the biomarkers for corpus atrophy were calculated, all cases with proton pump inhibitors (PPIs) use in the past three months were excluded as PPIs affect biomarker results. Factors with a statistically significant risk measured as odds ratios (OR) with 95 percent confidence interval where then analysed together with age and gender and the model was then, when applicable, reduced until only significant risks (and age and gender) remained. Variance, goodness of fit and interaction models were calculated when applicable and p values calculated were two-tailed; the alpha level of significance was set at 0.05.

4 RESULTS

4.1 LOW GASTRIC CANCER RISK WITH NEGATIVE *H. PYLORI* SEROLOGY (STUDY I)

Responders and non-responders
Every fourth non-responder to the initially mailed questionnaire (185 out of 738) was approached by telephone or mail and asked, besides completing a short key question interview, to give a blood sample for *H. pylori* serology. In total, 143 (77.3%) were reached and willing to answer, but only 19 agreed to give a blood sample. Of these, eight subjects (42%) had a positive test.

***H. pylori* test status and mucosal findings**
Of the 1000 investigated subjects, 336 (33.6%, 95% CI:30.7-36.5) were positive on culture, and 325 (32.5%, 95% CI:29.6-39.4) on histology (Table 2), with a kappa value of 0.96 (95%CI: 0.94-0.98) between the tests. Those who were positive on either histology or culture (n=339) were considered to be currently infected (i.e. true *H. pylori* positive or gold standard). There were no significant differences in sex distribution (in any of the three *H. pylori* status groups shown in Table 3).

Table 2. The proportion (n=1000) of subjects positive or negative for *H. pylori* by culture and/or histology.

	Culture positive	Culture negative	Total
Histology positive	322	3	675
Histology negative	14	661	325
Total	336	664	1000

Table 3. Sociodemographic characteristics of the three main *H. pylori* infectious status groups.

	n	**%**	**95% CI**	**Mean age**	**% women**	**% high education**
H. pylori true positive	339	33.9	31.0-36.8	58.4	50.4	27.1
Serology positive only	93	9.3	7.5-11.1	57.0	49.5	24.7
H. pylori true negative	568	56.8	53.7-59.9	50.0	51.2	51.9
All Groups	**1000**			**53.5**	**51.2**	**41.0**

Serology vs. mucosal findings
Of the EGD-group, 430 (43.0%, 95% CI:39.9-46.1) had positive serology for *H.pylori* . In the sero-positive group, 93 (21.6% of all sero-positive, and 9.3% of the entire population) were negative on both histology and culture. Negative serology but a positive result on either culture or histology was found for two subjects.

Consequently, 568 subjects tested negative for *H. pylori* according to culture, histology and serology, and thus had no sign of the infection (true *H. pylori* negative). They were significantly younger than both the true *H. pylori* positive group and the group positive on serology only ($p<0.0001$, respectively).

Accuracy of *H. pylori* serology. In individuals positive for *H. pylori* on either histology or culture or on both as the gold standard, serology had the following accuracy: sensitivity 99 %, specificity 86 %, positive predictive value (PPV) 78 % and negative predictive value (NPV) 100%.

Education level
Those with lower education had a higher risk of having a positive test for *H. pylori* infection (Table 3). The OR was 1.9 (95% CI 1.4-2.5) when comparing the true neagative group with those positive on serology (plus the two with a false negative serology test),taking age and genderinto account, and 1.8 (95% CI 1.2-2.4) when comparing the true neagative group with those with current *H.pylori* infection.

Gastritis signs and *H. pylori* status
Histology status of the three main *H. pylori* infectious status groups split by *H. pylori* status is shown in Table 4. Among the 93 subjects who were positive on serology only, 10 subjects showed neutrophil granulocyte activity in the corpus (n=9) and/or in the antrum (n=4). Eight of those cases had taken PPI therapy during the past three months, three of them continuously.

The degree of atrophy and of IM in the corpus was significantly higher in those positive for *H.pylori* on serology,but only among those with current infection ($p=0.001$ and $p=0.005$, respectively). The mean age of those with corpus atrophy and current infection, was 67.8 years vs. 65.9 years among those positive on serology only (ns). The corresponding figures for IM were 66.2 and 65.1 years (ns), respectively.

Corpus atrophy and IM were significantly more common with rising age ($p<0.0001$) as well as among those with signs of current or past infection. All subjects with corpus atrophy and all but one with IM were 52 years or older. Altogether, 9.5% of the subjects had gastric atrophy: 6.7% in the corpus, 3.7% in the antrum and 0.9% in both locations. IM was seen in 16.9%: 7.9% of the subjects had IM in the corpus, 12.6% in the antrum and 3.6% in both locations.

Corpus dominant gastritis (CDG) (inflammatory activity in corpus is higher than in the antrum)) was diagnosed in 41 cases (4.1%, 95% CI: 2.9-5.3), all of those were sero-positive and had a mean age of 64.2 years (all but three were >52 years). In 39 cases, the infection was confirmed by culture and in 35 by histology (all cultured positive). In the remaining six cases, the inflammation suggested active *H. pylori* gastritis on histology. Totally, four individuals had had PPI therapy (two of them continuously) and two had had NSAID during the past three months. None of these 41 cases had atrophy of the antrum, while nine (three with pre-autoimmune gastritis) had atrophy (slight=4, moderate=5) of the corpus. When compared with all currently infected subjects, gastric atrophy was significantly more

common in the corpus (but not in the antrum) among those with CDG (p=0.02). Intestinal metaplasia was shown in the antrum in 14 (34%) cases (slight n=10, moderate n= 3, marked n= 1), and in the corpus in 18 (44%) cases (slight n=11, moderate n= 4, marked n= 3). IM was also significantly more common (p=0004) in the corpus of those with CDG, than in all currently infected. No such difference was seen in the antrum.

Lymphoid follicles or lymphatic aggregates were found in 89% (46% in the corpus, 78% in the antrum of those with a current infection, in 37% (29% and 9%, respectively) of those positive on serology only and in 13% (8% and 6%, respectively) among those with no signs of *H. pylori* infection. All differences were statistically significant (p<0.0001), except for antral changes, comparing those positive on serology only vs. those who were truly *H. pylori* negative.

Table 4. Density of infiltration of lymphocytes and granulocytes, and atrophy and intestinal metaplasia (% of all cases per location) in corpus and antrum at different *H. pylori* status

	True *H. pylori* negative (n=568)					**True *H. pylori* positive (n=339)**					**Positive on serology only (n=93)**				
	Lympho[1]	Granulo[2]	Atrophy[3]	IM[4]	Hp+[5]	Lympho[1]	Granulo[2]	Atrophy[5]	IM[4]	Hp+[5]	Lympho[1]	Granulo[2]	Atrophy[3]	IM[4]	Hp+[5]
Corpus															
0	81	98	98	98	0	0.3	1	90	88	6	54	90	82	79	0
1	18	2	0	1	0	36	71	2	7	38	33	5	1	11	0
2	1	0	0.4	0	0	62	23	6	3	41	12	3	4	4	0
3	0	0	1	1	0	2	6	2	2	15	1	1	12	5	0
Antrum															
0	39	99	100	98	0	0	5	94	73	14	26	96	97	89	0
1	59	1	0	1	0	9	23	2	18	25	67	4	1	7	0
2	1	0.2	0	0	0	87	67	1	6	46	8	0	1	2	0
3	0	0	0	1	0	4	4	2	3	15	0	0	1	2	0
Missing data: corpus n=2, antrum n=7						corpus n=6, antrum n=2					corpus n=1, antrum n=3				

[1].Grade of infiltration of lymphoplasmatic cells: 0=none, 1=slight, 2=moderate, 3=high grade

[2] Activity of neutrophilic granulocytes: 0=none, 1=slight, 2=moderate, 3=high grade

[3] Body of glands: 0=normal, 1=slight , 2=moderate, 3=high grade atrophy

[4]Complete intestinal metaplasia: : 0=none, 1=slight, 2=moderate, 3=high grade

[5] *H. pylori* density: 0=none, 1=

Autoimmune gastritis
Autoimmune gastritis of the corpus mucosa was found in 28 individuals (2.8%, 95% CI: 1.8-2.8). Six of those were true *H. pylori* positives (and were also positive on serology), and another 13 were positive on serology only. Nine cases were negative for *H. pylori* in all tests performed. *H. pylori*, age and gender were not found to be a significant risk factors for autoimmune gastritis in the logistic regression analysis (neither when measured for those with a true H. pylori infection only, or when combined with those positive on serology only).

A further 12 cases had pre -autoimmune gastritis: eleven had current *H.pylori* infection, and the other were positive on *H.pylori* serology only.

Other aspects
In 16 cases, the histology showedtypical chronic active gastritis, consistent with a diagnosis of active *H. pylori*-gastritis, although no bacteria were found. Of these, 10 cases were positive on culture and 13 on serology, and three cases were negative on both culture and serology. Four of the 16 admitted NSAID use, all four of them were positive on culture and serology, and one stated PPI use the past three months, also positive on culture and serology.

In three cases, *H. pylori* was found on histology of the cardia biopsies only and in a further three cases, only in the angulus biopsies. *Helicobacter heilmannii* was found by light microscopy in one subject with negative serology *H. pylori* culture.

Histology, *H. pylori* and risk assessment.
Of those endoscoped 116 individuals (11.6%, 95% CI: 9.6-13.6) had a potentially premalignant condition, either atrophy or IM in the corpus mucosa (or in 5 cases in corpus mucosa from the angulus biopsies). Of those, 83 (72%) were true *H. pylori* positives, 22 (19%) were positive on serology only and 11(9%) were true *H. pylori* negatives. Therefore, 1.9% of those true *H. pylori* negatives were at risk, compared with 24.5% among the true *H. pylori* positives and 23.7% among those positive on serology. The risk of having any of the three potentially pre-malignant findings in association with *H. pylori* infection compared with no infection, was calculated by logistic regression and gave an OR of 11.3 (95% CI: 6.2-22.9) when age, sex, education, NSAID and ASA was factored in. The only other significant risk factor was rising age (OR 7.5, 95%CI: 4.2-15.3). Considering only those with current infection, the corresponding ORs were 11.3 (95% CI: 6.1-23.1) and 8.7 (95% CI: 4.4-17.7), respectively. The results did not change when use of antacids, H_2RA or PPI was factored into the models. Of the 11 who were at risk, despite never being infected by *H.pylori*, nine (82%) had autoimmune gastritis and none had pre-autoimmune gastritis.

When those with pre-autoimmune or autoimmune gastritis and all with lymphoid follicles or lymphatic aggregates were added to those with corpus atrophy or corpus IM or CDG, 91% were at risk of having a potentially premalignant finding among the true *H. pylori* positives, 43% of those positive on serology and 13% of those who were true *H. pylori* negatives. The risk estimation of having any of all these potentially premalignant findings for those with current or past infection, compared to those having no sign of the infection, was OR 27.1 (95% CI: 19.2-38.9) with the same independent factors introduced (rising age not significant). When only those with confirmed *H. pylori* infection were compared with those confirmed *H. pylori* negative, the OR was 72.0 (95% CI: 45.7-117.7).

4.2 CANCER RISK FACTORS IN THE GASTRIC INCISURA AND CARDIA (STUDY II)

Incisura

The distribution of the types of epithelium in evaluable biopsies (i.e. those with sufficient material to *analyse* mucosal type) from the incisura is shown in Table 5; 45% had *antralization* of the incisura and 16% had transitional zone mucosa (mixed corpus and antral mucosa). Subjects with *antralization* were significantly older than those with corpus mucosa (p=0.006), whilst the numerical difference between transitional and corpus mucosa was not statistically significant. There was no significant difference in gender distribution. The difference in *H. pylori* infection rate between those with corpus and transitional mucosa type was not statistically significant, but there was a significant difference in *H. pylori* infection rate between those with corpus and antral mucosa (*antralization*) (p<0.0001).

Table 5. Characteristics of subjects with different types of mucosa at the incisura

	Corpus	Transitional	Antral ("*antralization*")
n=728	282 (39%)	114 (16%)	332 (45%)
Men %	52.9	52.6	46.4
Mean age	52.7	54.7	55.8
H. pylori infected	56 (19.9%)	16 (14.0%)	180 (54.2%)

Of the 332 subjects with *antralization* of the incisura, 71 (9.8%) had atrophy and/or intestinal metaplasia at this site. In 18 subjects (2.5%), this was the sole site of these precancerous lesions based on histology.

There was a significant association of *antralization* with corpus and antral atrophy and intestinal metaplasia elsewhere in the stomach (Table 6)

Table 6. Prevalence of histological atrophy and intestinal metaplasia (IM) in subjects with *antralization* and with normal (corpus mucosa)at the incisura

	Antralization	Corpus type	p
Corpus atrophy %	11.7	3.9	<0.0001
Antrum atrophy %	8.3	2.5	0.001
Corpus IM %	13.3	3.6	<0.0001
Antrum IM %	19.9	5.0	<0.0001

The independent risk factors, after adjusting for age and sex, for *antralization* at the incisura are shown in Table 7. Reflux symptoms, daily or anytime during past three months, dyspepsia, reflux esophagitis, Barrett´s esophagus, use of NSAID and/or aspirin, snuff use, alcohol use and level of education, did not come out statistically significant risk factors when adjusting for age and sex

Table 7. Independent risk factors (univariate and adjusted for age and sex) for histological *antralization* at the incisura versus normal (corpus) mucosa. Transitional cases excluded.

Risk factors	*Antralization* %	Corpus type %	p	OR Adjusted. for age & sex
Esophagitis	13.6	20.6	0.020	OR 0.65 (95%CI: 0.42 - 0.99)
H. pylori +	54.2	19.9	<0.0001	OR 4.70 (95%CI: 3.23 - 6.83)
Smoking	20.8	12.8	0.009	OR 1.88 (95%CI: 1.20-2.93)
Any atrophy or IM[#](H)*	21.7	6.7	<0.0001	OR 3.63 (95%CI: 2.07 6.36
Corpus atrophy or IM (H)	16.0	4.6	<0.0001	OR 3.51 (95%CI: 1,84 6.75
Corpus atrophy (S)*	11.9	3.7	<0.0001	OR 3.16 (95%CI: 1.52-6.54)
G-17 ≥5	38.6	27.31	0.003	OR 1.54 (95% CI: 1.11-2.23
PG I <25	10.3	3.2	<0.001	OR 3.12 (95%CI: 1.45-6.71)
PGI/PGII Ratio < 2.5	10.8	1.8	<0.0001	OR 6.04 (95%CI: 2.32-15.77)

Reflux symptoms daily or anytime during past three months, dyspepsia, esophagitis, Barrets esophagus, use of NSAID and/or aspirine, snuff use, alcohol use and level of education did not come out statistically significant when adjusting for age and sex.

[#] Antrum, incisura or corpus

* (H)= on histology, (S)=on serology,

All with a goodness of fit < 0.05

Reflux esophagitis was significantly associated with normal corpus type mucosa in the incisura. In a stepwise logistic regression analysis, with age and sex taken into account, smoking and corpus atrophy remained independent risk factors, irrespective of whether corpus atrophy was measured with histology (smoking: OR 1.90; 95% CI 1.21-3.00, corpus atrophy and/ or intestinal metaplasia: OR 3.63; 95% CI 1.89-6.98) or serology (smoking: OR; 1.87, 95% CI: 1.18-2.96, corpus atrophy: OR 3.15; 95% CI: 1.52-6.54). G-17, PG I, PG II and PGI/PGII ratio were not introduced to the models.

None of the factors listed in Table 7-10 consituted a significant risk or protective factor for transitional mucosa when corrected for age and sex .

Cardia

Of the 987 subjects with evaluable biopsies from the cardia (an additional 5 had only squamous mucosa) shown in Table 8, 915 (92.7%) had true cardia mucosa. Of those, 910 were available for analysis of the presence or absence of intestinal metaplasia and atrophy of the cardia, as shown in Table 9. The same risk factors as listed in (and below) Table 7 were analysed univariately (data not shown) and with logistic regression with correction for age and sex (for the latter analysis, atrophy in the antrum could not be analysed due to lack of cases). Only *H. pylori* infection (20.8% in corpus type mucosa, 34.9% in the cardia type mucosa, p=0.015) was a statistically significant, protective factor after correction for age and sex: OR: 0.52, 95% CI 0.28-0.94.

Table 8. Characteristics of the 987 subjects with different types of cardia mucosa

	Cardia mucosa	Corpus mucosa	p
n (%)	915(92.7%)	72 (7.3%)	
Men%	40.3	49.2	ns
Mean age	54.3	52.0	ns
H. pylori infected n (%)	319 (34.9)	15 (20.8)	<0.0001

Table 9. Association between histologically confirmed atrophy and intestinal metaplasia of the cardia mucosa versus normal cardia mucosa

	Cardia mucosa with atrophy and/or intestinal metaplasia	Cardia mucosa without atrophy and/or intestinal metaplasia	p =
n=910	94 (10.3)%)	816 (89.7%)	
Men%	48.9	49.2	ns
Mean age	62.2	53.4	<0.0001

Risk factors for atrophy and intestinal metaplasia of the cardia.

There were significantly more subjects with any grade of atrophy and/or intestinal metaplasia of the cardia among the 910 subjects with cardia mucosa (94/910; 10.3%) compared to those with corpus mucosa (2/72; 2.8%, p= 0.04). In these abnormal cases, presence of reflux esophagitis appeared to have a “protective” effect, as shown in Table 10, while presence of reflux symptoms during the past three months was not a statistically significant protective factor (29.8% vs. 40.0%, p=0.06, OR 0.67, 95% CI 0.42-1.08). The independent cancer risk factors of the cardia are shown in Tables 9 (age and gender) and 10. We tested for all factors listed in Table 7. As none of the cases had Barrett’s esophagus (compared to 1.8% of those without atrophy and/or intestinal metaplasia in the cardia mucosa (ns), a logistic regression analysis was not applicable.

Table 10. Risk factors for atrophy and/or intestinal metaplasia in cardia mucosa biopsies

	Cardia mucosa in cardia biopsies with atrophy and/or IM at cardia	Cardia mucosa in cardia biopsies without atrophy and/or IM at cardia	p =	OR CI
Esophagitis	6.4	16.4	0.01	OR 0.35 (95% CI 0.15-0.83)
H. pylori infected	57.5	31.9	<0.0001	OR 2.15 (95% CI1.37-3.37)
Atrophy and/ IM elsewhere in stomach[#](H)*	43.6	7.9	<0.0001	OR 6.31 (95% CI 3.74-10.65)
Corpus atrophy/IM (H)	42.4	5.1	<0.0001	OR 9.80 (95% CI 5.63-17.07)
Corpus atrophy (S)*	34.7	3.7	<0.0001	OR 8.60 (95% CI 4.78-15.48)
G-17 ≥5	53.8	30.7	<0.0001	OR 2.25 (95%CI 1.44-3.52)
G-17≥10	39.8	16.1	<0.0001	OR 2.87 (95%CI 1.79-4.60)
PG I <25	29.0	3.3	<0.0001	OR 7.85 (95%CI 4.21-14.65)
PGI/PGII Ratio < 2.5	28.0	3.3	<0.0001	OR 7.27 (95%CI 3.88-13.63)

[#]Antrum, incisura or corpus
***(**H)=on histology, (S) on serology

When current *H. pylori* infection and esophagitis was introduced into the logistic regression model, together with age and sex, the risk (*H.pylori*) and protective influence (esophagitis) of having atrophy and/or intestinal metaplasia remained (OR 2.05, 95% CI; 1.30 - 3.23 and OR 0.41, 95% CI; 0.17- 0.97, respectively). When corpus atrophy or any atrophy and/or intestinal metaplasia (either on serology or histology) was added to the regression model, these (*H.pylori* and esophagitis) factors were no longer statistically significant. However, when only the 807 subjects without any atrophy and/or intestinal metaplasia in the corpus, incisura or antrum were analysed, current *H. pylori* infection remained the only statistically significant risk factor (OR 1.94, 95%CI: 1.08 - 3.46), while the putative, "protective" effect of esophagitis did not remain statistically significant (OR 0.45, 95% CI: 0.16 - 1.30). Of the 108 cases with atrophy and/or intestinal metaplasia in the stomach distal to the cardia, 64 did not have intestinal metaplasia and/ or atrophy in the cardia.

The combined risks of atrophy and/or intestinal metaplasia of the cardia mucosa, with atrophy and/or intestinal metaplasia elsewhere in the stomach, male sex (50.0 vs. 53.2%, p=0.01), age (62.0 vs. 66.4 years<0.0001), and *H. pylori* (57.3 vs. 67.0%, p<0.0001) were all statistically significant cancer risk factors of the cardia.

4.3 SERUM BIOMARKERS AND DIAGNOSIS ATROPHIC GASTRITIS (STUDY III)

Of 1001 subjects who underwent endoscopy, biopsies from both the antrum and the corpus as well as blood serum were available in 976 subjects.
In delineation of different topographic types of atrophic gastritis with serological biomarkers (GastroPanel®, Biohit Plc, Helsinki, Finland), a previously determined algorithm and specific empirically determined cut-offs for serum values of the biomarkers were used [115, 116]. This algorithm and different phenotypes of gastritis and atrophic gastritis, delineated by the GastroPanel into four groups, are presented briefly in Table 11.

Table 11. Algorithm for interpretation of serum biomarkers.

Interpretation	**Serum biomarkers**
1:Normal (healthy gastric mucosa)	All biomarkers are normal
2:Non-atrophic *H. pylori* gastritis	Hpab (IgG) is 38 EIU or more; all other biomarkers are normal
3:Multifocal atrophic gastritis (moderate or severe) both in antrum and corpus – atrophic gastritis is likely *H. pylori* associated– ***acid output is low and the stomach can be achlorhydric***	PGI is less than 25 microg/l and/or PGI/PGII ratio is below 3. G-17 is below 5 pmol/l irrespective of the Hpab level (Hpab is an indicator whether the atrophic gastritis is associated with an on-going *H. pylori* infection or not)
4: Atrophic gastritis (moderate or severe) in corpus alone – ***acid output is low and the stomach can be achlorhydric. Atrophic gastritis is often "autoimmune".***	as above but G-17 is 5 pmol/l or more

Abbreviations: Hpab=serum antibody (IgG) level of *H. pylori*. G-17=fasting serum level of gastrin-17. PGI and PGII: fasting serum level of pepsinogens I and II.

As shown in Table 12, subjects with normal mucosa (n=351) were grouped together with those with *H. pylori*- negative, non-atrophic gastritis (n=259), assuming a common physiological function, and for the same reason those with *H. pylori*-positive , non- atrophic gastritis (n=280) were lumped together with those who had *H. pylori*-positive gastritis limited to the antrum (n=24), thus forming columns 1 and 2 in Table 13 (n=610 and 304, respectively).

Table 12. All cases where histologically classified into group 1-4, in accordance with the possible biomarker outcome (as shown in Table 11).

1: Normal or *H.pylori* negative gastritis/gastropathy (n=351+259=619) **:** *Either* completely *normal* antral and corpus mucosa without *H. pylori* in Warthin-Starry (WS) stain, no active gastritis and no *H. pylori* detected by WS stain, and without high grade atrophy either in corpus or antrum, and no high grade intestinal metaplasia in either location, *or H. pylori negative non-atrophic gastritis* defined by signs of chemical reactive gastritis (normal mucosa in antrum and corpus in association with slight but not active gastritis with foveolar hyperplasia, capillary ectasia and increase of ascending smooth muscle fibres in the lamina propria) [31, 117, 118] with no concomitant corpus or antrum atrophy as defined above (n=351) *or ex-H. pylori gastritis* with features of former *H. pylori* gastritis as diagnosed whenever basal lymphoid aggregates in antrum or corpus [102] were found besides slight chronic but not active gastritis (n=259).
2: *H.pylori* gastritis without corpus atrophy (n=304): *Non-atrophic gastritis* (n=280) was defined as chronic active gastritis with lymphocytes, plasma cells and neutrophils within the lamina propria and in some cases with leucopedesis into the surface epithelium, basal lymphoid aggregates or lymphoid follicles and morphological detection of *H. pylori* with the Warthin-Starry Silver stain (except for eight cases with typical signs of a infection but no bacteria seen on histology: six of these were also positive on culture and seven on serology). Also, slight atrophy in the antrum and/or corpus was permitted. *Antrum-limited atrophic gastritis* (n=24) was defined whenever granulocytosis was seen in the antrum but not in corpus (n=11/24) and/or moderate or high grade atrophy or moderate or high grade intestinal metaplasia was seen in the antrum but not in the corpus (n=23/24) in subjects *H. pylori* infection on histology
3. Multifocal (antrum and corpus atrophic) gastritis (n=15) was defined as moderate or high grade atrophy or moderate or high grade intestinal metaplasia in the antrum and in the corpus, irrespective of whether *H. pylori* infection is histologically proven or not (7/15 were Hp positive on histology, of the remaining eight, four were positive on culture, and of the still remaining four, all were seropositive and three of these were diagnoses as Ex-Hp on histology. Only one had no sign of current or previous *H. pylori* infection).
4. Corpus limited atrophic gastritis (n=47) was defined as moderate or high grade atrophy or moderate or high grade intestinal metaplasia only in the corpus, irrespective of whether or not *H. pylori* infection is histologically proven. 19/47 were Hp positive on histology, another four on culture, another 10 had signs of Ex-Hp infection [102] on histology (of whom six were seropositive), another seven were positive on serology, and seven had no signs at all of a current or past *H. pylori* infection**.**

Some of the cases were also diagnosed with **corpus –dominant, chronic active *H.pylori.* gastritis** (CDG), whenever the activity of the corpus gastritis scored one score or more higher than the antrum activity, and the antrum activity was scored at least as slight [103], or as **atrophic, autoimmune gastritis**, whenever there was a total loss of parietal and chief cells, often in combination with focal intestinal metaplasia and hyperplasia of the gastric ECL cells [31]. However, due to a suggested common physiological function,as a consequense of mucosal affection, those cases were not analyzed separately.

Table 13. Cross-tabulation of the diagnostic interpretations of the histological appearances and the serum biomarker assays (in **bold** the cases with corpus atrophy)

Histology **Biomarker assay with GastroPanel**	Normal or *H. pylori* negative gastritis/gastropathy	*H. pylori* gastritis without corpus atrophy	Multifocal (antrum and corpus atrophic gastritis)	Corpus limited atrophic gastritis	Total number of cases; histology
Normal	541	34	**0**	**4**	579
H. pylori gastritis without atrophy	64	255	**5**	**19**	333
Multifocal (antrum and corpus) atrophic gastritis	**2**	**3**	**3**	**3**	11
Corpus limited atrophic gastritis	**3**	**12**	**7**	**31**	53
Total number of cases; GastroPanel	610	304	15	47	976

A 4x4 cross-tabulation of the applicable diagnoses obtained from histology and those obtained by the serological biomarker assays is presented in Table 14, where corpus atrophy is shown in either group 3 or 4, depending on whether concomitant antral atrophy was found or not.

Diagnosis by serology detected corpus atrophy, with or without concomitant antral atrophy, in 3/351 subjects (0.8% 95% CI, 0-1.7%) with a normal stomach and in 2/259 subjects (0.8%; 95% CI: 0-1.9%) with *H. pylori* -negative gastritis, as assessed histologically, i.e. a total of 5/610 (0.8%; 95% CI: 0.1-1.5%) as shown in column 1 in Table 13. Moreover, column two in the same table shows that the same serological outcome was found in 15/304 subjects (4.9 %; 95% CI: 2.5-7.3 %) with histologically-assessed *H. pylori* non-atrophic gastritis (none had *H. pylori*-related antral atrophy). The difference between those 4.9% with histological *H. pylori* non-atrophic gastritis and the 0.8% (5/610) with normal histology or *H. pylori* negative non-atrophic gastritis was statistically significant ($p<0.0001$), as was the case for the two histological subgroups.

Of subjects with normal stomach by histology, 311/351 (89%, 95% CI: 86-92%) cases were correctly classified by the biomarker assays, which by definition, however, cannot separate "normal" histology from *H. pylori* –negative non -atrophic gastritis. 230 cases out of the 259 (89% (95% CI: 85-93%) cases diagnosed by histology were detected by the biomarkers. These two groups are thus shown merged in column 1 in Table 13.

Among the 304 cases in column 2, Table 13, biomarkers correctly classified 255 (84%; 95% CI: 80-88 %) of subjects with *H. pylori* non-atrophic or antrum-limited atrophic gastritis.
None of the 24 cases with solely *H. pylori*-induced antral atrophy (not shown separately in the table) was detected by the biomarker test, indicating its limitations in detecting changes confined to the antrum.

Columns 3 and 4 in table 13 lists the subjects with atrophic pangastritis (n=15) and those with corpus limited atrophic gastritis (n=47) according to the diagnosis obtained from the biomarker tests. All together, the biomarkers correctly diagnosed 44 (3+3+7+31) of these 62 (15+47) subjects, i.e. 71% (95% CI:60-82%) of the cases.

Using histology as gold standard and combining corpus atrophy, with or without concomitant antral atrophy, versus subjects without any corpus atrophy, as shown in Table 14, it appears that the overall agreement of the serological markers to diagnose corpus atrophy compared to histology is 96% (95%CI: 95-97), i.e. (44+894/976) from Table 14. In this comparison, the sensitivity and specificity of the markers for corpus atrophy is 71% (95%CI:68-74%) and 98% (95%CI: 97-99%;), respectively. This corresponds to a positive predictive value of 69% (CI 95%: 66-72%), and a negative predictive value of 98% (95% CI 97-99.%) for the serum biomarker test panel.

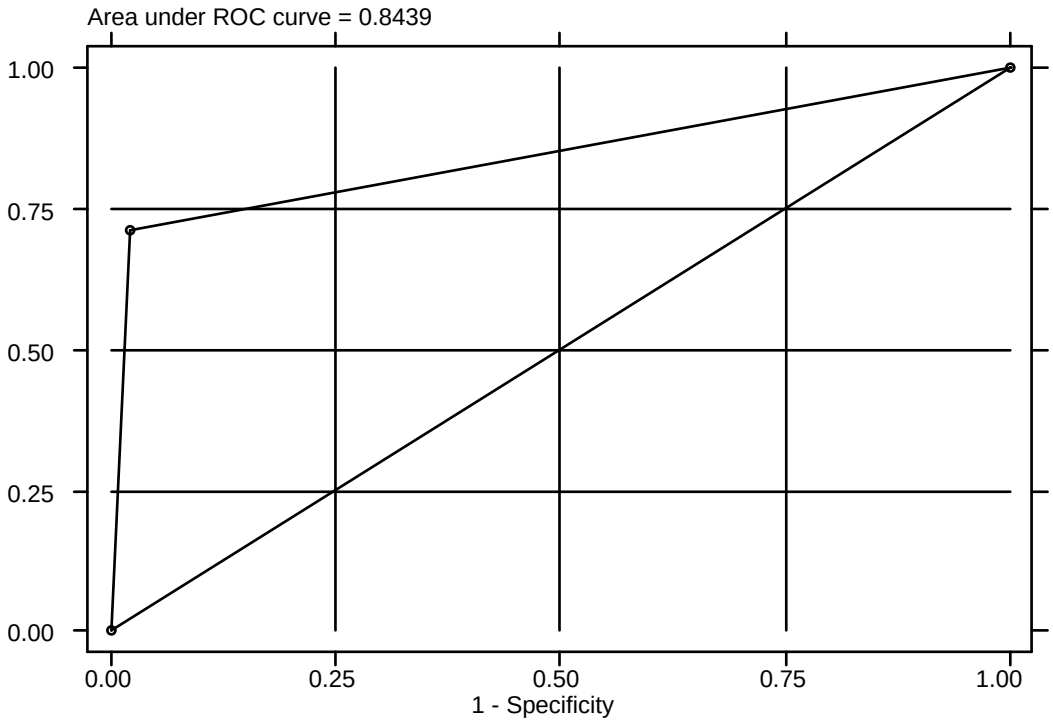
Table 14
Corpus atrophy with histology (“Histo+”) as gold standard versus serology (“Panel+”)

	Histo +	Histo -	
Panel +	44	20	64
Panel -	18	894	912
	62	914	976

The positive Likelihood ratio is 35.5 (95%CI: 35.0-36.0%;) and the area under the ROC is 0.84, as shown in Figure 3.

Figure 3

ROC for corpus atrophy as measured with serum biomarkers (GastroPanel) with histology as a gold standard



Of the 64 subjects classified to have corpus atrophy by serum biomarkers in Table 14, 20 were considered to be non-atrophic by histology. The rate of “false positive” results by the biomarker assay is therefore 31% (CI 95%: 28-34%). As 18 of the cases with atrophy on histology were negative for the biomarker, the “false negative” rate is 2.0% (CI 95%: 1.1-2.9%). Thus, of those with a positive biomarker for corpus atrophy, 69% were positive and 2% negative on histology ($p<0.0001$).

Among the 41 cases with corpus dominant gastritis, 17 (41%, 95%CI: 26-56) had corpus and/or antral atrophy diagnosed by the biomarker.
The 28 cases diagnosed as autoimmune gastritis by histology had atrophy in 24 cases (86%, 95%CI: 73-99) according to the biomarker.

In the blinded, second opinion, the kappa value for corpus atrophy was 0.71 (95% CI 0.52-0.90) and for intestinal metaplasia was 0.85 (95% CI± 0.65-1.0) between the two pathologists, and the overall agreement was 82%(95%CI: 75-89%) and 86% (95%CI: 79-93%), respectively, thus confirming the validity of histology for diagnosis in this study

4.4 ANTIMICROBIAL SUSCEPTIBILITY OF *H.PYLORI* , A POPULATION (STUDY IV)

Current *H. pylori* infection

Of the 1001 subjects that accepted an upper endoscopy, 1000 permitted biopsies to be taken.

Three hundred and thirty six subjects (33.6%) were shown *H. pylori* positive by culture (48.0% women), and among those, it was possible to test antimicrobial susceptibility in 333 subjects. Of those infected by *H pylori*, the mean age was 59 years compared to 52 among the uninfected (p<0.001) and 20.8% of the infected had passed higher education compared to 48.0% among the uninfected (p<0.001 univariate, p=0.005 when age was taken into account) .

Antimicrobial susceptibility

Antimicrobial susceptibility of *H. pylori* isolates to metronidazole, clarithromycin, tetracycline and amoxicillin was determined for the 333 subjects by agar dilution. Of those, none was resistant to amoxicillin, five persons (1.5 %,95%CI:0.2-2.8) were resistant to clarithromycin, one person (0.3 %,95%CI:0-0.9) was resistant to tetracycline and 48 persons (14.4 %,95%CI:10.6-18.2) were resistant to metronidazole. Two persons (0.6%;95%CI:0-1.4) had *H. pylori* strains resistant to both clarithromycin and metronidazole. The range and distribution of MICs of the isolates is shown in Table 15.

Table 15: MIC (µg/ml) distribution in *Helicobacter pylor*i isolates (n=333) Resistant in **bold**

	MIC								
	≤0.016	0,032	0,064	0,125	0,25	**8**	**12**	**32**	**64**
Clarithromycin									
n=333	7	140	141	39	1	**1**	**1**	**1**	**2**
%	2	42	42	12	0.3	0.3	0.3	0.3	1

	MIC					
	≤0,016	0,032	0,064	0,125	0,25	0,5
Amoxicillin						
n=333	100	119	68	31	11	4
%	30	35	20	9	3	1

	MIC							
	≤0,064	<0,125	0,125	0.25	0.5	1	2	**4**
Tetracycline								
n=333	12	3	58	181	61	15	2	**1**
%	4	1	17	54	18	4	1	0.3

	MIC								
	≤0,25	0,5	1	2	4	**8**	**16**	**32**	**>32**
Metronidazole									
n=333	5	23	100	118	33	**6**	**9**	**18**	**21**
%	2	7	30	35	10	2	3	5	6

Of all 336 subjects infected with *H. pylori*, detected by culture, 167 (49.7%) were women. Of the five subjects resistant to clarithromycin, two were women, while the only subject with resistance to tetracycline was a man. Of the 48 with resistance to metronidazole, 39 (81%) were women. The age distribution is shown in Table 16. Among those tested for metronidazole resistance (n=333, missing data n=3), 123 reported dyspeptic symptoms and 23 had peptic ulcer at endoscopy. The proportion of metronidazole resistance among those who reported dyspeptic symptoms was 18.9% compared to 11.9 % among those who did not (n.s.). The corresponding figures among those with and without peptic ulcer, was 21.7 and 13.9 % respectively (n.s.). The proportion of subjects that had passed higher education was 16.7% among those with metronidazole resistance compared to 13.6% for those without (n.s.)

Table 16. The age distribution in metronidazole resistance

Age (years)	20-34	35-49	50-64	>65
n	4	15	21	14
%	7.4	27.8	38.9	25.9

Use of antibiotics

Seven percent of the study subjects without resistance to metronidazole, stated that they had taken antibiotics in the past and eight percent among those with resistance (n.s.). The overall use of macrolides including clarithromycin in the Kalix-Haparanda area, Sweden and various European countries at the time of the study (defined as DDD/1000inhab./day) is summarized in Table 17.

Table 17: Prescription of all macrolides (including lincosamides) and clarithromycin only (where available) by means of DDD/1000 inhabitants/day in 1997.

	Macrolides & lincosamides.	Clarithromycin
Kalix & Haparanda	0.8	0.10
Sweden	1.1	0.20
France	5.98	
Italy	5.07	
Spain	5.87	
Portugal	3.69	
Finland	1.86	

Estimation of change in clarithromycin resistance in a test- and -treat scenario

A calculation comparing the estimated antibiotic use, expressed as DDD/1000 inhabitants/day [110, 119] with the “scope them all“ strategy compared to the more widely recommended “test- and- treat“ strategy in patients presenting with dyspeptic symptoms in primary care is thus of interest. In our study 376/1000 (37.6%) persons reported dyspeptic symptoms [120] and it can be estimated that 5% [121] of these, 19 persons, would consult annually. Of these 19 persons, one out of three would likely be *H. pylori* infected (as shown above) i.e. 6 persons, and approximately one of these (i.e. 1 out of the 1000) can be estimated to have a peptic ulcer at the time of consultation [122] and should thus receive eradication therapy, compared to 6 persons treated with the “test –and- treat“ strategy, (or 5 out of 1000 subjects more) if an accurate test on current infection (i.e. urea breath test or fecal H. pylori test) is used, as recommended in the literature [68].

This corresponds to a DDD/1000inhab/day of 0.02 if one out of six individuals is treated (9 000 000 inhabitants, 1 out of 1000 take 1 DDD during 7 days means that 9000 inhabitants consume 7 DDD per year or 63000 DDD). Translated to DDD/1000 inhabitants per day among 9 million people during a year gives (63000*1000)/(9000000*365)=0,02 DDD/1000 inhabitants/day. With a test –and- treat strategy, using recommended methods for detection of current infection [68], all six would be treated. This would increase the use of antibiotics to 6 x 0.02 = 0.12 DDD/1000 inhabitants/year, or about a tenth of the of the total macrolide consumption in Sweden, as shown in Table 17
.
The risk for the secondary resistance development to clarithromycin after triple therapy is, as discussed above, approximately 1.6% [123]. If we apply this to a population of 9 million people, where 1.6% out of 5/1000 (as calculated in the former paragraph) develops resistance, 720 subjects or 0.008% of the entire population will be affected annually due to a change in management from an endoscopy to a test- and- treat approach. The corresponding figures in a worst case scenario with for example 90% eradication rate [124] and 50% of the failures due to secondary clarithromycin resistance [125] would be 2250 subjects or 0.025% of the population.

5 DISCUSSION

To our knowledge, we have conducted one of the the largest population- based, upper endoscopic surveys with a random design internationally so far, showing that it is possible to perform an endoscopic evaluation of a random population sample with an acceptable methodology, high participation rate and good generalizability[126] in a valid sample of the studied population [96].

The Kalixanda study subjects constitute a representative population sample, 20 to 80 years old, from the reliable national Swedish population register, covering all inhabitants in the caption area without exception. The cohort is thus free from many, if not all, the potential biases, e.g. health care seeking behavior. Stratified sampling (every seventh) was used because it was available from the population register and in this case considered to be equivalent to random sampling because of the large sample size. All subjects were then randomized irrespectively of age and sex and given a unique ID number.

A strength of the Kalixanda study is thus that we succeeded in investigating a representative sample of the general population with endoscopy, took an extensive number of biopsies, validated symptom questionnaires and followed a strict, predefined endoscopy and histology protocol. The response rates, both in answering to the postal questionnaire and accepting the EGD (esophago-gastro-duodenoscopy), were acceptable, being 74% and 73%, respectively.

There were more contraindications to EGD than in patients usually referred for endoscopy, as the study subjects could not be put at risk in any way by participating in the study. Thus, subjects with unstable angina or pregnant subjects, for example, were not endoscoped. However, most influence in terms of excluded subjects with organic upper GI disorders would most probably have arisen from the exclusion of the 10 subjects (1% of the EGD study sample) with previous upper GI surgery [96].

The EGD study sample had a mean age which was about four years higher than that of the original Kalixanda study population and the general Swedish population, mainly due to a lower response rate among the symptom free youngest quarterile of the study sample. The difference in prevalence for the main GI symptom groups, i.e GERS (Gastro-Esophageal-reflux-Symptoms), dyspepsia, was in the range of 4-6% [96] and thus of minor clinical importance and also easy to control for.

The socio-economic status in the study catchment area was somewhat lower than the Swedish average [97, 98], however still negligible from an international perspective. Moreover, the prevalence of positive *H. pylori* serology of 42% is similar to other countries in Northern Europe [22, 23, 127]. The prevalence of 42% in *H. pylori* serology among the non-responders, despite the small sample size, contradicts eventual bias caused by socio-economic status. In our study, *H. pylori* status was the same in this group as the responders. Although the education level was slightly lower, the drop- outs were most probably reasonably equal to the responders, as the *H. pylori* infection rate is known to correlate with socio-economic welfare [128]. It is therefore unlikely that socio-economic factors have markedly influenced our results.

The three endoscopist who performed all the EGD:s were very experienced (2500 to 6000 endoscopies each). They took part in training sessions for landmarks at EGD and

endoscopic diagnosis [129-132] and were also tested for concordance in order to minimize the inter-observer variation with good agreement between the endoscopists [96, 133]. There were no significant difference in esophageal findings between the two endoscopy units [101, 133].

The histological evaluation was done by two experienced pathologists with a special interest in GI pathology, who were unaware of the clinical data and endoscopic findings. The kappa-value for agreement between observers was good. For example, in the evaluation of *H. pylori* infection it was 0.76 (95% CI 0.56-0.96) for the corpus and 0.78 (95% CI 0.59-0.98) for the antrum of the stomach [96].

The logistics of the study and the generalizable outcome has been described in details elsewhere before [96]. To conclude this part of my thesis, we consider the endoscoped sample as being well representative of Northern European caucasians regarding health and morbidity in the stomach.

In this study we explored the epidemiology of *H. pylori* infection and gastric histology, the distribution of the premalignant changes, i.e. gastric mucosal atrophy and IM, under different circumstances, the potential possibilities to evaluate the gastric mucosa with serological biomarkers and the prevalence of antibiotic resistance among *H. pylori* in the general, non-patient adult population.

Lately, the gastric cancer risk discussion has focused on the premalignant conditions gastric atrophy and intestinal metaplasia [60, 134-136], as we do in this report. The clinically important perspectives are whether eradication of *H. pylori* can prevent development of gastric cancer, and if it does, is the timing of the eradication before the development of atrophy and IM important, i.e. is there a mucosal “point of no return” after which eradication has no or minor effect on the morbidity in gastric cancer?

Despite the fact that *H. pylori* eradication cures active gastritis, its effect on gastric mucosal atrophy, IM and gastric cancer is still controversial [60, 135, 136]. In most studies, corpus atrophy seems to improve after eradication of *H.pylori,* while IM tends to persist [137]. Wong et al. [60] recently showed that those with corpus atrophy and IM at eradication, retained their cancer risk. These findings are supported by our results.

In our study, the proportion of subjects with atrophy and IM in the corpus was statistically even worse among those positive on serology only, than among those currently infected, so at least it did not decrease. Moreover, the prevalence of both corpus atrophy and IM increased with age among those infected, as has also been reported by others [138]. Those without any signs of current or past infection with *H.pylori* had almost no corpus atrophy or IM.

The persistence of these potentially premalignant changes in post- infectious, sero-positive cases should be considered when deciding on further investigation by endoscopy. Cag A antibodies, detected by immunoblot, can indicate past infection in situations were serology has became negative [42].

The literature gives support for a “point of no return” in the process of gastric mucosal inflammation [61, 139], i.e. if atrophic changes and intestinal metaplasia has developed, eradication of *H.pylori* can not cure the mucosa from these changes and the risk for gastric cancer remains virtually unchanged as our study also indicates. Although the literature is controversial, it seems that eradication of *H. pylori,* as early as possible, will have the

strongest impact on reduction of gastric cancer development, before premalignant changes have occurred [58, 59, 62].

Precancerous gastric lesions (atrophy and IM) are common in the junctional mucosa, i.e. the incisura angularis and cardia. *Antralization* of the incisura, following *H.pylori* infection, is thought to be an expansion of distal- type mucosa to replace the lost acid- secreting mucosa [77]. The primary sites for distal gastric cancer are the antrum and incisura, along the lesser curvature of the stomach at sites of intestinal metaplasia and atrophy [51-53].

The prevalence of antral-type mucosa of the incisura (*antralization*) and the precancerous lesions of the cardia in the general population is unknown, but its presence may indicate a higher cancer risk. In our study, the association of *H. pylori* infection with *antralization* was statistically significant (54% versus 20% of those with body- type mucosa) as was intestinal metaplasia and/or atrophy at this site.

In the cardia, only 10% had IM, but this was significantly associated with gastric IM and atrophy elsewhere in the stomach, in particular corpus atrophy. The major risk factor for development of IM and atrophy of the cardia, in our large series, was *H. pylori* infection. Whilst some cardia cancer studies do not show a positive association between *H.pylori* infection and cardia cancer, our study results suggests that this infection, in fact, is the major cause of precancerous lesions at this site. This is an important finding as IM, in the long term, at least by some have been proposed to reverse following eradication of *H.* pylori [140].

Obesity and tobacco have also been linked as risk factors to cardia cancers[141]as has tobacco to gastric cancer [142].Whilst smoking, in our study had a positive association with *antralization* of the incisura, we found no association with cardia atrophy or IM. Obesity was not linked to precancerous lesions in the cardia in our study, although obesity is linked to cardia cancer [86].

The gold standard for diagnosing atrophic gastritis and IM is histology [31], but it also has limitations. Accuracy for the diagnosis of gastritis and atrophic gastritis, based on histopathology of endoscopic biopsy specimens, is limited for several reasons. Firstly, inflammatory lesions, atrophy and intestinal metaplasia are often patchy and focal in the stomach and sampling errors occur readily. Secondly, inter-observer and intra-observer variations in interpretation of the biopsy appearances are well proven [143, 144] and thirdly, upper endoscopy might not be feasible in all clinical situations. Therefore, a blood-based, non-invasive test to diagnose and evaluate presence of gastritis and atrophic gastritis, without such limitations, would be ideal and it would also reflect the physiological function of the entire gastric mucosa.

Serological testing for *H. pylori* antibodies (Hpab) is the simplest non-invasive test for “gastritis”, but it is limited to the diagnosis of concurrent or recent *H.pylori* infection. Combined serum assays of pepsinogen I (PGI) and pepsinogen II (PGII), and the ratio (PGI/PGII), with assays of Hpab allow for the diagnosis of corpus atrophy and *H. pylori* gastritis [115, 116, 145]. Furthermore, combining gastrin, particularly the gastrin-17 (G-17) assay, with these biomarkers enables delineation of patients with atrophic pangastritis (of both antrum and corpus), which has the highest known risk for gastric cancer [146, 147], even after *H. pylori* eradication [148, 149]. In addition, low and high serum levels of G-17 can be used to delineate patients with antrum- predominant, atrophic gastritis and in the verification of atrophic gastritis limited to the gastric corpus [115, 116, 150]. Thus, combined assays of pepsinogens (PGs) and G-17 with Hpab, give a more accurate status of

the gastric mucosa than testing for *H. pylori* infection alone, and these assays reduce the risk and rate of false negative results obtained by using *H. pylori* tests alone, particularly amongst older people with more advanced atrophic gastritis and hypochlorhydric (low acidic) stomach [147, 151-153]. Some 5-10% of older people in the general population of the Nordic countries, have atrophic gastritis [154]. Also, due to the lower grade of colonization in such cases, direct *H. pylori* tests (breath test, stool antigen test, urease biopsy test, culture or biopsy microscopy) may give false negative results [41, 155-161].

Our study indicates that biomarkers can diagnose moderate or severe corpus atrophy with high accuracy when compared with a histological diagnosis. The apparently high "false positive" outcome of 31% may actually highlight the inadequacy of relying on histology when standard biopsy routines are used, as these may "miss" the appropriate tissue areas due to biopsy sampling error. The value of validating biomarkers for the diagnosis of gastritis and atrophic gastritis, is highly dependent on the validity and interpretation of biopsy histology. In our study, the excellent kappa values for interobserver observations on relevant corpus histology, ensures reliable histology data [162, 163] but does not exclude possible errors in biopsy sampling. A potential cause of bias in the histological assessment of *H. pylori* is PPI use. In our population sample, however, only 3,6% (2.2% continuously) had used PPI during the past week [120], thus not markedly influencing the results.

If the above mentioned aspects of *H. pylori*-infection and its treatment would be implemented, i.e. the existence of a mucosal "point of no return" and a wider indication for treatment, the use of antibiotics would increase and the risk for antimicrobial resistance could increase likewise, both against *H.pylori* and other bacterial species [58, 72, 94]. An increased prevalence of antibiotic resistance in *H pylori* strains has serious implications as, apart from patient compliance, antimicrobial resistance is the most important factor in determining the outcome of antibiotic treatment. The prevalence of antimicrobial resistance varies geographically and ranges from 10 to 90% for metronidazole and from 0 to 15% for clarithromycin [64]. Resistance against clarithromycin and metronidazole is also frequently associated and this is of particular clinical importance, as these drugs are used together in almost all current standard *H. pylori* eradication regimens [71]. Recent studies have shown that clarithromycin resistance, among *H. pylori* strains, is a predominant cause of therapy failure [164]. The resistance against antibiotics in current use has increased during the *H. pylori* treatment era, most probably because of liberal treatment with these antibiotics of other infections [64]. Moreover, it has also been shown that increased consumption of macrolides other than clarithromycin, such as erythromycin, might lead to increased clarithromycin resistance [64].

Most knowledge of antibiotic resistance comes from patient stomach samples, as it requires endoscopy to obtain specimens from the gastric mucosa. Information about the background antimicrobial susceptibility of *H. pylori* strains in the community (also in non-patients) is important, however, as treatment of *H. pylori* infection is often based on an empirical knowledge in primary and secondary care, without an initial susceptibility test. Information on antimicrobial susceptibility in the community to commonly used drugs is therefore critical for sustainable *H. pylori* treatment.As we could show in study IV, the prevalence of antimicrobial in our study population was low.

The internal validity of the methods used to detect current *H. pylori* infection seems to be accurate, since the culture method used showed excellent overall agreement and high kappa values (98.3% and 0.96, respectively) when compared with *H. pylori* detection by histology: when a positive histology and/or culture result was compared with serology, the sensitivity of the latter was 99%. In only three cases out of 336 was the susceptibility

impossible to detect. The inflammatory parameters on histology also correlated excellently [165].

Bacterial resistance is most common to metronidazole, with worldwide rates ranging from 10 to 90% [64]. In our study, 16.1 % of the isolates were resistant to metronidazole, which is lower than previous findings in patients referred for endoscopy [70]. The high prevalence of metronidazole resistance might also be due to frequent use of metronidazole for other intestinal and gynecological problems, which is supported by the fact that 74 % of those resistant to metronidazole were women in our study. However, previous studies have shown that even if a strain has been shown to be metronidazole resistant *in vitro,* that does not negatively influence treatment outcome when using triple-therapy including metronidazole [166].

Amoxicillin resistance has been negligible (<1%) so far [64, 123] which was also the case in our study. Occasionally higher rates have been reported however, such as 38% in a Brazilian study [167] but such figures have to be interpreted with caution until more data are available.

Clarithromycin is widely used to treat patients with duodenal ulcer, functional dyspepsia and abdominal discomfort without diagnosis of *H. pylori* infection. Data on clarithromycin resistance in Sweden are relatively limited. Around 2 % of the isolates in the present study were clarithromycin resistant, and it might be important to monitor any trend in this resistance in Sweden, because prescription of this drug for other infections might increase.Our data are also seven years old. In a previous eradication study on patients with duodenal ulcers, the primary resistance was only 3% [123], thus diminishing the worry of high resistance rate in such “pure“ patients.

Secondary resistance to clarithromycin is also a major concern, although data from Lind et al [123] suggest the development of a secondary resistance rate of only 1.6% (4/254 off all subjects included in an intention- to- treat analysis in the study, or 4/29 with unsuccessful eradication) among all patients treated with a clarithromycin-based therapy combined with a PPI and never treated before for duodenal ulcer.

The “scope them all” strategy was the clinical approach of choice for patients under 50 years of age consulting for uninvestigated dyspepsia at the time of this study (and still is), in opposition to recommended guidelines in many other countries, where “test –and- treat” is the first choice [168]. One reason for this is the fear for antibiotic resistance. We have shown however that the introducing the latter strategy would affect the antibiotic resistance to clarithromycin, the real fear, only marginally, at most 0.025% of the population would be annually affected. A screening situation would though dramatically change this figure.

6 CONCLUSIONS

This, the Kalixanda study is, to our knowledge the largest, population-based, endoscopic study in a non-biased cohort of adults, representative of the entire adult population,and with a high participation rate. In our study, the prevalence of *H.* pylori infection and *H. pylori*-related histological changes in the gastric mucosa has been well described, as well as a non-invasive method for diagnosing atrophic changes in the gastric mucosa. We have also described the prevalence of antimicrobial resistance towards commonly used antimicrobial agents used for eradication of *H. pylori* and how widened indications for *H. pylori* eradication could affect the antimicrobial resistance situation.

We believe that the discussions needs to continue concerning when and how to diagnose *H. pylori* infection, as well as about the Swedish guidelines for eradication of *H. pylori*. The concept of test- and- treat for *H.pylori* infection, could safely be applied to patients younger than 45-50 years consulting for dyspeptic symptoms rather than refer for endoscopy and thus free up resources for other more pressing needs of endoscopy, for example screening for colorectal precancerous lesions and malignancies.

Study I
One third of the subjects who underwent endoscopy had ongoing *H. pylori* infection, and a further 10% had signs of past *H.pylori* infection. Corpus -dominant gastritis was found mostly among the former, while corpus atrophy and IM mainly occurs in the latter group. Sero-negativity for *H.pylori* excludes presence of precancerous conditions with high probability in a screening situation.

Study II
Precancerous lesions in the gastric junctional mucosa (incisura and gastro-esophageal junction) of a normal population are rather common and principally associated with *H. pylori* as well as IM and/or atrophy elsewhere in the stomach. Smoking is also an additional risk factor for *antralization*

Study III
Non-invasive, serological biomarker assay shows high accuracy for the diagnosis of corpus atrophy, which is common in the general population.

Study IV
The resistance to the tested and commonly used antibiotics for eradication of *H. pylori,* was found to be lower in our study than expected from previous patient sample studies, especially for clarithromycin. This most probably reflects the restrictive prescription policy for this antibiotic in the study area. Introduction of a test -and -treat strategy for *H.pylori* infection in Sweden would only marginally affect the resistance to clarithromycin in the society.

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