Helicobacter pylori and its Association with Gastric and Oesophageal Carcinomas

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Helicobacter pylori **and its Association with Gastric and Oesophageal Carcinomas**

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To my family

"The greatest obstacle to discovery is not ignorance - it is the illusion of knowledge."

Daniel J. Boorstin (1914-2004)

1. ABSTRACT

Helicobacter pylori is one of the most common infections in man. The infection is often acquired during childhood and usually results in a chronic life-long inflammation in the gastric mucosa. The aim of our studies was to investigate the association between *H. pylori* seropositivity and the development of gastric and oesophageal carcinomas. Nested case-control studies were performed in the Malmö Preventive Medicine cohort consisting of 32,906 subjects. Tumour cases were identified by the Swedish National Cancer Registry. *H. pylori* infection was identified serologically by an in-house ELISA and a commercial Western blot method, Helicoblot 2.1. The more virulent *H. pylori cag*A-positive strain was identified by the CagA band in Helicoblot 2.1. We found that *H. pylori* seropositivity was associated with a higher risk of non-cardia gastric adenocarcinoma. *H. pylori* seropositivity was a risk factor for non-cardia gastric adenocarcinoma among both smoking and non-smoking subjects. CagA seropositivity was a risk factor for non-cardia gastric adenocarcinoma in the *H. pylori* seropositive subgroup. The size of our material did not allow the estimation of the association between *H. pylori* seropositivity and oesophageal adenocarcinoma or oesophageal squamous cell carcinoma, however, there was an inverse tendency associated with oesophageal squamous cell carcinoma. The Helicoblot 2.1 CagA band was evaluated in subjects with no known gastric or oesophageal malignancy. The CagA band had a bimodal peak intensity distribution. The changes in seroprevalence with year of birth suggested that CagA seropositivity was false-positive in a major proportion of *H. pylori* seronegative subjects when identified by Helicoblot 2.1.

Keywords:

Gastric adenocarcinoma, Oesophageal carcinoma, *Helicobacter pylori*, CagA, Western blot

2. CONTENTS

3. ABBREVIATIONS AND DEFINITIONS

AP-1	Activator protein 1
APC	Adenomatous Polyposis Coli
BMI	Body Mass Index
BSA	Bovine Serum Albumin
*cag*A	*cytotoxin associated gene* A
CagA	the protein of the *cytotoxin associated gene* A
cag-PAI	*cytotoxin associated gene* Pathogenecity Island
CCUG	Culture Collection, University of Göteborg
CI	Confidence Interval
CIM	Current Infection Marker
DCC	Deleted in Colorectal Cancer gene
DNA	Deoxy Nucleotide Acid
EHSG	European *Helicobacter* Study Group
EIA	Enzyme ImmunoAssay
ELISA	Enzyme-Linked ImmunoSorbent Assay
EpC	Centre for Epidemiology, National Board of Health and Welfare
EPIYA	denotes the amino acid sequence Glu-Pro-Ile-Tyr-Ala
ERK	Extracellular signal-Regulated Kinase
FAP	Familial Adenomatosis Polyposis syndrome
Gy	Gray
h	hour
HDGC	Hereditary Diffuse type Gastric Cancer
HGF	Hepatocyte Growth Factor
HNPCC	Hereditary Non-Polyposis Colorectal Cancer
HM-CAP	High Molecular Cell Associated Protein
HP-NAP	*H. pylori* Neutrophil Activating Protein
IARC	International Agency for Research on Cancer
ICD-9	International Classification of Disease, Ninth Revision
ICD-O	International Classification of Disease for Oncology
IgG	Immunoglobuline G
INF-γ	Interferon γ
IL	InterLeukin

iNOS	inducible Nitric Oxide Synthase
IS*605*	Insertion Sequence *605*
JAM	tight Junctional Adhesion protein
kDa	kiloDalton
LPS	LipoPolySacharides
Mm-MAST	Malmö modified Michigan Alcohol Screening Test
MALT	Mucosa Associated Lymphoid Tissue
MEK	Mitogen activated protein kinase kinase
MPM	Malmö Preventive Medicine
MRSA	Multiresistant *Staphylococcus aureus*
NF-κB	Nuclear Factor κB
NSAID	Non-Steroidal Anti-Inflammatory Drug
OR	Odds Ratio
ORF	Open Reading Frame
PAH	Polycyclic Aromatic Hydrocarbons
PBS	Phosphate Buffered Saline
P	P-value
ras	retrovirus-associated DNA sequences (gene family)
ref.	reference
s.d.	standard deviation
s.e.	standard error
SHP-2	Src Homology 2 domain-containing protein tyrosine Phosphatase
SNCR	Swedish National Cancer Registry
TLR	Toll Like Receptor
TNF-α	Tumour Necrosis Factor Alpha
TP53	Tumour Protein p53
UK	United Kingdom
USA	United States of America
*vac*A	*vacuolating toxin* A
VacA	the protein of the gene *vacuolating toxin* A
WHO	World Health Organisation
ZO-1	Zonula Occludens-1

4. ORIGINAL PAPERS

This thesis is based on the following papers which will be referred to in the text by their Roman numerals:

I. Simán JH, Forsgren A, Berglund G, Florén C-H. Association between *Helicobacter pylori* and gastric carcinoma in the city of Malmö, Sweden. A prospective study. Scand J Gastroenterol. 1997;32:1215-21.[*]

II. Simán JH, Forsgren A, Berglund G, Florén C-H. Tobacco smoking increases the risk for gastric adenocarcinoma among *Helicobacter pylori*-infected individuals. Scand J Gastroenterol. 2001;36:208-13.[*]

III. Simán JH, Forsgren A, Berglund G, Florén C-H. *Helicobacter pylori* infection is associated with a decreased risk of developing oesophageal neoplasms. Helicobacter. 2001;6:310-6.[†]

IV. Simán JH, Engstrand L, Berglund G, Florén C-H, Forsgren A. Evaluation of western blot CagA seropositivity in *Helicobacter pylori*-seropositive and -seronegative subjects. Clin Diagn Lab Immunol. 2005;12:304-9.[‡]

V. Simán JH, Engstrand L, Berglund G, Forsgren A, Florén C-H. *Helicobacter pylori* and CagA Seropositivity and its Association with Gastric and Oesophageal Carcinoma. (manuscript).

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5. INTRODUCTION

5.1. *Helicobacter pylori*

5.1.1. History of *H. pylori*

In 1983, Robin Warren and Barry Marshall published their first paper in Lancet on unidentified curved bacilli on gastric epithelium in active chronic gastritis (Warren, 1983; Marshall, 1983). Dr Warren, a pathologist at the Royal Perth Hospital, had for several years observed a high occurrence of small curved and S-shaped bacilli and a closely associated granulocyte infiltration in gastric biopsies. He believed that they played a role in gastric disease. Dr Marshall, a gastroenterology fellow, was attracted by the hypothesis and persuaded Dr Warren to make further investigations. They noted that, although the bacilli could not be classified by reference to *Bergey's Manual of Determinative Bacteriology,* they resembled campylobacters more than spirochetes. By using microaerophilic campylobacter isolation techniques, Warren and Marshall achieved the first ever culture of the bacterium from human gastric biopsies in their 35th attempt in 1982. Because of a MRSA epidemic at the Royal Perth Hospital this *H. pylori* culture was not read until after the Eastern weekend (Kidd and Modlin, 1998; Barry Marshall, personal communication).

Bacterial colonisation of the stomach had already been observed a century earlier. This was however mostly unknown to clinicians and pathologists, because of the prevailing dogma that peptic ulceration was caused by gastric acidity. Kidd and Modlin (1998) have in a review described the warren of early investigations and the marshalling of the new paradigm. Bottcher (1875) and Letulle, gastric bacteriologists, studied gastric ulceration in the mammalian stomach and were able to demonstrate bacterial colonies in the ulcer floor and in its mucosal margins. The pathologist Klebs (1881) described a bacillus-like organism in the lumen of and between the cells of the human gastric gland, with corresponding small round cell infiltration. Jaworski (1889), professor of Medicine at the Jagiellonian University of Cracow, described in detail a spiral organism in human gastric contents, which he named *Vibrio rugula.* It was however argued that similar structures could be found after mixing acid with pharyngeal or even bronchial mucus (Boas, 1907). Salomon (1896) found spirochetes in the gastric mucosa of dogs, cats and rats and reported colonisation of the gastric pits of white mice that had been fed gastric mucus from dogs. Krienitz (1906) reported spirochetes in the gastric contents of a patient with gastric carcinoma. Hoffman (1925) inoculated guinea pigs with 5 ml of gastric contents from a patient with duodenal ulcer. The guinea pigs developed gastric ulcers. Gram-negative, fine slender rods were recovered from these guinea pigs and were inoculated into another guinea pig, which also

developed a gastric ulcer. Doenges (1938) reported the presence of spirochetes in every single *Macacus rhesus* monkey that he studied, but only in 11 out of 103 human gastric mucosa specimens. Freedberg and Barron (1940), with experience from a silver staining method used in investigations of dogs, were however disappointed with their finding of spirochetes in only 53% of surgery specimens from patients with gastric ulcer (14% in specimens from non-ulcerating stomachs). There were also apparent histological differences between the organisms found in humans and those found in the *Macacus rhesus* monkey. They concluded that no absolute ethiopathologic role could be predicted for these organisms. Palmer (1954) used a vacuum tube to obtain gastric mucosal biopsies from 1,180 subjects and with the use of standard histological staining techniques, instead of the silver staining method, he failed to detect any spirochetal organisms. He concluded that earlier results could be explained by post-mortem colonisation.

In 2005 the Nobel Prize in Physiology or Medicine was awarded to Robin Warren and Barry Marshall for having "with tenacity and a prepared mind challenged prevailing dogmas" (Nobel Assembly, 2005). They demonstrated irrefutably that the bacterium *Helicobacter pylori* can cause peptic ulcer disease and they made the bacterium available to scientific study by developing culture techniques. Their achievement was reached with generally available techniques, such as fibre endoscopy, silver staining of histological sections and culture techniques for microaerophilic bacteria.

There is now firm evidence that *H. pylori* causes more than 90% of duodenal ulcers and up to 80% of gastric ulcers (Coghlan *et al.,* 1987; Tatsuta *et al.,* 1990; Karita *et al.,* 1994; Labenz and Borsch, 1994; Seppälä *et al.,* 1995). Treatment of *H. pylori* infection may cure two thirds of early stages of the rare gastric mucosa associated lymphoid tissue (MALT) lymphoma (Farinha and Gascoyne, 2005). The discovery that peptic ulcer disease has a microbial cause has stimulated the search for microbes as possible causes of other chronic inflammatory conditions, such as Crohn's disease, ulcerative colitis, rheumatoid arthritis and atherosclerosis.

5.1.2. Microbiology

The new genus *Helicobacter* was described in October 1989 and the originally named *Campylobacter pyloridis,* later *Campylobacter pylori*, was finally renamed *H. pylori* (Goodwin *et al.,* 1989). *H. pylori* is a curved or spiral-shaped, unipolar, multiflagellated, Gram-negative rod, 2.5 - 4.0 μm long and 0.5 - 1.0 μm wide. The covering glycocalyx has a thickness of up to 40 nm. There are 3 - 6 sheathed flagella with a membranous terminal bulb attached to one pole enabling motility in viscous

solutions. Each flagellum has a length of 2.5 μm and a thickness of 30 nm (Goodwin and Worsley, 1993). *H. pylori* has a circular genome consisting of 1.60 - 1.73 megabases and 1,590 open reading frames (ORFs) (Taylor *et al.,* 1992; Tomb *et al.,* 1997). The average guanine and cytosine (G+C) content is 39%.

H. pylori colonises the gastric epithelium and its overlaying mucus layer (Hazell *et al.,* 1986). Less than 1% of the bacteria are estimated to adhere to the epithelium. The bacterial density of the mucus gel is 10^4 - 10^5 per cubic mm as compared to 10^1 - 10^3 per cubic mm adherent to the gastric epithelium (Kirschner and Blaser, 1995; Atherton *et al.,* 1996; Nowak *et al.,* 1997; Falk *et al.,* 2000). In Mongolian gerbils, the highest density of *H. pylori* is found in the 25 μm part of the mucus layer that is closest to the epithelium, whereas the remaining 75 μm of luminal mucus is virtually free of *H. pylori* (Schreiber *et al.,* 2004). The pH gradient in the mucus, but not the bicarbonate/CO_2 gradient or the urea/ammonium gradient, maintains the distribution pattern of *H. pylori* in the mucus (Schreiber *et al.,* 2004).

The enzyme urease constitutes about 10 to 15% of all protein synthesised by *H. pylori* (Scott *et al.,* 1998). The ammonia produced by the enzyme from urea available in the gastrointestinal tract protects the bacteria from the acidic gastric environment. This protection is most important during transmission, before the bacteria have reached their protective niche in or below the gastric mucus layer. Additionally, ammonia improves bacterial survival by protecting them from pepsin, which has its optimum activity at low pH in the gastric lumen (Schreiber *et al.,* 2005). It has been suggested that proton neutralisation occurs either in the bacterial cytoplasma by ammonia from intra-cytoplasmic urease with extrusion of charged ammonium ions, in the periplasmic space after diffusion of intra-cytoplasmic non-charged ammonia to this space or in the extra-cytoplasmic space in clouds of ammonia surrounding the bacteria, where the clouds of ammonia are due to an altruistic autolysis mechanism of the *H. pylori* (Stingl *et al.,* 2002).

H. pylori only colonises gastric mucosa, present in the stomach or as gastric metaplasia in the duodenum or elsewhere in the gastro-intestinal tract (Johan *et al.,* 1990; Kestemberg *et al.,* 1993). The colonisation is usually most prominent in the antrum and cardia and lower in the acid-producing corpus. In conditions with lower acidic output however, antral colonisation may decrease and corpus colonisation increase (Dixon, 1991). Subjects with antrum-predominant gastritis run a higher risk of developing duodenal ulcer, whereas subjects with corpus-predominant gastritis run a higher risk for gastric ulcer and intestinal gastric carcinoma (Dixon, 2001).

H. pylori causes a chronic inflammation in the gastric mucosa and escapes eradication by dampening the adaptive and innate immune response (Pinto-Santini and Salama, 2005). *H. pylori* induces a Th1 immune response with release of interleukins 8, 12 and 18 (IL-8, IL-12 and IL-18), tumour necrosis factor alpha (TNF-α) and interferon gamma (INF-γ) from epithelial cells and attracted leukocytes. Activation of regulatory T cells downregulates the adaptive immune response (Lundgren *et al.,* 2003). The innate immune response is probably induced by toll-like receptor 2 (TLR2) rather than toll-like receptor 4 (TLR4), resulting in a low-level gastric inflammation (Lepper *et al.,* 2005). Similarly, chronic infections with *Borrelia burgdorferi* (Lyme disease) and *Mycoplasma spp.* activate TLR2 rather than TLR4 (Hirschfeld *et al.,* 2001). The flagellin subunit of *H. pylori* is at the same time less potent in activating TLR5 (Gewirtz *et al.,* 2004).

H. pylori may attain coccoidal forms after prolonged culture or under stressful environmental conditions (Sato, 2000). It is not known however, whether the bacterium is able to revert to its normal morphology and regain its capacity of replication (Goodwin and Worsley, 1993; Nilius *et al.,* 1993). Two types of coccoidal forms have been described, one with electron-loosened cytoplasm and enlarged periplasmatic space and another one with electron-dense cytoplasm and intact cytoplasmatic membranes (Goodwin and Worsley, 1993; Saito *et al.,* 2003).

H. pylori may have been colonizing the human stomach since time immemorial. An attempt to estimate the minimal time since the last common ancestor of *H. pylori* has suggested 2,500 - 11,000 years. The estimate is based on the synonymous mutation clock rate, which is based on nucleotide mutations that do not change the amino acid sequence (Falush *et al.,* 2001).

5.1.2.1. Cytotoxin Associated Gene *Pathogenecity Island*

H. pylori virulence has been associated with presence of a *cytotoxin-associated gene* pathogenecity island (*cag*-PAI). The *cag*-PAI has a G+C content of 35%, which is different from the rest of the *H. pylori* genome, suggesting that *cag*-PAI has been acquired by horizontal transfer (Censini *et al.,* 1996). The *cag*-PAI is inserted in the glutamate racemase gene of the *H. pylori* chromosome and contains up to 31 ORFs. The organisation of the *cag*-PAI differs between *H. pylori* strains. The *cag*-PAI may be a single uninterrupted unit or divided into *cag*I and *cag*II by either one copy of IS*605* or a large piece of chromosomal DNA, flanked on both sides by IS*605* (Censini *et al.,* 1996). The gene *cag*A belongs to *cag*I and its protein CagA is highly immunogenic.

There are significant homologies between six of the *cag*-PAI ORFs and genes belonging to the Type IV secretion system of *Agrobacterium tumefaciens, Bordetella pertussis* and *Legionella pneumophilia* (Backert *et al.,* 2000). Type IV secretion systems form molecular syringes that inject specific substrates into the cytosol of the target cell. Recent studies have shown that CagA is translocated into the gastric epithelial cells where it is tyrosine-phosphorylated into $CagA^{P\text{-}Tyr}$ by Src-like protein-tyrosine kinases (Odenbreit *et al.,* 2001; Selbach *et al.,* 2002*b*). Phosphorylation occurs at an EPIYA motif representing a 5-amino-acid sequence. The flanking sequences show a geographical variation, with Western CagA being associated to lower grades of inflammation, activity of gastritis and atrophy than East-Asian CagA (Higashi *et al.,* 2002; Hatakeyama, 2004).

Virulence of *cag*-PAI may not be limited to Type IV secretion mechanisms. Host cell secretion of interleukin-8 (IL-8), a potent neutrophil and T-cell chemoattractor and activator, has been associated with *cag*-PAI. IL-8 secretion however, is not affected by knockout mutations of genes belonging to the Type IV system (Selbach *et al.,* 2002*a*).

About 50% of *H. pylori* strains express the vacuolating toxin gene *vac*A. The proteins VacA and CagA are commonly found simultaneously, although *cag*A and *vac*A are not located together in the *H. pylori* genome. The vacuolating toxin VacA consists of 87 kDa monomers that, when assembled, form a flower-shaped oligomer in eukaryotic cells. The oligomer acts as a porin and forms large vacuoles *in vitro* (Reyrat *et al.,* 2000).

5.1.3. Culture

Microaerophilic culture conditions are necessary for the growth of *H. pylori* (Warren, 1983). The optimal oxygen concentration is between 2 and 8%. Maximal growth occurs at 37 °C, neutral pH and with addition of 8 - 10% carbon dioxide (Mégraud *et al.,* 1985; Goodwin and Worsley, 1993). Agar supplemented with 5 - 10% horse or sheep blood is suitable for culture (Glupczynski, 1996). The bacteria are urease-, oxidase- and catalase-positive (Goodwin *et al.*, 1989; Goodwin and Worsley, 1993).

5.1.4. Epidemiology

Acquisition of *H. pylori* occurs predominantly in childhood and the infection is life-long in most subjects. Intrafamilial spread, especially from mother to child, seems to play an important role. In developing countries the prevalence is high already in early adulthood and remains high during adult life (Frenck and Clemens, 2003). By contrast, in developed countries there is a gradual

increase in prevalence with age (Rothenbacher and Brenner, 2003). This has been attributed to a birth cohort effect (Replogle *et al.,* 1996). Subjects born at the same time have a similar prevalence of *H. pylori*, irrespective of their age, when measured in longitudinal studies, whereas subjects born later but with the same age at measurement have a lower *H. pylori* prevalence (Roosendaal *et al.,* 1997; Gause-Nilsson *et al.,* 1998; Rehnberg-Laiho *et al.,* 2001; Perez-Perez *et al.,* 2002). Studies following children during childhood and adolescence have shown a peek in *H. pylori* incidence below the age of five (Mitchell *et al.,* 1992; Russell *et al.,* 1993; Ashorn *et al.,* 1995; Granström *et al.,* 1997; Granquist *et al.,* 2002; Malaty *et al.,* 2002). The prevalence of *H. pylori* infection world-wide is about 50% (Torres *et al.,* 2000). A Swedish study reported a prevalence of 17% among subjects aged 30 - 39 years and of 47% among subjects aged 60 - 69 years in the mid-1990s (Bergenzaun *et al.,* 1996). Several developing countries have a prevalence above 80% among the adult population (Frenck and Clemens, 2003).

H. pylori infection in the mother has repeatedly been shown to be an independent risk factor for *H. pylori* infection in children (Malaty *et al.,* 1991; Elitsur *et al.,* 1999; Han *et al.,* 2000; Rothenbacher *et al.,* 2002; Kivi *et al.,* 2003; Rocha *et al.,* 2003; Escobar and Kawakami, 2004; Aguemon *et al.,* 2005; Farrell *et al.,* 2005). Fingerprinting methods have shown that children often harbour the same strain as their mother (Han *et al.,* 2000; Kivi *et al.,* 2003; Konno *et al.,* 2005). Bed sharing is a risk factor for *H. pylori* infection (Aguemon *et al.,* 2005; Farrell *et al.,* 2005) and the same strain has also been found among siblings (Han *et al.,* 2000; Kivi *et al.,* 2003). However, the reinfection rate among children over the age of five is low, despite a high prevalence among parents and siblings (Rowland *et al.,* 1999; Rowland, 2000). Transmission from the father has been documented but seems to occur to a lesser degree (Malaty *et al.,* 1991; Aguemon *et al.,* 2005; Farrell *et al.,* 2005). Spouses may become infected with the same strain (Malaty *et al.,* 1991; Kivi *et al.,* 2003). Interestingly, smoking among mothers protects children from contracting the infection (Brenner *et al.,* 2000). Low socioeconomic status is frequently found to increase the risk of being infected (Malaty *et al.,* 2001; Moayyedi *et al.,* 2002). A higher prevalence has been observed in institutionalised patients and in military service staff, emphasizing that crowding might be a risk factor for becoming infected (Kyriazanos *et al.,* 2001; Morad *et al.,* 2002; Wallace *et al.,* 2004). The spreading of *H. pylori* infection does not correspond to the fecal-oral spreading of Hepatitis A, to the waterborne *Corynebacterium parvum* or to the oral-oral spreading Epstein-Barr virus (Luzza *et al.,* 2000; Malaty *et al.,* 2003; Steinberg *et al.,* 2004). Swedish backpackers, most of whom reported having had an episode of diarrhoea, had no increased risk of *H. pylori* infection (Lindkvist *et al.,* 1995). Vomiting among siblings has been described as a risk factor, suggesting a gastro-oral

transmission route (Luzza *et al.,* 2000). *H. pylori* has been cultured from samples of experimentally induced vomitus (Parsonnet *et al.,* 1999).

5.2. Gastric Malignant Tumours

Table 5.2.1.1. Estimated gastric cancer incidence in different populations in the year 2000 (Ferlay *et al.*, 2001)

Population	Age standardised rate* (per 100,000)	
	Men	Women
Eastern Asia	42.58	19.57
Eastern Europe	34.05	14.54
South America	23.14	11.69
Central America	18.64	13.14
Middle Africa	16.99	14.06
Southern Europe	19.48	9.74
Polynesia	12.97	9.16
Caribbean	14.46	7.20
Western Europe	13.84	7.03
Northern Europe	12.74	6.08
Western Asia	11.17	6.09
Australia/New Zealand	9.79	5.00
Eastern Africa	7.07	6.71
South-Eastern Asia	8.68	4.82
Southern Africa	8.56	3.65
Northern America	7.76	3.68
Micronesia	5.22	5.16
South Central Asia	6.61	3.45
Melanesia	5.91	3.77
Western Africa	5.36	3.90
Northern Africa	5.56	3.32
Sweden	8.83	4.66

* adjusted to world standard population.

Gastric adenocarcinomas, the most common gastric cancer, comprise about 90% of all gastric malignancies (Kelley and Duggan, 2003). Most other gastric malignancies are gastric non-Hodgkin lymphomas, leiomyosarcomas, gastric carcinoids and gastrointestinal stroma tumours. Rarely occurring gastric cancers include adenosquamous, squamous or undifferentiated carcinomas and choriocarcinomas. Very rare primary gastric malignancies are rhabdomyosarcomas, hemangiopericytomas and Kaposi´s sarcoma (Kelley and Duggan, 2003).

5.2.1. Incidence

Gastric cancer is the 4th most common cancer worldwide after lung cancer, breast cancer and colorectal cancer. An estimated 934,000 new cases of gastric cancer were diagnosed in the year 2002 compared to 1,350,000 new cases of lung cancer. Gastric cancer was, after lung cancer, the second largest cause of cancer-related deaths, the estimated mortality was 700,000 cases in 2002 (10.4% of cancer deaths). The total burden of cancer incidence and cancer mortality in that year was estimated to 10.9 million cases and 6.7 million cases, respectively (excluding non-melanoma skin cancer) (Parkin *et al.,* 2005). The incidence of gastric cancer is decreasing, but as a result of world population growth and ageing the absolute number of gastric cancer cases is increasing. In 1980, when gastric cancer was ranked as the most common cancer, there were 669,400 new cases worldwide (Parkin *et al.,* 1988).

The highest gastric cancer incidences are found in Japan, North Korea and South Korea (annual incidence 70.2 - 69.2/10^5 in men and 28.6 - 25.7/10^5 in women, age-standardised). High incidence rates are also seen in Eastern Asia, Eastern Europe, Central and South America, while the rates are low in eastern and northern Africa, North America and southern Asia (Ferlay *et al.,* 2001) (Table 5.2.1.1.). The gastric cancer incidence is almost twice as high in men as in women (Ferlay *et al.,* 2001).

In Sweden 602 new male and 388 new female cases of gastric malignancies were diagnosed in the year 2003. The gastric cancer incidence this year, according to the national cancer registry (The Swedish Cancer Registry, 2005), was 15.1/10^5 in men and 7.6/10^5 in women, when adjusted to the Swedish population of the year 2000 and 6.7/10^5 in men and 3.3/10^5 in women, when adjusted to world standard population. The Swedish gastric cancer incidence has continuously declined since cancer registration in Sweden begun in 1958. This year there were 1526 male and 911 female new cases of gastric cancer in Sweden. The crude incidences in 1958 were 41.3/10^5 in men and 24.5/10^5 in women (The Swedish Cancer Registry, 1960).

5.2.2. Classification

The International Classification of Diseases for Oncology (ICD-O) by the World Health Organisation (WHO) classifies gastric tumours anatomically as cardia, fundus, body, antrum or pyloric (Percy *et al.,* 1990). Tumours of the cardia include tumours of the cardia orifice, the cardio-oesophageal junction, the gastro-oesophageal junction and tumours overlapping the oesophagus and the stomach. Studies on the association of gastric cancer with *H. pylori* have generally distinguished between cardia and non-cardia gastric adenocarcinoma. Most of the decreasing incidence and geographic variation of gastric cancer relate to non-cardia gastric adenocarcinoma (Kelley and Duggan, 2003), while the incidence of cardia gastric adenocarcinoma are more uniform and have been reported to increase (Blot *et al.,* 1991; Powell and McConkey, 1992; Hansson *et al.,* 1993*b*; Pera *et al.,* 1993; Zheng *et al.,* 1993; Lord *et al.,* 1998)

Histologically, gastric adenocarcinomas are usually classified according to Laurén, into intestinal or diffuse type adenocarcinomas. Intestinal adenocarcinomas have glandular lumina with polarised columnar cells or form solid components with distinct epithelial tracts. In contrast, diffuse adenocarcinomas grow infiltratively, the cells are smaller, indistinctly defined and uniform and are solitary or form small clusters or masses of loosely attached cells (Laurén, 1965). Intestinal gastric adenocarcinoma develops through a sequence of precursor lesions from

superficial gastritis, gastric atrophy, intestinal metaplasia, dysplasia to adenocarcinoma. Each precursor lesion occurs in a gastric mucosa in which the preceding lesion is present (Correa *et al.,* 1990*a*; Correa *et al.,* 1990*b*). In contrast, diffuse gastric adenocarcinoma develops without corresponding precursor lesions.

5.2.3. Etiology

H. pylori infection, host genetic factors, high intake of salt and low intake of fruit and vegetables, particularly citrus fruits, alliums and raw vegetables, have been associated with an increased risk of gastric cancer (El-Omar *et al.,* 2000; Gonzalez, 2002; Tsugane, 2005). Better living conditions with less crowding, better hygiene and refrigeration (Graham *et al.,* 1990; La Vecchia *et al.,* 1990; Lee *et al.,* 1995) with all year availability of fruit, vegetables and non-preserved food are believed to explain the decrease of gastric cancer incidence during the last half century.

Fruit and vegetables have been associated with a weaker protective effect in recent cohort studies than anticipated from earlier case-control studies (Riboli and Norat, 2003). The difference may be due to recall bias or due to changed dietary pattern because of preclinical symptoms (Botterweck *et al.,* 1998). A meta-analysis of tobacco smoking has showed an increased risk of gastric cancer (Tredaniel *et al.,* 1997). Alcohol consumption, however, has not been associated with gastric cancer (Franceschi and La Vecchia, 1994). Gastric ulcer, but not duodenal ulcer, has been associated with gastric cancer (Lee *et al.,* 1990; Molloy and Sonnenberg, 1997). The risk is continuously elevated compared to the standardised incidence ratio, but highest during the first years after the gastric ulcer diagnosis (Hansson *et al.,* 1996). Partial gastrectomy approximately doubles the risk for gastric cancer in the gastric remnant (Tersmette *et al.,* 1990). The risk may be reduced during the initial 10 years after the operation, because of the removal of the most cancer-prone distal part of the stomach and then increases to a 4-5-fold risk after 15 - 25 years (Tersmette *et al.,* 1995). Pernicious anemia increases the risk for gastric cancer (Hsing *et al.,* 1993). Therapeutic ionic radiation of 15 - 30 Gy directed at the stomach increased the risk 2-4-fold (Griem *et al.,* 1994). Non-cardia gastric adenocarcinoma is associated with low socioeconomic status, in contrast to cardia gastric adenocarcinoma (Powell and McConkey, 1992; Brewster *et al.,* 2000).

Host genetic factors associated with gastric cancer development have been found in poly-morphisms of the IL-1 gene cluster and of the tumour necrosis factor alpha genes (TNF-α-308)

(El-Omar *et al.,* 2003; Machado *et al.,* 2003). Pro-inflammatory alleles seem to predispose to gastric cancer.
Familial clustering may occur due to *H. pylori* infection or blood group A (Kelley and Duggan, 2003). Germline mutations in the E-cadherin gene for cell adhesion is an underlying factor in the rare hereditary diffuse type gastric cancer syndrome (HDGC) (Keller *et al.,* 2004). Gastric cancer has been associated with other cancer syndromes mainly affecting other organs, such as the hereditary nonpolyposis colorectal cancer syndrome (HNPCC; germline mutation in DNA mismatch repair genes), the Li Fraumeni syndrome (germline mutation in TP53 tumour suppressor gene), the familial adenomatosis polyposis syndrome (FAP; germline mutation in APC gene) and the Peutz-Jeghers syndrome (Lindor and Greene, 1998).

Migrant studies have shown that subjects moving from high-risk to low-risk areas for stomach cancer retain a higher risk, more similar to their homeland population (Howson *et al.,* 1986). Second generation migrants from Japan to Hawaii instead ran a lower risk, more like the native Hawaii population (Haenszel *et al.,* 1972). Environmental factors early in life may therefore be of etiologic importance.

Although *N*-nitroso compounds are carcinogenic, high dietary nitrate intake has not been associated with an excess risk for gastric cancer in epidemiological studies (Gonzalez *et al.,* 1994; Pobel *et al.,* 1995). Dietary nitrate comes mainly from green and root vegetables and water and vegetarians do indeed have a risk for gastric cancer that is lower than the standardised incidence ratio (McKnight *et al.,* 1999). Likewise, in spite of increasing nitrate concentrations in ground water, as a secondary effect of leaching of fertilizers (Addiscott, 1996), the incidence of gastric cancer is decreasing.

Salivary glands secrete about 25% of ingested nitrate at up to 10 times the plasma concentration, creating an enterosalivary circulation of nitrate (Edwards *et al.,* 1954; McKnight *et al.,* 1999). Oral *Staphylococcus sciuri* and *S. intermedius* reduce nitrate to nitrite. *N*-nitroso compounds are then formed from nitrite through acid-catalyzed reactions or bacterial nitrosation (Mirvish, 1995). *N*-nitroso compounds may also be formed directly from nitric oxide released in inflammatory processes. The gastric epithelium is protected by the covering mucus gel and by ascorbic acid secreted in the gastric juice (Sidebotham *et al.,* 1991; Sobala *et al.,* 1991*a*; Banerjee *et al.,* 1994). It has been suggested that the entero-salivary circulation improves the host defence against

gastrointestinal infections, as nitrogen compounds formed after acidification of nitrate increase microbial killing at the higher postprandial gastric pH (Dykhuizen *et al.,* 1996; Xu *et al*., 2001).

5.2.3.1. H. pylori

H. pylori was classified as a Group 1 human carcinogen in 1994 by IARC (International Agency for Research on Cancer) (IARC, 1994). The IARC classification was primarily based on data showing the association between *H. pylori* infection and gastric cancer in case-control studies nested within prospective cohorts (Forman *et al.,* 1991; Parsonnet *et al.,* 1991; Nomura *et al.,* 1991; Lin *et al.,* 1995), in retrospective case-control studies (Talley *et al.,* 1991; Sipponen *et al.,* 1992; Archimandritis *et al.,* 1993; Blaser *et al.,* 1993; Estevens *et al.,* 1993; Hansson *et al.,* 1993*a*; Kuipers *et al.,* 1993; Lin *et al.,* 1993*a*; Lin *et al.,* 1993*b*) and in geographical correlation studies (Correa *et al.,* 1990*c*; Forman *et al.,* 1990; Sierra *et al.,* 1992; EUROGAST, 1993; Palli *et al.,* 1993). Data showing that *H. pylori* was associated with gastritis, *i.e.* a consistent association with gastritis (Dixon, 1991; Kuipers *et al.,* 1995), gastritis developing after *H. pylori* ingestion (Ramsey *et al.,* 1979; Marshall *et al.,* 1985; Morris and Nicholson, 1987; Graham *et al.,* 1988; Sobala *et al.,* 1991*b*) and the disappearance of *H. pylori* after successful treatment (Rauws *et al.,* 1988; Genta *et al.,* 1993) contributed to the classification by IARC, as well as data in follow-up studies showing progression from non-atrophic gastritis to atrophic gastritis (Ihamäki *et al.,* 1985; Correa *et al.,* 1990*b*; Kuipers *et al.,* 1995). The carcinogenic mechanism of *H. pylori* was judged unknown.

In a collaborative meta-analysis of 12 case-control studies nested in prospective cohorts, the risk of developing non-cardia gastric adenocarcinoma associated with *H. pylori* was estimated to 3.0 (95% CI: 2.3 - 3.8) (*Helicobacter* and Cancer Collaborative Group, 2001). When restricted to cases with more than 10 years of follow-up from blood sampling to tumour diagnosis, the risk was 5.9 (3.4 - 10.3). It was suggested that the lower risk found in subjects with shorter follow-up was due to gastric atrophy with concomitant loss of *H. pylori* infection during the course of intestinal gastric adenocarcinoma development. No risk was found for gastric adenocarcinoma of the cardia, odds ratio 1.0 (95% CI: 0.7 - 1.4). Subjects younger than 60 years had a 2.2 (95% CI: 1.3 - 3.7) times higher risk for non-cardia gastric adenocarcinoma. There were no variations in risk with gender or between intestinal versus diffuse histological subtypes. The risk for non-cardia gastric adenocarcinoma has also been addressed in another meta-analysis, with an odds ratio of 3.1 (95% CI: 1.8 - 5.3) (Huang *et al.,* 1998). The overall risk for gastric adenocarcinoma has been addressed in another two meta-analyses, with odds ratios of 2.5 (95% CI: 1.9 - 3.4) and 2.0 (95% CI: 1.7 - 2.4), respectively (Danesh, 1999; Eslick *et al.,* 1999). A funnel plot showed no publication

bias (Eslick *et al.,* 1999). Instead, studies with positive results were generally of good quality, whereas studies with negative results were of poor or moderate quality. These meta-analyses, combining prospective and retrospective studies and not always distinguishing between cardia and non-cardia adenocarcinomas, may however have underestimated the risk for gastric non-cardia adenocarcinoma associated with *H. pylori*.

Table 5.2.3.1.1. Odds ratios of the association between *H. pylori* and non-cardia gastric adenocarcinoma from 12 prospective case-control studies included in the *Helicobacter* and Cancer Collaborative Group meta-analysis

Country*	Median	Cases		Controls		Odds ratio	
	interval (y)	N	*H. pylori* pos	N	*H. pylori* pos	OR	95% CI
UK	8.7	9	67 %	27	44 %	2.9	0.5 - 16.5
USA I	15.0	62	89 %	62	61 %	5.2	1.8 - 15.3
USA II	13.8	74	96 %	74	76 %	8.5	2.0 - 36.8
Taiwan	2.0	21	71 %	160	60 %	1.7	0.6 - 4.5
Finland I	5.1	75	88 %	130	82 %	1.7	0.8 - 4.0
China I	4.8	114	88 %	331	82 %	1.5	0.8 - 2.9
Sweden	5.1	27	89 %	108	49 %	11.1	2.5 - 49.4
Japan	3.6	38	90 %	190	74 %	3.0	1.0 - 8.9
Norway	12.0	132	90 %	614	63 %	5.2	2.8 - 9.4
Iceland	15.0	35	77 %	176	69 %	1.5	0.7 - 3.5
Finland II	4.1	93	94 %	204	71 %	4.7	2.0 - 11.3
China II	3.6	82	62 %	174	51 %	1.8	1.0 - 3.2
Overall	6.0	762	86 %	2250	69 %†	3.0	2.3 - 3.8

* *References*: UK (Forman *et al.,* 1991; Wald *et al.,* 1997), USA I (Parsonnet *et al.,* 1991), USA II (Nomura *et al.,* 1991), Taiwan (Lin *et al.,* 1995), Finland I (Aromaa *et al.,* 1996), China I (Webb *et al.,* 1996; Yuan *et al.,* 1999), Sweden (Simán *et al.,* 1997), Japan (Watanabe *et al.,* 1997), Norway (Hansen *et al.,* 1999), China II (Limburg *et al.,* 2001). Data from Finland II and Iceland are unpublished.

† Weighted by the number of cases in each study to allow for the different matching ratios of controls to cases.

5.2.4. Carcinogenic Mechanisms

Chronic inflammation is believed to be of primary importance in gastric carcinogenesis (Correa *et al.,* 1975; Correa, 2004). Inflammatory cells may damage the gastric mucosa by long-standing release of proteases and reactive oxygen and nitrogen species (Dallegri and Ottonello, 1997). Mucosal damage results in apoptosis and increased gastric epithelial cell proliferation (Xia and Talley, 2001). DNA damage may occur because of reactive oxygen and nitrogen species or increased cell proliferation (Preston-Martin *et al.,* 1990; Nguyen *et al.,* 1992; Papa *et al.,* 2002). Acquired mutations alter the cell phenotype to precancerous stages that ultimately leads to cancer (Tahara, 2004). Bacterial overgrowth seems however, not to be a prerequisite as nitrosation may

be acid-catalyzed and the concentrations of nitrite and total *N*-nitroso compounds in the normal and the atrophic stomach do not differ (Sobala *et al.,* 1991*a*).

H. pylori is thought to have a central role in gastric carcinogenesis. *H. pylori* causes a chronic mucosal inflammation, increases apoptosis and cell proliferation, releases enzymes and cytotoxins causing mucosal damage and interacts directly with the host cell through the type IV secretion system of the *cag*-PAI.

Polymorphonuclear and mononuclear leucocytes are attracted to the gastric mucosa by IL-8 release from gastric epithelial cells, by macrophage-derived IL-1 and TNF-α and by several factors derived from *H. pylori* including lipopolysacharides (LPS), urease and neutrophil activating protein (HP-NAP) (Crabtree, 1996; Correa, 2004). *H. pylori*, especially *cag*-PAI containing strains, induces the IL-8 release from the gastric epithelial cells. *H. pylori* increases the expression of inducible nitric oxide synthase (iNOS) in attracted macrophages and neutrophils (Correa, 2004; Mannick *et al.,* 1996). These endogenously formed reactive nitrogen species may result in mucosal and DNA damage (Hahm *et al.,* 1997).

H. pylori induces apoptosis, a genetically regulated form of programmed cell death, primarily by the Fas Ag pathway (Moss *et al.,* 1996; Stoicov *et al.,* 2004). Metaplastic and dysplastic cells may however become resistant to Fas-mediated apoptosis (Lee *et al.,* 2003). Cell proliferation may increase to compensate for apoptosis and epithelial damage, but could be directly stimulated by gastrin, cytokines and reactive oxygen metabolites (Nakajima *et al.,* 1997; Peek *et al.,* 2000). Cell proliferation is reduced in the normal mucosa after *H. pylori* eradication (Lynch *et al.,* 1995). Cell proliferation is higher in intestinal metaplasia and dysplasia (Cahill *et al.,* 1996).

H. pylori disrupts the gastric mucus gel and decreases the concentration of ascorbic acid, a reactive species scavenger, in gastric juice (Sidebotham *et al.,* 1991; Sobala *et al.,* 1991*b*; Banerjee *et al.,* 1994). *H. pylori* may therefore facilitate carcinogenesis by *N*-nitroso compounds and other gastric luminal carcinogens.

The *H. pylori* release of the enzymes urease, phospholipases and peroxidases may contribute to mucosal damage (Xia and Talley, 2001). *H. pylori* urease may induce DNA damage through monochloramine (NH_2Cl) produced from ammonia (NH_3) and hypochlorous acid (HClO), the latter a reactive oxygen metabolite from activated neutrophils (Suzuki *et al.,* 1998).

Direct interactions of *H. pylori* with the gastric epithelial cells have been suggested as carcinogenic mechanisms. Upregulation of IL-8 and neutrophil chemotactic chemokines may occur after physical contact between *cag*-PAI containing *H. pylori* strains and the gastric epithelial cells, with induction of nuclear factor κB (NF-κB) and the activator protein 1 (AP-1) (Naumann *et al.,* 1999; Naumann, 2001; Crabtree, 2001). Translocated CagA in the gastric epithelial cells may interact with intracellular signalling pathways (Naumann, 2005). Phosphorylated CagA has been shown to interact with SHP-2, which plays an important role in hepatocyte growth factor (HGF) induced cellular changes (Kodama *et al.,* 2000). CagA translocation causes *in vitro* actin-cytoskeletal rearrangements and the formation of the "hummingbird" phenotype (Higashi *et al*., 2002). Phosphorylated CagA may enhance cell scattering by binding to the cytoplasmic part of the activated c-Met receptor (Churin *et al*., 2003). Co-localisation of CagA with tight junctional adhesion protein (JAM) and the scaffolding zonula occludens-1 protein (ZO-1) may disrupt epithelial tight junctions and promote a motogenic response (Amieva *et al.,* 2003). CagA may modulate the Ras/MEK/ERK signalling cascade leading to the activation of nuclear transcription factors fos, jun and myc and eventually induce cell scattering and proliferation (Gale *et al.,* 1993; Mimuro *et al.,* 2002). These interactions constitute plausible carcinogenic mechanisms, although their precise role in gastric carcinogenesis remains to be clarified.

The carcinogenic mechanisms of tobacco smoke in gastric cancer are still unclear. Tobacco smoke does however contain a multitude of carcinogenic compounds. The aerosol contains about 4,800 compounds and about 10^{10} particles/ml (Pfeifer *et al*., 2002). The particular phase constitutes 10% of smoke weight and contains carcinogenic compounds such as polycyclic aromatic hydrocarbons (PAH), *N*-nitroso compounds, aromatic amines and metals (Hoffmann *et al*., 2001). Long-living reactive species in the particular phase may be swallowed and lead to the formation of DNA adducts and oxidative DNA damage in the gastric mucosa (Dyke *et al*., 1992; Pryor, 1997). Lower plasma levels of reactive species scavengers (ascorbic acid and ß-carotene) have been observed among smokers (Buiatti *et al*., 1996).

Gastric cancer does not seem to have the regular sequence of mutations seen in colorectal cancer. Common mutations in gastric cancer occur in the tumour suppressor genes TP53, the adenomatous polyposis coli gene (APC) and the deleted in colorectal cancer gene (DCC) (Tahara, 2004).

5.3. Oesophageal Malignant Tumours

Squamous cell carcinoma of the oesophagus is the predominant form of oesophageal malignancy. Adenocarcinoma of the oesophagus, the other main oesophageal malignancy, occurred in less than 5% of oesophageal carcinoma cases before the mid 1970s (Lagergren, 2005). The incidence has however increased rapidly and the incidence of oesophageal adenocarcinoma has in some countries surpassed that of squamous cell carcinoma (Devesa *et al.,* 1998). Melanomas, leiomyosarcomas, small cell carcinomas and lymphomas occur rarely (Enzinger and Mayer, 2003; Wu *et al.,* 2004).

5.3.1. Incidence

Oesophageal cancer is the eighth most common cancer worldwide and the sixth most common cause of death from cancer. In 2002 there were 462,000 newly diagnosed cases and the number of deaths from oesophageal cancers was 386,000 (5.7% of total number of cancer deaths) (Parkin *et al.,* 2005). The worldwide incidence in 2002, age standardised to world standard population, was $11.5/10^5$ in men and $4.7/10^5$ in women (Parkin *et al.,* 2005). High risk areas are China (males 27.4, females $12.0/10^5$), southern Africa, eastern Africa and Japan. Low risk areas are middle Africa and western Africa (males $1.3/10^5$ and females $0.6/10^5$).

There were 302 new male and 103 new female cases of oesophageal malignancy in Sweden in the year 2003. The incidence of oesophageal malignancy this year were $7.3/10^5$ in men and $2.0/10^5$ in women, when adjusted to the Swedish population of the year 2000 and $3.6/10^5$ in men and $0.9/10^5$ in women, when adjusted to the world standard population (The Swedish Cancer Registry, 2005). The Swedish incidence of oesophageal malignancies in males and females increased between 1958 and 1970, but has not changed since (The Swedish Cancer Registry, 2005). In 1958 the crude incidence was $3.2/10^5$ in men and $1.8/10^5$ in women, corresponding to 119 male and 66 female cases of oesophageal malignancy in Sweden this year (The Swedish Cancer Registry, 1960).

There was a significant increase in annual incidence for oesophageal adenocarcinoma in Sweden from 1980 to 1995 (annual percent change (a.p.c): males 2.3%, females 3.1%), whereas squamous cell carcinoma has begun to decline among males. More dramatic increases in annual male incidence rate have however been observed in the USA (a.p.c: white: 8.6%, black: 4.1%), Norway (a.p.c: 8.3%), Denmark (a.p.c: 7.9%), Iceland (a.p.c: 7.7%), Finland (a.p.c: 7.2%) and South Australia (a.p.c: 6.4), whereas modest changes have been seen in France (a.p.c: 2.8%) and the

Netherlands (a.p.c: -1.6%) (Vizcaino *et al.,* 2002). Similar increases among females have been seen in the USA (a.p.c: white: 6.8%, black: 13.8%), Scandinavia (a.p.c: 3.1 - 6.1%) and in the Neatherlands (a.p.c: 0.8%). The increase in incidence has persisted during a period of no or minimal changes in diagnostic methods (Lagergren, 2005).

5.3.2. Classification

Adenocarcinomas arising within 5 cm of the gastro-oesophageal junction are classified according the system proposed by Siewert and Stein (Siewert and Stein, 1998). Type I is "adenocarcinomas of the distal oesophagus which usually arises from an area with specialized intestinal metaplasia of the oesophagus (*i.e.* Barrett's oesophagus) and which may infiltrate the gastro-oesophageal junction from above". Type II is "true carcinoma of the cardia arising from the cardiac epithelium or short segments with intestinal metaplasia at the gastro-oesophageal junction; this entity is also often referred to as 'junctional carcinoma'". Type III is "subcardial gastric carcinoma which infiltrates the gastro-oesophageal junction and distal oesophagus from below". In a study by Lagergren *et al.*, (1999*a*) adenocarcinomas with their centre within 2 cm proximal to or 3 cm distal to the gastro-oesophageal junction were classified as gastric cardia adenocarcinomas, unless they occurred adjacent to Barrett's oesophagus. These were instead classified as oesophageal adenocarcinomas. Barrett's oesophagus is a specialised columnar-cell metaplasia that replaces the normal oesophageal squamous cell epithelium. Pedrazzani *et al.* (2005) have classified adenocarcinomas with their centre located 1 cm above to 2 cm below the gastro-oesophageal junction as type II, *i.e.*, gastric cardia adenocarcinoma. The ICD-O recommends that oesophageal tumours be classified either as cervical, thoracic or abdominal or by thirds (upper, middle or lower) (Percy *et al.,* 1990). These two classifications are non-analogous. Adenocarcinomas are further classified as intestinal or diffuse using Laurén's classification, originally specified for gastric adenocarcinomas (Laurén, 1965).

5.3.3. Etiology

Chronic irritation seems to increase the risk of oesophageal squamous cell carcinoma. High alcohol consumption and tobacco smoking may account for more than 90% of oesophageal squamous cell carcinoma (Brown *et al.,* 2001). The incidence increases with poverty (Brewster *et al.,* 2000). The male to female incidence ratio is 3-4:1 (Vizcaino *et al.,* 2002). Other causes of this carcinoma are achalasia, oesophageal diverticulas, caustic injury and frequent ingestion of extremely hot beverages (Enzinger and Mayer, 2003). The Plummer Vinson syndrome has been associated with squamous cell carcinoma (Larsson *et al.,* 1975). The only associated genetic

disorder is non-epidermolytic palmoplantar keratoderma (tylosis), which confer a 90% risk for oesophageal squamous cell carcinoma before the age of 70 (Risk *et al.,* 1999).

Caucasian males have a preponderance for oesophageal adenocarcinoma. The male to female ratio, 7-10:1, is one of the highest for cancers that can occur in both sexes (Vizcaino *et al.,* 2002; Forman, 2004*a*). In contrast to squamous cell carcinoma, there is no association to socioeconomic status (Brewster *et al.,* 2000). Barrett's oesophagus, a condition secondary to gastro-oesophageal reflux, in which the squamous epithelium of the distal oesophagus is replaced with columnar epithelium, is the strongest risk factor for the development of oesophageal adenocarcinoma, with an excess risk ranging from 30-fold to 400-fold (Spechler *et al.,* 1984; Cameron *et al.,* 1985; Robertson *et al.,* 1988; Hameeteman *et al.,* 1989; Ovaska *et al.,* 1989; Van Der Veen *et al.,* 1989; Drewitz *et al.,* 1997). Gastro-oesophageal reflux per se has been shown to increase the risk, with an odds ratio ranging from 2.0 to 43.5 depending on severity and duration of reflux symptoms (Lagergren *et al.,* 1999*a*; Chow *et al.,* 1995). In population-based studies, a high BMI has been associated with an increased risk, with an odds ratio of 16 when subjects with BMI > 30 are compared to subjects with BMI < 22 (Chow *et al.,* 1998*b*; Lagergren *et al.,* 1999*b*; Wu *et al.,* 2001). No association has been found between alcohol consumption and oesophageal adenocarcinoma. Tobacco smoking and drugs relaxing the lower oesophageal sphincter may increase the risk, whereas the use of non-steroidal anti-inflammatory drugs (NSAID) may reduce it.

A history of radiotherapy targeted at mediastinum, such as in the treatment of breast cancer or lymphoma, increases the risk of both histological types of oesophageal cancer (Enzinger and Mayer, 2003).

6. AIMS

The overall aim of this thesis was to estimate the risks of gastric and oesophageal carcinomas associated with *H. pylori* infection.

The specific aims were:

- to estimate the risk for gastric adenocarcinoma associated with *H. pylori* seropositivity in different locations of the stomach,

- to investigate the risk for gastric adenocarcinoma related to tobacco smoking in *H. pylori* seropositive and *H. pylori* seronegative subjects,

- to estimate the risk for oesophageal adenocarcinoma and oesophageal squamous cell carcinoma associated with *H. pylori* infection,

- to evaluate a western blot method for the detection and interpretation of CagA seropositivity in a middle-aged population,

- to investigate how the presence of CagA seropositivity influence the risk of gastric and oesophageal carcinoma.

7. MATERIALS AND METHODS

7.1. Study Design

The risk of developing gastric cardia adenocarcinoma, gastric non-cardia adenocarcinoma, oesophageal adenocarcinoma and oesophageal squamous cell carcinoma associated with *H. pylori* infection, CagA and tobacco smoking was investigated by nested case-control studies in the health-screening cohort of Malmö Preventive Medicine, University Hospital Malmö (Berglund *et al.*, 2000).

7.2. Population

The health-screening cohort of Malmö Preventive Medicine, University Hospital Malmö, consists of 32,906 middle-aged subjects in the city of Malmö (23,104 men, 9,802 women). The subjects belong to birth year cohorts that were invited in their entirety (males born 1921, 1926-1942, 1944, 1946, 1948 and females born 1928, 1930-1936, 1938, 1941) and they were enrolled from Sept 1st, 1974 until Feb 28th, 1992. Among 44,078 eligible citizens the participation rate was 75%, ranging from 62% to 85% in different birth cohorts. Another 2,222 subjects belonging to birth year cohorts of the Malmö Preventive Medicine cohort of which only a part of the birth year cohort was invited (males born 1949; females born 1926, 1942 and 1949) were not included in the studies of this dissertation. At enrolment two plasma samples and one serum sample were taken from each participant and frozen at -20 ºC. Each participant was asked to answer a questionnaire about life style, which included questions on alcohol and tobacco consumption.

7.3. Identification of Gastric and Oesophageal Malignancies

Gastric (ICD-9 151) and oesophageal (ICD-9 150) malignancies were identified through the Swedish National Cancer Registry (SNCR) up to Dec 31st, 1997 and through the Department of Pathology, University Hospital Malmö up to Dec 31st, 2000 (Papers I, II: SNCR up to Dec 31st, 1992; Paper III: SNCR up to Dec 31st, 1995 and the Department of Pathology up to Apr 30th, 1999; Papers IV, V: SNCR up to Dec 31st, 1997 and the Department of Pathology up to Dec 31st, 2000). The tumour diagnosis was confirmed by reviewing medical records of pathology, autopsy, gastroscopy, x-ray and operation history reports. Cases with gastric cardia and non-cardia adenocarcinoma, oesophageal adenocarcinoma and oesophageal squamous cell carcinoma were included. Cases with non-malignant disease, primary tumour outside the stomach or the oesophagus, other gastric malignancies, tumour diagnosis before enrolment, non-performed

histology investigation, missing blood samples or missing medical records were excluded. Cases with missing data on occupation, tobacco or alcohol consumption were excluded in Paper III.

7.4. Classification of Gastric and Oesophageal Adenocarcinomas

A non-cardia gastric adenocarcinoma was defined as an adenocarcinoma occurring in the stomach with its center at least 3 cm distal to the gastrooesophageal junction (Misumi *et al.*, 1989; Siewert and Stein, 1998; Lagergren *et al.*, 1999*a*). A cardia adenocarcinoma was defined as occurring within 2 cm proximal to and 3 cm distal to the gastro-oesophageal junction in the absence of Barrett's metaplasia (Misumi *et al.*, 1989; Siewert and Stein, 1998; Lagergren *et al.*, 1999*a*). An oesophageal adenocarcinoma was defined as occurring 2 cm proximal to the gastro-oesophageal junction (Z-line) or within 2 cm proximal to and 3 cm distal to the gastro-oesophageal junction if located adjacent to Barrett's metaplasia. Barrett´s metaplasia had to be described macroscopically in the gastroscopy report, or macroscopically or microscopically in the histology report of the resected tissue sample. The classification of large tumours depended on the location of their main tumour mass. Squamous cell carcinomas at the gastro-oesophageal junction were classified as oesophageal. Non-cardia gastric adenocarcinomas up to Dec 31st, 1992, were categorised as either corpus-fundus, corpus-antrum or antrum. A tumour with its mass equally divided between corpus and antrum was put in the corpus-antrum category.

7.5. Control Population

Four control subjects were selected for each case in the case-control study, matched by sex, date of birth (± 6 months) and date of enrolment (± 6 months). Only control subjects for whom there were available blood samples were eligible. Already matched control subjects for whom blood samples were not available for complementary CagA analysis were excluded from the studies of Papers IV and V. Control subjects who were deceased at the time of tumour diagnosis in corresponding matched cases were excluded from nested case-control studies in Papers III and V, but included in the serological evaluation population of Paper IV. Control subjects for whom there were no data on occupation, tobacco or alcohol consumption were excluded in Paper III.

7.6. Serological Evaluation Population

The serological analyses of Paper IV were evaluated in a serological evaluation population with no known gastric or oesophageal carcinomas, consisting of the matched control population described above. A set of correction factors were used to make the selected material

representative of the Malmö Preventive Medicine cohort. For each cell, consisting of subjects of a single sex, born in a single year and the size of which was determined by the number of subjects belonging to the cell, a correction factor was created. The correction factor for each cell was calculated as the quotient of the relative cell size of the Malmö Preventive Medicine cohort by the relative cell size of the study samples. Corrected sums of seropositive subjects and cell sizes were calculated and corrected seroprevalences were calculated as the quotients of these sums.

7.7. Serological Analyses

7.7.1. ELISA

H. pylori seropositivity according to ELISA (enzyme-linked immunosorbent assay) was determined through an in-house method developed by Gnarpe *et al.* (1988), based upon IgG antibodies against the *H. pylori* reference strain CCUG 17874. ELISA was used for all papers.

The reported sensitivity and specificity were 0.98 and 0.81 respectively, when culture from antrum biopsies was used as a golden standard. Antigen coated 96-well plates were prepared in advance. On analysis each 96-well plate contained a low positive control sample of a known optical density (0.75, s.d. 0.075), a high positive control sample (optical density 1.31, s.d. 0.041) and a negative control sample. Each sample was analyzed in duplicate. The mean optical density was corrected for the low positive control sample of each 96-well plate. Samples with a corrected optical density greater than 0.700 were regarded as seropositive. Those with a corrected optical density less than or equal to 0.700 were regarded as seronegative. The case-control status of the samples was not known to the laboratory technician.

Coated 96-well plates were prepared from the *H. pylori* reference strain CCUG 17874, which was grown on GAB agar plates (agar supplemented with horse blood, horse serum, isovitalex and cystein) at +37 °C in a microaerophilic environment for three days. The colonies were harvested and fixed in formaldehyde 4%. PBS with sodium azide 0.1% was added. The bacterial preparation was ultrasonicated, its concentration determined, added to the wells and incubated for 2 h. The wells were then blocked with PBS pH 7.2/Tween® and BSA at +37 °C for 10 min. There were two coated wells and one uncoated well for each sample on the 96-well plate. The 96-well plates were stored at +4 °C. It is possible to store the 96-well plates for several months.

Sera from stored samples were diluted 1:100, added to the wells and incubated at +37 °C for 90 min. Anti-human IgG conjugate (D0336, Dako Denmark A/S, Denmark) was added and the

plate was incubated at +37 °C for 90 min. Finally, the substrate (Sigma 104, Sigma-Aldrich, St. Louis, USA) was added and the 96-well plates were scanned after 15 min in a microtiter scanning photometer (Multiscan PLUS, Labsystems, Vantaa, Finland).

7.7.2. Western Blot

CagA seropositivity and *H. pylori* seropositivity were determined with the commercial western blot assay Helicoblot 2.1 (Genelabs Diagnostics, Singapore). Western blot was used for Papers IV and V.

The Helicoblot 2.1 assay consists of nitrocellulose membrane strips containing an electrophoretically separated bacterial lysate from an ulcer forming *H. pylori* strain and a separate 'current infection marker' (CIM) of recombinant *H. pylori* antigen slotted onto the nitrocellulose membrane. Each test kit also included reactive and non-reactive control sera, and a photocopy of the positive reactive control strip, with markers for *H. pylori* specific bands and their molecular weights. The bacterial lysate and the positive control of all kits in our studies belonged to a single batch (Matthew Maks, Genelabs diagnostics, Singapore, personal communication). The manufacturer reports a sensitivity of 96% and specificity of 95% as compared to histology, culture, the rapid urease test and/or the urea breath test.

Sera were diluted 1:100 and added for 1 h at room temperature, followed by incubation with in kit included goat anti-human IgG conjugated with alkaline phosphatase for 1 h at room temperature. The strips were then developed with 5-bromo-4-chloro-2-indolyl-phosphate and nitroblue tetrazolium for 15 min (Figure 7.7.2.1.).

Developed immunoblot strips were scanned at a resolution of 600 dots per inch with no filter (filter setting: 'white'; scanner: Model GS-700 Densitometer, Bio-Rad Laboratories, Hercules, California). A band analysis computer program (Quantify One, Bio-Rad Laboratories, Hercules, California) was used for manual identification of the bands. The computer program contained tools for magnification and contrast enhancement of scanned strips (Figure 7.7.2.2.). Our definition of a band was based on the shape of an area with increased intensity, from its maximum intensity level to the lower intensity level at which it reached across the strip. In order to be a valid band, the area of increased intensity had to reach across the strip and had to be more prominently extended across the strip than along the strip at all intensity levels. A positive

current infection marker had to have a detectable increase of intensity in at least half of the rectangular CIM area and well defined edges.

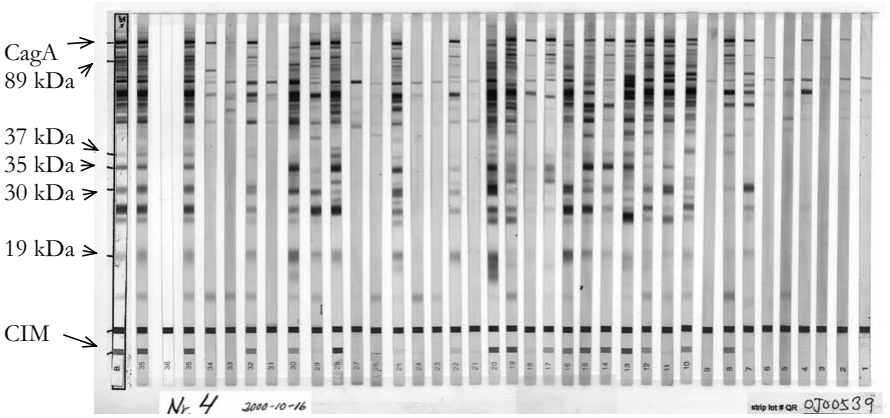

Figure 7.7.2.1. Developed immunoblot strips of Helicoblot 2.1. The first strip from the left is the photocopy of the positive control with marked molecular weight. The following two strips contain the reactive and non-reactive control sera. The rest are immunoblot strips of study subjects. (CIM - current infection marker).

The Quantify One program contained tools to facilitate molecular weight determination. The lane of each immunoblot strip was defined manually by marking the beginning and the end of the electrophoretic part of the strip. The program used a standard pattern with manually defined molecular band weights and the band pattern of the positive reactive control of each kit to suggest molecular weights of the bands of individual immunoblot strips. Each suggested band molecular weight was manually verified or adjusted according to the band pattern of the specific strip.

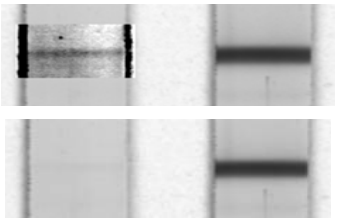

Figure 7.7.2.2. The upper left part of the picture shows a band detected with magnification and contrast enhancement tools.

The peak intensity of *H. pylori* specific bands was determined after subtraction of background intensity (background intensity subtraction setting: rolling disc size 10) and adjustment by the band intensity of the corresponding positive reactive control.

The five criteria for *H. pylori* seropositivity were, as recommended by the manufacturer: the combination of the 116 kDa band (CagA) with the current infection marker (CIM), the combination of the bands 19.5 kDa and 30 kDa, or at least one of the bands 89 kDa, 37 kDa, or 35 kDa.

We defined CagA seropositivity as a detectable 116 kDa band according to the method described above. A CagA band with an intensity greater than 50 arbitrary intensity units was defined as being a high intensity CagA seropositive band. A detectable CagA band with an intensity less than the stated value was defined as a low intensity CagA seropositive band.

All samples were interpreted by a single observer (H.S.). The case-control status of the samples was not revealed until the *H. pylori* and CagA serostatus had been determined.

7.8. Tobacco Consumption

The state of tobacco smoking at the time of enrolment was classified by the questionnaire as current smokers, ex-smokers, never smokers or unknown smoking status. The effect of tobacco consumption was studied in Papers II, III and V.

There were 33 questions on tobacco smoking (questions 030101 - 030121, 030134 - 030139, 100104) and one question on tobacco sniffing (question 030122) in the Malmö Preventive Medicine questionnaire (Table 7.8.1.). During the enrolment period, the wording of eight smoking questions were changed slightly (questions 030111 - 030115, 030120, 030104 - 030105 replaced by 030134 - 030135), seven questions on the amount of smoking (questions 030112 - 030114, 030116 - 030118, 030120) were replaced by three new questions (questions 030136 - 030139), one question on the amount of smoking was omitted (question 030115), five questions on type of tobacco used were omitted (questions 030116 - 030120) and two new questions on snuff dipping/chewing tobacco and smoking on the enrolment day smoking were added (questions 030122 and 100104).

Table 7.8.1. Questions in the Malmö Preventive Medicine questionnaire addressing the use of tobacco, including the time period during which each question was used (translated from Swedish for the purpose of this presentation only)

Code	Question	Time period
030101	Have you at any time in your life been smoking daily for half a year?	Not changed
030102	Do you smoke?	Not changed
030103	Do you inhale the smoke?	Not changed
030104	Have you been smoking for more than one year?	Start - 830404
030105	Have you been smoking for more than five years?	Start - 830404
030106	Have you been smoking for more than ten years?	Not changed
030107	Have you substantially reduced your smoking in the last six months?	Not changed
030108	Have you given up smoking during the last year?	Not changed
030109	Did you give up smoking between 1 and 5 years ago?	Not changed
030110	Did you give up smoking more than 10 years ago?	Not changed
030111.a	Do you smoke cigarettes daily?	Start - 830404
030111.b	Do you smoke daily?	830405 - End
030112.a	Do you smoke more than 10 cigarettes per day?	Start - 800330
030112.b	Do you smoke 10 cigarettes or more per day?	800331 - 830404
030113.a	Do you smoke more than 20 cigarettes per day?	Start - 800330
030113.b	Do you smoke 20 cigarettes or more per day?	800331 - 830404
030114.a	Do you smoke more than 30 cigarettes per day?	Start - 800330
030114.b	Do you smoke 30 cigarettes or more per day?	800331 - 830404
030115.a	Do you smoke more than 40 cigarettes per day?	Start - 800330
030115.b	Do you smoke 40 cigarettes or more per day?	800331 - 830404
030116	Do you smoke 1 cigar or 3 cigarillos per day?	Start - 830404
030117	Do you smoke 2-3 cigars or 4-6 cigarillos per day?	Start - 830404
030118	Do you smoke at least 3 cigars or at least 6 cigarillos per day?	Start - 830404
030119	Do you smoke a pipe?	Start - 830404
030120.a	Do you smoke more than 1 packet of pipe tobacco per week?	Start - 800330
030120.b	Do you smoke 1 packet of pipe tobacco or more per week?	800331 - 830404
030121	Do you want to give up smoking?	Not changed
030122	Do you use snuff or chewing-tobacco?	780302 - 800330
030134	Have you been smoking for a period of 5 - 9 years?	830405 - End
030135	Have you been smoking for a period of 1 - 4 years?	830405 - End
030136	Do you smoke 30 cigarettes or more per day or 15 cigars or more per day or 200 g tobacco or more per week?	830405 - End
030137	Do you smoke 20 cigarettes or more per day or 10 cigars or more per day or 150 g tobacco or more per week?	830405 - End
030138	Do you smoke 10 cigarettes or more per day or 5 cigars or more per day or 75 g tobacco or more per week?	830405 - End
030139	Do you smoke 20 g tobacco or more per day of different kinds of tobacco (1 cigarette = 1 g, 1 cigarillo = 2 g, 1 cigar = 5 g, 1 packet of tobacco = 50 g)?	830405 - End
100104	Have you smoked anything this morning, before the examination here today?	780302 - End

Current smokers were defined as subjects giving affirmative answers to any of the questions beginning with 'Do you smoke …' (questions 030102, 030111 - 030120, 030136 - 030139) or to the question about smoking on the day of enrolment (question 100104). Subjects giving negative answers to these questions but giving affirmative answers to any other smoke-related question (questions 030101, 030103 - 030110, 030121, 030134 - 030135) were classified as ex-smokers. Never smokers were subjects giving negative answers to all of these questions.

Duration was classified into less than 5 years, 5 to 10 years and more than 10 years of tobacco smoking according to the answers to questions 030104 - 030106 and questions 030134 - 030135.

The daily amount of tobacco consumption among current smokers was classified into less than 10 g tobacco per day, 10 to 20 g, 20 to 30 g and more than 30 g tobacco per day. A consumption of more than 20 g tobacco per day was classified as heavy smoking, less than 20 g per day as light smoking. The total consumption of cigarettes, cigars, cigarillos and pipe tobacco was considered (questions 30112 - 30118, 30120, 30136 - 30139). One cigarette was regarded as 1 g of tobacco, one cigarillo as 2 g, one cigar as 5 g and one packet of tobacco as 50 g. The daily amount of smoking could only be determined among current smokers. Pack years of smoking among current smokers was calculated from the amount and duration of tobacco smoking.

Subjects that were snuff dipping or using chewing-tobacco were identified by question 030122.

In Paper II, non-current smokers included all but the current smoker category and never smokers included the unknown smoking category. In Paper III, subjects with missing data on smoking status were excluded. In Paper V, the categories current smokers, ex-smokers, never smokers and unknown smoking status were used.

7.9. Alcohol Consumption

Alcohol consumption at the time of enrolment was classified as high or normal based on the questionnaire. The effect of alcohol consumption was studied in Paper III.

High alcohol consumption was identified by an adapted version of the Michigan Alcohol Screening Test (Selzer, 1971; Luczak *et al.*, 2001), the Malmö modified Michigan Alcohol Screening Test (Mm-MAST) (Kristenson and Trell, 1982). The Mm-MAST consists of nine questions on the usage of alcohol (Table 7.9.1.), and has been evaluated in the Malmö Preventive

Medicine cohort. Affirmative answers in two or more questions identified heavy drinkers (mean alcohol consumption of 84 g/day, range 30 - 300 g/day) with a sensitivity of 66% and specificity of 95% (Kristenson and Trell, 1982). During the enrolment period, three Mm-MAST questions were omitted (questions 40146, 40150, 40151).

The criterion for high alcohol consumption was affirmative answers to two out of nine (until Apr 4th, 1983) or six questions (after Apr 4th, 1983) of the Mm-MAST questionnaire.

Subjects with missing data on alcohol consumption were excluded.

Table 7.9.1. The questions belonging to the Malmö modified Michigan Alcohol Screening Test (Mm-MAST) in the Malmö Preventive Medicine questionnaire, including the time period during which each question was used (translated from Swedish for the purpose of this presentation only)

Code	Question	Time period
40142	Do you usually take a drink before going to a party?	760412 - End
40143.a	Do you regularly drink a bottle of wine on weekends?	760412 - 780301
40143.b	Do you regularly drink alcohol, e.g. o bottle of wine or similar, on weekends?	780302 - End
40144.a	Do you drink a few beers every day to ease up?	760412 - 830404
40144.b	Do you drink a few beers, some glasses of wine or a drink every day in order to ease up?	830405 - End
40145	Are you more tolerant to alcohol today than 10 years ago?	760412 - End
40146	Do you have to concentrate on keeping pace when you drink alcoholic beverages in company?	760412 - 830404
40148	Has it happened that you can not remember how you got to bed after moderate partying?	760412 - End
40149	Do you usually feel remorseful after partying?	760412 - End
40150	Do you usually follow up with a beer the day after a party?	760412 - 830404
40151	Do you sometimes try to avoid drinking alcoholic beverages for some time, e.g. during a week ?	760412 - 830404

7.10. Socio-economic Status

Occupational data were included as an indicator of socio-economic status and was provided by the National Registry. Occupational data were used in Papers II, III and V.

National Registry data were collected from 1960, 1970, 1980, 1985 and 1990. The most recent data for each subject were used. Occupation was categorized into the main groups of the Swedish socio-economic classification (Reports on Statistical Co-ordination, 1982): blue-collar workers, white-collar workers, self-employed and unknown occupation. Blue-collar workers included

trained and untrained workers in goods and service production; white-collar workers included both lower levels and leading positions; self-employed included business leaders, freelancing academic professionals and farmers and unknown occupation included people without gainful employment and subjects with missing data.

7.11. Mortality Data

Mortality data, used to determine the time of death of control subjects who were matched to tumour cases, were collected from the Centre for Epidemiology (EpC), National Board of Health and Welfare. Mortality data were used for Papers III and V.

7.12. Statistic Analyses

Relative risks were estimated as odds ratios using conditional logistic regression for matched sets (Breslow and Day, 1980). The Mantel-Haenzel formula and homogeneity tests were used for Paper I. Multiple conditional logistic regression and the conditional likelihood ratio statistic for homogeneity and interaction were used for Papers II, III and V (LogXact™, Cytel Software Corporation, Cambridge, USA). The logistic regression was asymptotic in Papers I - III, with exact algorithms used when there were no asymptotic estimates. Exact algorithms were used for Paper V because of sparse data for the analysis of gastric cardia adenocarcinoma, oesophageal adenocarcinoma and squamous carcinoma. Exact algorithms were also used for Paper V because of imbalanced data in the analysis of CagA seropositivity in the subgroup of *H. pylori* seropositive non-cardia gastric adenocarcinoma subjects. Exact algorithms in logistic regression calculates the confidence interval and the P-value of a parameter using the exact permutation distribution of the parameter, as defined from the regression model and from the observations made for the other parameters of the model, *i.e.* the exact permutation distribution of the parameter conditional on the sufficient statistics of other parameters. Point estimates in an exact logistic regression are either maximal likelihood estimates or median unbiased estimates.

Multiple linear regression models using the computer program SAS release 6.12 (SAS Institute Inc., Cary, NC, USA) were used for Paper IV to determine changes in prevalence with time.

McNemars test was used to compare the estimated seroprevalences of different laboratory methods measured on a single sample.

The expected number of tumour cases in the Malmö Preventive Medicine cohort was estimated using national data on tumour occurrences in 5-years age cohorts, tumour occurrences in the city of Malmö, national population size and population size of the city of Malmö, available for each year and sex from 1974 to 1997. The cohort was assumed to have similar tumour incidence as the city of Malmö and a similar age distribution of tumour incidence as the national population.

$P < 0.05$ was regarded as significant, 95% confidence interval was calculated, two-sided tests were used.

8. RESULTS

8.1. Study Population

The Swedish National Cancer Registry (SNCR) and the Pathology Department, University Hospital Malmö, identified 137 (120 by SNCR) gastric and 54 (44 by SNCR) oesophageal tumours occuring after enrolment until Dec 31st 2000. All cases were verified by medical records. Included and excluded cases are shown in Tables 8.1.1-2. Characteristics at the time of enrolment for case subjects and control subjects are shown in Tables 8.1.3-4.

Table 8.1.1. Cases included in Papers I - III and V

	Papers I, II		Paper III		Paper V	
	Cases	Controls	Cases	Controls	Cases	Controls
Gastric adenocarcinoma						
Non-cardia	27	108	-	-	67	250
Cardia	13*	52	-	-	24	88
Unclassified location	6†	24	-	-	-	-
Prior partial gastrectomy	10	40	-	-	-	-
Oesophageal malignancy						
Adenocarcinoma	-	-	7	24	12	47
Squamous cell carcinoma	-	-	29	94	37	129
Unclassified histology	-	-	8	31	-	-
Total	**56**	**224**	44	149	140	514

* Four cardia cases were reclassified into two oesophageal adenocarcinoma cases and two non-cardia adenocarcinoma cases in Paper V.

† Unclassified cases were reclassified as non-cardia adenocarcinoma cases in Paper V.

The number of control subjects excluded was 27 in Paper III (12 subjects for not being at risk, *i.e.* deceased at the time of tumour diagnosis in the matched case) and 52 in Paper V (46 subjects not at risk).

Table 8.1.2. Cases excluded from Papers I - III and V

Exclusion criteria	Papers I,II	Paper III	Paper V
Non-malignant disease	2	1	4
Malignancy outside the gastro-oesophageal tract	-	-	3
Oesophageal malignancy	1	-	-
Gastric malignancy other than adenocarcinoma	6	-	13
Tumour diagnosis before enrolment	9	-	10
Missing histology investigations	1	-	1
Missing blood samples	6	1	8
Missing medical record	-	-	1
Missing data on occupation, tobacco or alcohol	-	3	-
Prior partial gastrectomy	-	-	11
Total	25	5	51

Table 8.1.3. Characteristics at the time of enrolment for gastric adenocarcinoma cases and control subjects

Gastric adenocarcinoma	Non-cardia				Cardia			
	Case		Control		Case		Control	
	Mean	(s.d.)	Mean	(s.d.)	Mean	(s.d.)	Mean	(s.d.)
Year of birth*	1931	(6.1)	1931	(6.2)	1930	(6.0)	1931	(5.8)
Enrolment age*	50.7	(6.6)	50.6	(6.7)	51.4	(6.2)	51.0	(6.1)
Age at diagnosis	60.0	(7.7)			63.5	(8.2)		
Years of follow-up*	9.2	(6.1)			12.1	(4.4)		

* For the entire Malmö Preventive Medicine cohort, the mean year of birth was 1934 [range 1921-1948], the mean age at enrolment was 47.9 [range 29.2 - 63.6] years and the mean follow-up time from enrolment until Dec 31st 2000 was 18.3 [range 9.1 - 26.3] years.

The number of tumour cases in the cohort that was identified by the SNCR was comparable to the expected number of gastric (123) and oesophageal tumours (50) estimated from the age-adjusted incidence in the city of Malmö, $P > 0.78$ and $P > 0.36$, respectively.

The SNCR identified all cases until Dec 31st 1997. The Department of Pathology found 85% of these tumour cases. Another five tumour cases were estimated to have occurred without our knowledge outside the catchment area of the Department of Pathology from Jan 1st 1998 to Dec 31st 2000.

There were five cases of gastric non-cardia adenocarcinoma, no cases of gastric cardia adenocarcinoma, no cases of oesophageal adenocarcinoma and one case of squamous cell carcinoma with a shorter follow-up time than two years.

Table 8.1.4. Characteristics at the time of enrolment for oesophageal carcinoma cases and control subjects

Oesophageal carcinoma	Adenocarcinoma				Squamous cell carcinoma			
	Case		Control		Case		Control	
	Mean	(s.d.)	Mean	(s.d.)	Mean	(s.d.)	Mean	(s.d.)
Year of birth*	1932	(5.6)	1932	(5.4)	1931	(6.7)	1931	(6.5)
Enrolment age*	49.3	(6.7)	49.2	(6.5)	50.6	(7.5)	50.2	(7.4)
Age at diagnosis	61.9	(5.1)			61.9	(7.5)		
Years of follow-up*	12.6	(5.1)			11.3	(4.8)		

* For the entire Malmö Preventive Medicine cohort, the mean year of birth was 1934 [range 1921-1948], the mean age at enrolment was 47.9 [range 29.2 - 63.6] years and the mean follow-up time from enrolment until Dec 31st 2000 was 18.3 [range 9.1 - 26.3] years.

8.2. Risk Factors

The *H. pylori* seropositivity in cases and control subjects of Paper V is shown in Tables 8.2.1-2.

H. pylori seropositivity was more prevalent among cases of non-cardia gastric adenocarcinoma than among the matched control subjects, both for the 27 non-cardia adenocarcinoma cases diagnosed until Dec 31st 1992 (Paper I), which were analysed by ELISA (24 of 27 (89%) cases compared to 53 of 108 (49%) control subjects) and for the 67 cases diagnosed until Dec 31st 2000 (Paper V), which were analysed by ELISA (85% compared to 48%) and by Helicoblot 2.1 (97% compared to 59%) (Table 8.2.1.). CagA seropositivity was present in all *H. pylori* seropositive cases (65 of 65, 100%) and in 133 of 147 (90%) *H. pylori* seropositive matched control subjects.

Gastric cardia adenocarcinoma cases had similar *H. pylori* seroprevalences as control subjects, this similarity was found by both ELISA and Helicoblot 2.1 (Table 8.2.1).

Table 8.2.1. *H. pylori* and CagA serostatus among gastric adenocarcinoma cases and matched control subjects*

Gastric adenocarcinoma	Non-cardia				Cardia			
	Cases		Controls		Cases		Controls	
	n	(%)	n	(%)	n	(%)	n	(%)
Helicoblot 2.1 *H. pylori* pos								
ELISA *H. pylori* pos								
CagA pos	57	(85.1)	110	(44.0)	13	(54.2)	41	(46.6)
CagA neg	0	(0.0)	9	(3.6)	0	(0.0)	4	(4.5)
ELISA *H. pylori* neg								
CagA pos	8	(11.9)	23	(9.2)	4	(16.7)	7	(8.0)
CagA neg	0	(0.0)	5	(2.0)	0	(0.0)	2	(2.3)
Helicoblot 2.1 *H. pylori* neg								
ELISA *H. pylori* pos								
CagA pos	0	(0.0)	1	(0.4)	0	(0.0)	0	(0.0)
CagA neg	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
ELISA *H. pylori* neg								
CagA pos	1	(1.5)	59	(23.6)	3	(12.5)	13	(14.8)
CagA neg	1	(1.5)	43	(17.2)	4	(16.7)	21	(23.9)
Total	**67**	**(100.0)**	**250**	**(100.0)**	**24**	**(100.1)**	**88**	**(100.1)**
Subtotals								
Helicoblot 2.1 *H. pylori* pos	65	(97.0)	147	(58.8)	17	(70.8)	54	(61.4)
ELISA *H. pylori* pos	57	(85.1)	120	(48.0)	13	(54.2)	45	(51.1)
CagA pos	66	(98.5)	193	(77.2)	20	(83.3)	61	(69.3)

* *H. pylori* serostatus was analysed by Helicoblot 2.1 or ELISA. CagA serostatus was analysed by Helicoblot 2.1.

Cases of oesophageal malignancy, irrespective of histology and diagnosed until Apr 31st 1999, had lower *H. pylori* seroprevalence by ELISA than the matched control subjects (10 (23%) *H. pylori* seropositive cases as compared to 67 of 149 (45%) control subjects; Paper III). The difference in seroprevalence among cases diagnoses up to Dec 31st 2000 was more pronounced in cases of oesophageal adenocarcinoma than in cases oesophageal squamous carcinoma (Table 8.2.2.).

Table 8.2.2. *H. pylori* and CagA serostatus among oesophageal carcinoma cases and matched control subjects

Oesophageal carcinoma	Adeno-carcinoma				Squamous cell carcinoma			
	Cases		Controls		Cases		Controls	
	n	(%)	n	(%)	n	(%)	n	(%)
Helicoblot 2.1 *H. pylori* pos								
ELISA *H. pylori* pos								
CagA pos	1	(8.3)	17	(36.2)	12	(32.4)	55	(42.6)
CagA neg	0	(0.0)	0	(0.0)	0	(0.0)	3	(2.3)
ELISA *H. pylori* neg								
CagA pos	2	(16.7)	4	(8.5)	3	(8.1)	8	(6.2)
CagA neg	1	(8.3)	3	(6.4)	0	(0.0)	2	(1.6)
Helicoblot 2.1 *H. pylori* neg								
ELISA *H. pylori* pos								
CagA pos	0	(0.0)	0	(0.0)	0	(0.0)	0	(0%)
CagA neg	0	(0.0)	0	(0.0)	0	(0.0)	0	(0%)
ELISA *H. pylori* neg								
CagA pos	3	(25.0)	11	(23.4)	9	(24.3)	19	(14.7)
CagA neg	5	(41.7)	12	(25.5)	13	(35.1)	42	(32.6)
Total	**12**	**(100.0)**	**47**	**(100.0)**	**37**	**(99.9)**	**129**	**(100.0)**
Subtotals								
Helicoblot 2.1 *H. pylori* pos	4	(33.3)	24	(51.1)	15	(40.5)	68	(52.7)
ELISA *H. pylori* pos	1	(8.3)	17	(36.2)	12	(32.4)	58	(45.0)
CagA pos	6	(50.0)	32	(68.1)	24	(64.9)	82	(63.6)

* *H. pylori* serostatus was analysed by Helicoblot 2.1 or ELISA. CagA serostatus was estimated by Helicoblot 2.1.

All subjects who proved *H. pylori* seropositive by ELISA also proved seropositive by Helicoblot 2.1, with the exception of one control subject. The seroprevalence of *H. pylori* analysed by Helicoblot 2.1 was significantly higher than the *H. pylori* seroprevalence analysed by ELISA, P<<0.001. The *H. pylori* seroprevalences analysed by Helicoblot 2.1 were also higher than those analysed by ELISA in all case and control groups, as shown in Tables 8.2.1-2. However, only 12 out of 72 (17%) subjects that were *H. pylori* seropositive by Helicoblot 2.1 but not by ELISA, depended on the combination of the CagA band and the current infection marker (CIM) for Helicoblot 2.1 seropositivity. In the study of non-cardia gastric adenocarcinoma, 2 out

of 8 (25%) cases and 5 out of 28 (20%) control subjects were solely dependent on the combination of the CagA band and the CIM for *H. pylori* seropositivity as analysed by Helicoblot 2.1.

Almost all *H. pylori* seropositive subjects analysed by Helicoblot 2.1 were also CagA seropositive. There was only one exceptive case of oesophageal adenocarcinoma among the cases. Approximately half of the subjects that were *H. pylori* seronegative by both methods were CagA seropositive.

Table 8.2.3. Smoking and occupation among gastric adenocarcinoma cases and matched control subjects

Gastric adenocarcinoma	Non-cardia				Cardia			
	Cases		Controls		Cases		Controls	
	n	(%)	n	(%)	n	(%)	n	(%)
Smoking	P = 0.026*				P = 0.37*			
Current Smoker	30	(44.8)	66	(26.4)	11	(45.8)	24	(27.3)
Ex-Smoker	22	(32.8)	91	(36.4)	7	(29.2)	31	(35.2)
Non-Smoker	12	(17.9)	78	(31.2)	4	(16.7)	27	(30.7)
Unknown	3	(4.5)	15	(6.0)	2	(8.3)	6	(6.8)
Occupation	P = 0.084*				P = 0.19*			
Self-employed	4	(6.0)	21	(8.4)	3	(12.5)	5	(5.7)
White-collar worker	19	(28.4)	108	(43.2)	7	(29.2)	44	(50.0)
Blue-collar worker	35	(52.2)	100	(40.0)	13	(54.2)	32	(36.4)
Unknown	9	(13.4)	21	(8.4)	1	(4.2)	7	(8.0)

* Case compared to control subjects, likelihood ratio statistics.

Current smoking was significantly more common among cases of non-cardia gastric adenocarcinoma ($P < 0.03$) and squamous cell carcinoma ($P = 0.0001$) as compared to the matched control subjects (Table 8.2.3-4.).

Table 8.2.4. Smoking and occupation among oesophageal carcinoma cases and matched control subjects

Oesophageal carcinoma	Adeno-carcinoma				Squamous cell carcinoma			
	Cases		Controls		Cases		Controls	
	n	(%)	n	(%)	n	(%)	n	(%)
Smoking	P = 0.28*				P = 0.0001*			
Current Smoker	7	(58.3)	13	(27.7)	19	(51.4)	28	(21.7)
Ex-Smoker	2	(16.7)	16	(34.0)	15	(40.5)	48	(37.2)
Non-Smoker	1	(8.3)	9	(19.1)	2	(5.4)	37	(28.7)
Unknown	2	(16.7)	9	(19.1)	1	(2.7)	16	(12.4)
Occupation	P = 0.80*				P = 0.69*			
Self-employed	4	(33.3)	10	(21.3)	4	(10.8)	12	(9.3)
White-collar worker	4	(33.3)	16	(34.0)	16	(43.2)	48	(37.2)
Blue-collar worker	3	(25.0)	16	(34.0)	13	(35.1)	55	(42.6)
Unknown	1	(8.3)	5	(10.6)	4	(10.8)	14	(10.9)

* Case compared to control subjects, likelihood ratio statistics.

Alcohol consumption was significantly more common in cases of oesophageal malignancy diagnosed until Apr 31st 1999 (Table 8.2.5.) than in matched control subjects. This difference did however turn out non-significant in the multiple regression model, when analysed in combination with tobacco smoking (see 8.5. below, Table 8.5.2.).

Table 8.2.5. Alcohol consumption among oesophageal malignancy cases and matched control subjects in Paper III

Oesophageal malignancy	Cases		Controls	
	n	(%)	n	(%)
Alcohol Consumption		P = 0.017*		
High	21	(47.7)	42	(28.2)
Normal	23	(52.3)	107	(71.8)

* Case compared to control subjects, likelihood ratio statistics.

8.3. *H. pylori* Associated Risks for Gastric and Oesophageal Carcinoma

The risks associated with *H. pylori* seropositivity were significantly different for cardia and non-cardia gastric adenocarcinoma ($P < 0.01$ in cases diagnosed until Dec 31st 1992 with *H. pylori* serostatus analysed by ELISA).

The risk of developing non-cardia gastric adenocarcinoma was associated with *H. pylori* seropositivity. The odds ratio was 17.8 (4.2 - 74.8) as compared to *H. pylori* seronegative subjects when analysed with Helicoblot 2.1 and after adjustment for occupation and tobacco consumption (Table 8.3.1). The corresponding odds ratios with ELISA were 5.4 (2.5 - 11.6) for all cases until Dec 31st 2000 and 11.1 (95% CI: 2.4 - 71.8) when restricted to cases diagnosed until Dec 31st 1992 (no adjustment). When the serological tests for ELISA and CagA were combined and *H. pylori* seropositive subjects were compared to subjects seronegative for both *H. pylori* and CagA, the odds ratio was 17.7 (95% CI: 2.3 - 137). A similar high odds ratio was found for CagA seropositive subjects as compared to CagA seronegative subjects (Table 8.3.1). Within the *H. pylori* seropositive subgroup, CagA seropositivity was associated with the odds ratio 9.7 (1.5 - ∞). Subjects with high intensity CagA seropositivity had higher risks for non-cardia gastric adenocarcinoma compared to CagA seronegative subjects in the whole cohort and among *H. pylori* seropositive subjects (Table 8.3.2.). There were no significant risks associated with low intensity CagA seropositivity, but the point estimates was in-between the risks associated with high intensity CagA seropositivity and CagA seronegativity. Similar risks associated with *H. pylori* seropositivity and CagA seropositivity were found after exclusion of cases occurring within 2 years of enrolment (data not shown). There was no significant difference between the non-cardia

locations corpus-fundus, corpus-antrum and antrum in risk associated with *H. pylori* seropositivity in cases diagnosed until Dec 31st 1992 (no adjustment), P > 0.40 (Table 8.3.3.).

Table 8.3.1. Odds ratios associated with *H. pylori* seropositivity (Helicoblot 2.1 and ELISA), CagA and CagA among *H. pylori* seropositive subjects, unadjusted and adjusted for occupation and tobacco consumption

	Gastric adenocarcinoma		Oesophageal carcinoma	
Logistic Regression Model	Non-cardia OR* (95% CI)	Cardia OR†(95% CI)	Adeno-carcinoma OR† (95% CI)	Squamous cell carcinoma OR† (95% CI)
A: Helicoblot 2.1:				
H. pylori pos				
adjusted	17.8 (4.2 -74.8)	1.5 (0.51 -4.8)	0.46 (0.07 -2.6)	0.44 (0.15 -1.2)
unadjusted	22.4 (5.3 -93.8)	1.5 (0.54 -4.6)	0.50 (0.10 -2.1)	0.56 (0.24 -1.3)
H. pylori neg	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)
B: ELISA:				
H. pylori pos				
adjusted	5.4 (2.5 -11.6)	1.3 (0.48 - 3.9)	0.22 (0.00 - 1.8)	0.38 (0.1 - 1.04)
unadjusted	6.3 (3.0 -13.3)	1.2 (0.43 - 3.2)	0.18 (0.00 - 1.4)	0.52 (0.21 -1.2)
H. pylori neg	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)
C: CagA:				
CagA pos				
adjusted	16.8 (2.2 - 130)	2.3 (0.66 - 12)	0.32 (0.03 - 2.1)	1.0 (0.35 -3.1)
unadjusted	20.1 (2.7 - 148)	2.2 (0.65 - 9.7)	0.45 (0.10 - 2.1)	1.0 (0.42 -2.5)
CagA neg	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)
D: CagA among *H. pylori* seropositive subjects (Helicoblot 2.1):				
CagA pos and *H. pylori* pos				
adjusted	9.7 (1.5 - ∞)†	2.7 (0.38 - ∞)	0.38 (0.02 -24)	2.0 (0.24 -∞)
unadjusted	9.6 (1.5 - ∞)†	2.5 (0.36 - ∞)	0.50 (0.03 -30)	1.2 (0.16 -∞)
CagA neg and *H. pylori* pos				
	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)

* Asymptotic logistic regression.
† Exact logistic regression.

No association was found between gastric cardia adenocarcinoma and *H. pylori* seropositivity or CagA seropositivity (Table 8.3.1.).

The combined risk of all oesophageal malignancies diagnosed until Apr 30th 1999, irrespective of histology, was lower in subjects who were *H. pylori* seropositive according to ELISA, odds ratio 0.29 (95% CI: 0.12 - 0.67, with adjustment). Although there were tendencies of inverse associations, no significant associations between *H. pylori* or CagA seropositivity and oesophageal or squamous cell carcinomas were found (Table 8.3.1). Similar estimates were found after exclusion of cases occurring within 2 years of enrolment (data not shown).

Table 8.3.2. Odds ratios for gastric non-cardia adenocarcinoma associated with high- and low-intensity CagA seropositivity, unadjusted and adjusted for occupation and tobacco consumption (Helicoblot 2.1)

	Gastric non-cardia adenocarcinoma	
Logistic Regression Model	adjusted OR (95% CI)	unadjusted OR (95% CI)
A: CagA:		
High intensity CagA pos*	20.6 (2.7 - 160)	26.6 (3.6 - 200)
Low intensity CagA pos*	4.8 (0.54 - 43)	5.5 (0.64 - 47)
CagA neg	1.0 (ref.)	1.0 (ref.)
B: CagA among *H. pylori* seropositive subjects (Helicoblot 2.1):		
High intensity CagA pos†	9.5 (1.5 - ∞)	9.9 (1.6 - ∞)
Low intensity CagA pos†	5.0 (0.53 - ∞)	3.8 (0.42 - ∞)
CagA neg and *H. pylori* pos	1.0 (ref.)	1.0 (ref.)

* Asymptotic logistic regression.
† Exact logistic regression.

The combined risk of gastric adenocarcinomas and oesophageal carcinomas was significantly increased in *H. pylori* seropositive subjects, odds ratio 1.7 (95% CI: 1.1 - 2.6).

The resemblance between adjusted and unadjusted risk estimates was good (Table 8.3.1-2.).

Table 8.3.3. The risks of gastric adenocarcinoma at different tumour locations associated with *H. pylori* seropositivity

Gastric adenocarcinoma diagnosed up to Dec 31st, 1992	No. of cases	Odds ratio	(95% CI)
All subjects	56	5.0	(2.2 - 11.5)
No prior gastric surgery	46	3.9	(1.7 - 9.2)
Cardia	13	0.92	(0.23 - 3.7)
Non Cardia	27	11.1	(2.4 - 71.8)
Corpus-Fundus	11	5.4	(0.94- 39.9)
Corpus-Antrum	6	∞	(0.71 - ∞)
Antrum	10	∞	(1.01 - ∞)

8.4. Tobacco and *H. pylori* Associated Risks of Gastric Adenocarcinoma

The risks associated with tobacco smoking were estimated for the 56 cases of adenocarcinoma diagnosed until Dec 31st 1992 and their matched control subjects (Paper II). ELISA was used to determine *H. pylori* serostatus.

Current smoking was associated with an odds ratio of 2.2 (95% CI: 1.2 - 4.2) of gastric adenocarcinoma compared to non-current smokers at the time of enrolment, when adjusted for *H. pylori* seropositivity and occupation. The risk for *H. pylori* seropositive current smokers was 11.0 (95% CI: 3.4 - 35.6) compared to non-current *H. pylori* seronegative subjects. There was no significant interaction between *H. pylori* seropositivity and current smoking, *i.e.* the risk associated with current smoking was similar in both *H. pylori* seropositive and seronegative subgroups (Table 8.4.1.).

The odds ratios of current smoking when stratified upon location were for cardia gastric adenocarcinoma 1.9 (95% CI: 0.48 - 7.8) and 2.7 (95% CI: 0.93 - 8.1) for non-cardia gastric adenocarcinoma, respectively.

Table 8.4.1. Odds ratios in conditional logistic regression analyses for the association of gastric adenocarcinoma with *H. pylori* infection and tobacco smoking, with cases diagnosed until Dec 31st 1992 and adjusted for occupation as an indicator of socio-economic status

	Current smoking	*H. pylori* infection	Matched odds ratio	(95% CI)
Relative risk in each category				
	No	No	1	(ref.)
	Yes	No	2.1	(0.56 - 8.2)
	No	Yes	4.9	(1.5 - 15.4)
	Yes	Yes	11.0	(3.4 - 35.6)
With *H. pylori* seropositive non-current smokers as reference category				
	No	Yes	1	(ref.)
	Yes	Yes	2.3	(1.1 - 4.7)
Without interaction term*, risk relative to each risk factor				
Current smokers			2.2	(1.2 - 4.2)
H. pylori			5.0	(2.2 - 11.2)

* The regression model was not improved by the interaction term, P > 0.94.

When both current smokers and ex-smokers were included in the regression model, their odds ratios for developing gastric adenocarcinoma were 1.85 (95% CI: 0.85 - 4.0) and 0.67 (95% CI:

0.24 - 1.8), respectively, as compared to never-smokers, with adjustment for *H. pylori* and occupation.

The risks were re-estimated for gastric adenocarcinoma cases diagnosed until Dec 31st 2000 and their control subjects. The *H. pylori* seropositivity was now analysed by Helicoblot 2.1, the regression model included an adjustment for occupation, stratification was done for cardia and noncardia location and the smoking categories current smokers, ex-smokers, never smokers and unknown smoking status were used. With these new cases and the updated regression model the odds ratios for current smokers and ex-smokers compared to never smokers were 2.1 (95% CI: 1.1 - 4.0) and 1.3 (95% CI: 0.7 - 2.7), respectively. There was no difference between gastric cardia and non-cardia adenocarcinoma in the risks associated with current or ex-smoking, $P > 0.91$. The risks of non-cardia gastric adenocarcinoma were 25.2 (95% CI: 3.3 - 190) for current smokers seropositive for *H. pylori* and 12.7 (95% CI: 1.6 - 100) for never smokers seropositive for *H. pylori*, as compared to never smokers seronegative for *H. pylori*. The interaction between *H. pylori* seropositivity and current smoking was not significant, $P > 0.55$.

8.5. Tobacco and *H. pylori* Risks for Oesophageal Malignancies

The odds ratio for developing an oesophageal malignancy was 17.3 (95% CI: 3.0 - 99.5) for current smokers and 5.9 (95% CI: 1.15 - 29.9) for ex-smokers, as compared to never smokers, with adjustment for *H. pylori* seropositivity and alcohol consumption, when studied among cases diagnosed until Apr 31st 1999. The difference between current smokers and ex-smokers was significant, $P < 0.03$. The corresponding odds ratios for oesophageal squamous cell carcinoma were 25.2 (95% CI: 2.2 - 295) and 8.2 (95% CI: 0.94 - 72.0) for current smokers and ex-smokers, respectively.

Table 8.5.2. Relative risks of oesophageal malignancy associated with *H. pylori* seropositivity, smoking and alcohol, estimated with multivariate conditional logistic regression analysis in cases diagnosed until Apr 31st 1999 and adjusted for occupation as an indicator of socio-economic status

	Odds ratio	(95% CI)
H. pylori	0.29	(0.12 - 0.67)
Smoker	17.3	(3.0 - 99.5)
Ex-smoker	5.9	(1.15 - 29.9)
High alcohol consumption*	1.22	(0.46 - 3.2)

* Mm-MAST: >84 g/day.

8.6. Evaluation of Serological Methods

8.6.1. ELISA

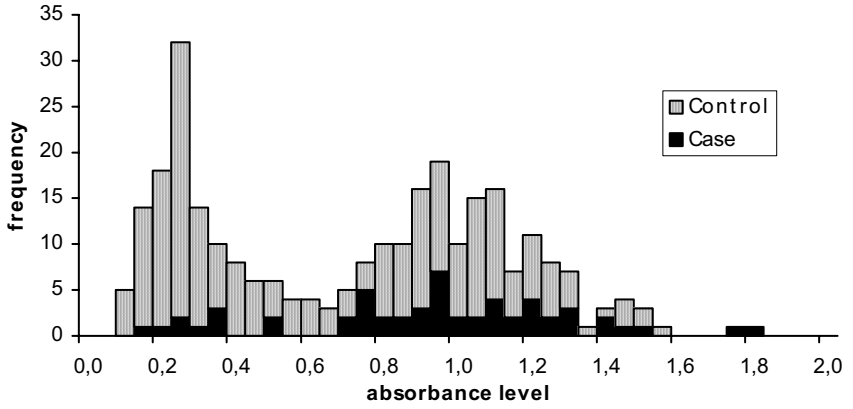

Figure 8.6.1.1. Frequency distribution of *H. pylori* IgG-antibody absorbance levels determined by the ELISA method.

The distribution of absorbance values from the enzyme-linked immunosorbent assay (ELISA) measuring IgG antibodies to *H. pylori* was evaluated for the 56 gastric adenocarcinoma cases identified until Dec 31st 1992 and their 224 matched control subjects. The distribution graph showed two well separated populations of near-Gaussian distributions. The cut-off value of 0.700 pre-determined by Gnarpe *et al.* (1988) was located in-between the two populations (Figure 8.6.1.1.).

Antibody levels may be affected by prolonged freezing times. The antibody protein may undergo some degradation over time or evaporation through insufficiently tightened caps may increase antibody levels. No association was however seen between *H. pylori* IgG antibody levels and freezing time when absorbance values were plotted against date of blood sampling (Figure 8.6.1.2.). The peak absorbance values of the seropositive and seronegative Gaussian populations were constant over time.

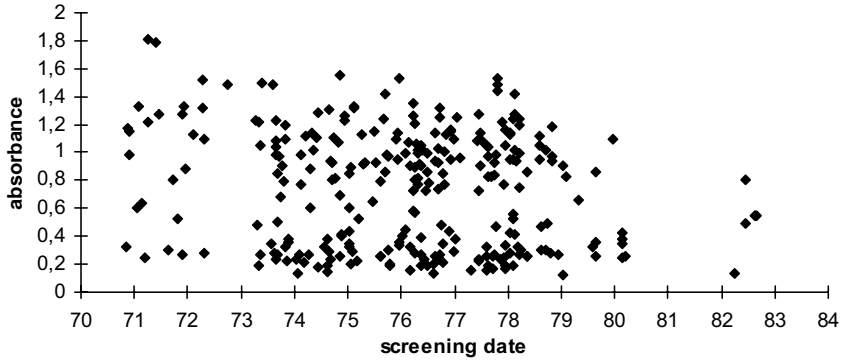

Figure 8.6.1.2. *H. pylori* IgG-antibody absorbance levels determined by the ELISA method at different screening dates.

8.6.2. Western Blot CagA

CagA seropositivity was evaluated in a serum evaluation population consisting of 650 study subjects, originally chosen as matched control subjects of cases identified until Dec 31st 2000. Therefore the male sex and older birth cohorts were slightly over-represented as compared to the Malmö Preventive Medicine (MPM) cohort (Table 8.6.2.1.). Correction factors could however be calculated for all sex and birth year cells. For this reason the seroprevalences are presented as crude values or as values adjusted for MPM.

Table 8.6.2.1. Matching criteria of the 650 study subjects of the serum evaluation population who were originally chosen as matched control subjects for case-control studies of gastric and oesophageal carcinoma

	Serum evaluation population	Malmö Preventive Medicine cohort
Year of birth	1931 (s.d.: ± 6.1)	1934 (s.d.: ± 6.3)
Year of enrolment	1981 (s.d.: ± 3.0)	1982 (s.d.: ± 3.5)
Males (percent)	85	70

CagA seroprevalence was significantly higher in *H. pylori* seropositive subjects (91%; adjusted for MPM) compared to *H. pylori* seronegative subjects (42%; adjusted for MPM), $P < 0.001$ (*H. pylori* serostatus analysed by Helicoblot 2.1) (Table 8.6.2.2).

The peak intensity of the Western blot CagA band ranged from 0 to 215 arbitrary units, with a visibility to the naked eye corresponding to about 10 arbitrary units. The frequency distribution

graph showed a bimodal distribution of the peak intensity (Figure 8.6.2.1.). Almost all (91%; adjusted for MPM) subjects with high peak intensity CagA bands were *H. pylori* seropositive (analysed by Helicoblot 2.1) and about three-fourths (74%; adjusted for MPM) of subjects with low peak intensity CagA bands were *H. pylori* seronegative.

Table 8.6.2.2. *H. pylori* seropositivity and CagA seropositivity of high and low intensity among matched control subjects

Matched control subjects	Prevalences			
	Crude		Adjusted to MPM*	
	n	(%)	n	(%)
***H. pylori* seropositive subjects†**				
High intensity CagA	305	(81.1)	260	(79.5)
Low intensity CagA	41	(10.9)	38	(11.6)
CagA seronegative	30	(8.0)	29	(8.9)
Total	**376**	**(100.0)**	**327**	**(100.0)**
***H. pylori* seronegative subjects†**				
High intensity CagA	27	(9.9)	26	(8.0)
Low intensity CagA	96	(35.0)	109	(33.7)
CagA seronegative	151	(55.1)	188	(58.2)
Total	**274**	**(100.0)**	**323**	**(99.9)**

* MPM: Malmö Preventive Medicine cohort.
† Analysed by Helicoblot 2.1.

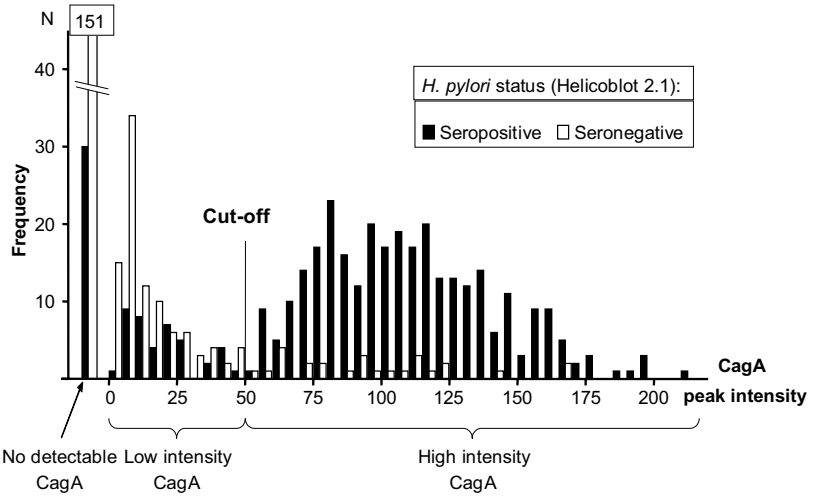

Figure 8.6.2.1. Frequency distribution of CagA peak intensity (crude). The peak intensity of the CagA band was higher in *H. pylori* seropositive subjects compared to *H. pylori* seronegative subjects. There was a bimodal distribution of CagA peak intensity in *H. pylori* seropositive subjects. Correction for sex and birth year did not have a substantial impact on the CagA peak intensity distribution graph (data not shown).

The prevalence of *H. pylori* seropositivity was higher in older subjects and decreased continuously with increasing year of birth (Figure 8.6.2.2., Table 8.6.2.3.). The prevalences of *H. pylori* seropositive subjects with high intensity CagA seropositivity and *H. pylori* seropositive subjects with low intensity CagA seropositivity also decreased continuosly with increasing year of birth. In contrast, the prevalence of *H. pylori* seronegative subjects with low intensity CagA seropositivity increased with increasing year of birth. On the other hand, the proportions of high and low intensity CagA seropositivity were constant in both *H. pylori* seropositive and *H. pylori* seronegative subgroups (Figure 8.6.2.3., Table 8.6.2.3.).

Table 8.6.2.3. Changes with increasing year of birth of seroprevalence and of proportion within *H. pylori* seropositive and *H. pylori* seronegative subgroups of *H. pylori* seropositivity and high or low intensity CagA seropositivity

	Change of seroprevalence per year of birth			Change of proportion within *H. pylori* seropos/neg subgroups per year of birth		
	percent unit	(s.e.)	P	percent unit	(s.e.)	P
H. pylori seropos						
Helicoblot 2.1	-1.37	(±0.32)	0.0001			
ELISA	-1.14	(±0.32)	0.0005			
H. pylori seropos (Helicoblot 2.1)						
High intensity CagA	-1.06	(±0.32)	0.0012	+0.10	(±0.35)	0.78
Low intensity CagA	-0.35	(±0.16)	0.029	-0.38	(±0.28)	0.18
H. pylori seroneg (Helicoblot 2.1)						
High intensity CagA	+0.18	(±0.13)	0.17	+0.09	(±0.30)	0.29
Low intensity CagA	+0.54	(±0.23)	0.021	-0.14	(±0.48)	0.77

There was no correlation between CagA band peak intensity and freezing time, r=0.0011.

The other immunoblot bands, 89 kDa, 37 kDa, or 35 kDa, 30 kDa and 19.5 kDa, for determination of *H. pylori* seropositivity in Helicoblot 2.1 had a unimodal distribution (data not shown).

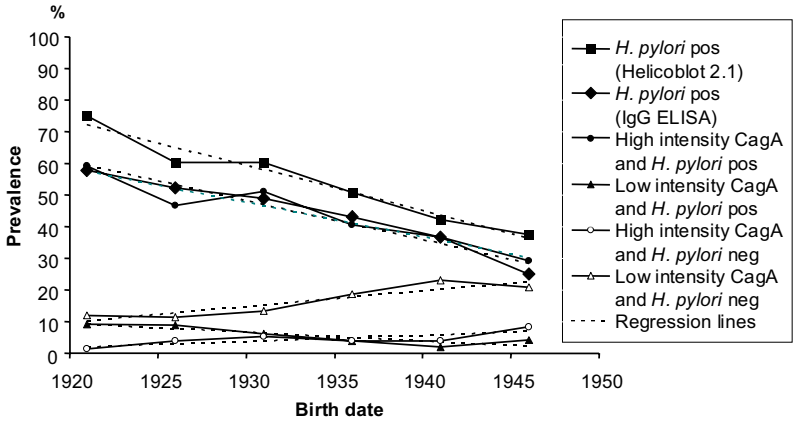

Figure 8.6.2.2. Prevalence per year of birth of different serological markers of *H. pylori*. Seroprevalences of *H. pylori* seropositivity (analysed by Helicoblot 2.1 and ELISA) and of high and low intensity CagA seropositivity in *H. pylori* seropositive and seronegative subgroups (analysed by Helicoblot 2.1).

Figure 8.6.2.3. Proportion of high and low CagA intensity subjects within *H. pylori* seropositive and *H. pylori* seronegative groups (analysed by Helicoblot 2.1). The proportions of high and low intensity CagA were constant in different birth cohorts in both *H. pylori* seropositive and seronegative subjects.

9. DISCUSSION

9.1. Overview

The studies presented in this thesis showed that non-cardia gastric adenocarcinoma was associated with *H. pylori* infection that was detected serologically prior to the development of the tumour. No significant association was found between *H. pylori* seropositivity and cardia gastric adenocarcinoma. *H. pylori* strains containing the *cag*A gene, identified by serological methods, were associated with a higher risk of non-cardia gastric adenocarcinoma than *H. pylori* strains that did not contain *cag*A. Tobacco smoking increased the risk for both cardia and non-cardia gastric adenocarcinoma and increased the risk in both *H. pylori* seropositive and seronegative subjects. The risk of gastric adenocarcinoma was higher among current smokers than among ex-smokers. Tobacco smoking did substantially increase the risk of squamous cell carcinoma, which similarly occurred more often among current smokers than among ex-smokers. Squamous cell carcinoma and adenocarcinoma of the oesophagus both tended to occur less often among *H. pylori* infected compared to uninfected subjects, although these results were not significant. There was no association between oesophageal squamous cell carcinoma and the *cag*A strain of *H. pylori*. The association between *H. pylori* and non-cardia gastric adenocarcinoma was stronger when the *H. pylori* infection was detected by the Western blot method Helicoblot 2.1, than by the in-house ELISA. The prevalence of subjects who were both CagA seropositive and *H. pylori* seronegative was higher in subjects born in more recent years, despite a lower *H. pylori* seroprevalence among these subjects. On the other hand, the proportion of CagA seropositive subjects in the *H. pylori* seronegative subgroup was the same irrespective whether the subjects belonged to early or late birth cohorts. As outlined in 9.2.8. below, these data were consistent with the finding that the majority of *H. pylori* seronegative but CagA seropositive subjects had a false CagA seropositive reaction in Helicoblot 2.1.

9.2. Interpretation

9.2.1. *H. pylori* Seropositivity and Non-cardia Gastric Adenocarcinoma

Subjects who were *H. pylori* seropositive prior to receiving their tumour diagnosis were found to have a higher risk for non-cardia gastric adenocarcinoma, as compared to *H. pylori* seronegative subjects. The association was found by using two different serological methods, ELISA and Helicoblot 2.1. Several biologically plausible carcinogenic mechanisms involving *H. pylori* infec-

tion have been described (see 5.2.4.). The finding is consistent with the literature on the subject (see 5.2.3.1) (*Helicobacter* and Cancer Collaborative Group, 2001; Huang *et al.*, 1998; IARC, 1994).

The association between *H. pylori* seropositivity and non-cardia gastric adenocarcinoma was stronger when *H. pylori* seropositivity was identified by the Western blot method Helicoblot 2.1, odds ratio 17.8 (4.2 - 74.8), than when identified by our in-house ELISA. The same stronger association was however also found in subjects identified to be seropositive by ELISA, when compared to subjects fulfilling the more stringent criterion of not being *H. pylori* infected, *i.e.* being both *H. pylori* and CagA seronegative. Furthermore, the same magnitude of the association was found when CagA seropositivity was used as the sole marker of *H. pylori* infection. Adjustment for tobacco smoking and occupation as a measure of socio-economic status did only have a small influence on this association. The association between non-cardia gastric adenocarcinoma and *H. pylori* seropositivity was stronger than in most studies that have used an ELISA method. Our finding was however consistent with results published by Ekström *et al.* (2001) and Brenner *et al.* (2004) and presented by Forman *et al.* (2004*b*) at the EHSG meeting in Vienna, September 22th-24th, 2004.

Ekström *et al.* (2001) have suggested that CagA antibodies may be retained longer after spontaneous *H. pylori* eradication than *H. pylori* antibodies identified by conventional ELISA and indicate a past *H. pylori* infection. By comparing *H. pylori* seropositive subjects with subjects lacking antibodies to both *H. pylori* and CagA, they found an odds ratio of 21.0 (95% CI, 8.3 - 53.4) for non-cardia gastric adenocarcinoma in their retrospective population-based study. Brenner, *et al.* (2004) excluded cases in which clearance of *H. pylori* infection may have occurred in the course of gastric cancer development, such as cases of advanced gastric cancer, cases with blood sampling more than 3 months after gastrectomy and cases that were CagA seropositive but *H. pylori* seronegative. Similarly, they found in their retrospective study an odds ratio of 18.3 (95% CI: 2.4 - 136.7) for non-cardia gastric adenocarcinoma. Forman, *et al.* (2004*b*) used Helicoblot 2.1 in a prospective cohort and found the odds ratio 15.9 (95% CI: 3.6 - 69.6) for the association of *H. pylori* seropositivity to non-cardia gastric adenocarcinoma. *H. pylori* seropositivity detected by ELISA, on the other hand, had an odds ratio of 2.2 (95% CI: 1.0 - 4.7) in the same material.

Helicoblot 2.1 was seropositive in a significantly greater number of subjects than ELISA in our studies. The stronger association between *H. pylori* seropositivity and non-cardia gastric adenocarcinoma found with Helicoblot 2.1 suggests that this method may lead to fewer

misclassifications than our in-house ELISA method. Further data are needed in order to generalize this result to Western blot and ELISA methods in general. Most studies and meta-analyses on *H. pylori* infection and non-cardia gastric adenocarcinoma have however used ELISA methodology. The strength of this association may therefore have been underestimated.

Presence of the CagA band together with the current infection marker (CIM) is one of five criteria for *H. pylori* seropositivity by Helicoblot 2.1. The ability to detect CagA seropositivity explained however only a part of the difference between Helicoblot 2.1 and ELISA. The CagA band together with the CIM was the sole criterion for *H. pylori* seropositivity in only two non-cardia gastric adenocarcinoma cases.

There was no significant difference between the non-cardia locations corpus-fundus, corpus-antrum and antrum regarding the risk associated with *H. pylori* seropositivity in the small material studied until Dec 31st, 1992 (Table 8.3.2.).

The difference between the odds ratios shown by ELISA in Paper I, 11.1 (95% CI: 2.4 - 71.8) and in Paper V, odds ratio 6.3 (95% CI: 2.9 - 15.1) was due to a small difference in *H. pylori* seroprevalence among cases, whereas control subjects had similar seroprevalences.

9.2.2. *H. pylori* Seropositivity and Cardia Gastric Adenocarcinoma

There was no evidence of an association between *H. pylori* seropositivity, as detected by ELISA or Helicoblot 2.1, and cardia gastric adenocarcinoma. Our finding is consistent with findings in the literature (*Helicobacter* and Cancer Collaborative Group, 2001). However, the number of cases limited the statistical power to detect an association between cardia gastric adenocarcinoma and *H. pylori* seropositivity.

Another difficulty of investigating diseases in the gastric cardia is that different authorities have defined different boundaries for this region (Ectors *et al.*, 2005). The lack of an association between *H. pylori* infection and cardia gastric adenocarcinoma may therefore be due to misclassification (Ekström *et al.*, 1999), especially as *H. pylori* infection has been associated with a lower risk of oesophageal adenocarcinoma (de Martel *et al.*, 2005).

We have applied the classification criteria used by Lagergren *et al.* (1999*a*), which separates oesophageal adenocarcinoma from cardia gastric adenocarcinoma in tumours occurring in the

gastro-oesophageal junction depending on the presence of Barrett's oesophagus adjacent to the tumour. The classification was modified by us only to enable use of written gastroscopy and histology reports. The Siewert classification Type I principally corresponds to oesophageal adenocarcinoma and Type II to cardia gastric adenocarcinoma (Siewert and Stein, 1998).

9.2.3. CagA Seropositivity and Gastric Non-cardia Gastric Adenocarcinoma

CagA seropositivity was a risk factor in the subpopulation of *H. pylori* seropositive subjects. *H. pylori* strains with *cag*-PAI have been shown to induce a stronger IL-8 release from gastric epithelial cells and to be associated with a stronger inflammatory reaction (Crabtree, 1996). CagA may be translocated into the gastric epithelial cells and interact with intercellular pathways that may be carcinogenic (Odenbreit *et al.,* 2001; Selbach *et al.,* 2002*b*). Our findings support these proposed mechanisms associated with *cag*-PAI.

The risk of non-cardia gastric adenocarcinoma associated with CagA seropositivity among *H. pylori* seropositive subjects is consistent with earlier studies. Parsonnet *et al.* (1997) and Blaser *et al.* (1995) found in their prospective nested case-control studies odds ratios of 3.3 (95% CI: 1.6 - 6.5) and 1.8 (95% CI: 0.9 - 3.9), respectively. The odds ratio was 1.18 (95% CI: 0.56 - 2.53) in the Linxian intervention study (Limburg *et al.*, 2001). Population-based and hospital-based retrospective case-control studies have shown odds ratios ranging from 1.61 (95% CI 1.06 - 2.45) to 7.3 (95% CI 1.7 - 30.6) (Rudi *et al.*, 1997; Vaucher *et al.*, 2000; Ekström *et al.*, 2001; Brenner *et al.*, 2002; Tatemichi *et al.*, 2003; Wu *et al.*, 2003; Held *et al.*, 2004; Lopez-Carrillo *et al.*, 2004), whereas two studies on Asian subjects have shown no association (Kikuchi *et al.*, 1999; Tatemichi *et al.*, 2003). A meta-analysis has found a risk of 2.01 (95% CI, 1.21 - 3.32) for non-cardia gastric adenocarcinoma associated with the *cag*A strain of *H. pylori* in *H. pylori* infected subjects (Huang *et al.*, 2003).

The Helicoblot 2.1 CagA band was found to have a bimodal intensity distribution (Figure 8.6.2.1.). Subjects with a high intensity CagA seropositivity had a risk for non-cardia gastric adenocarcinoma more or less similar to the risk associated with CagA seropositivity without regard to intensity. There were no significant risks associated with low intensity CagA seropositivity. The point estimates were however located in-between the risks of high intensity CagA seropositivity and CagA seronegativity. This intermediate risk corresponds well with the results from the evaluation of the CagA band in Paper IV (see below 9.2.8.).

Subjects who were *H. pylori* seropositive but CagA seronegative may have only a modest increase of risk, as the risk associated with CagA seropositivity among *H. pylori* seropositive subjects was about half the risk associated with *H. pylori* seropositivity alone.

There were only two *H. pylori* seronegative cases, only one of which was CagA seropositive (Table 8.2.1.). Therefore it was not possible to estimate the risk of isolated CagA seropositivity, using logistic regression models. Isolated CagA seropositivity however has a prevalence of about 20% in our control population. If isolated CagA according to Helicoblot 2.1 had been a risk for non-cardia gastric adenocarcinoma, more cases would have been expected. Our control population was reasonably representative of the study population, the Malmö Preventive Medicine cohort, although older men were slightly overrepresented due to matching.

CagA seropositivity alone had a similar magnitude of risk for non-cardia gastric adenocarcinoma as *H. pylori* seropositivity alone.

9.2.4. *H. pylori* Seropositivity and Oesophageal Malignancies

H. pylori seropositivity was found to reduce the combined risk of oesophageal and squamous cell carcinoma. Tendencies to inverse associations with *H. pylori* seropositivity were found for each of them, oesophageal and squamous cell carcinoma, although these associations were not significant. Our material did not however have the potential to detect associations weaker than a 10-fold reduction in oesophageal adenocarcinoma and a 3-fold reduction in squamous cell carcinoma (see below 9.3.2.).

Oesophageal adenocarcinoma has earlier been shown to be inversely associated with *H. pylori* infection. A prospective nested case-control study at Kaiser Permanente Medical Care Program, northern California, on oesophageal adenocarcinoma showed that *H. pylori* seropositive subjects had a reduced odds ratio of 0.37 (95% CI: 0.16 - 0.88) (de Martel *et al.*, 2005). Two population-based, retrospective studies have shown a reduced risk at an odds ratio of 0.4 (95% CI: 0.2 - 0.8) and 0.3 (95% CI: 0.2 - 0.6), respectively (Chow *et al.*, 1998*a*; Ye *et al.*, 2004), whereas a third study has not, at an odds ratio of 1.01 (95% CI: 0.58 - 1.77) (Wu *et al.*, 2003). A large study of pathology specimens found a lower prevalence of histologically assessed *H. pylori* in the stomach among 138 adenocarcinoma cases occurring in Barrett's oesophagus, compared to 712 gastric biopsies from non-ulcer dyspepsia patients, corresponding to an odds ratio of 0.48, $p < 0.001$ (Vieth *et al.*, 2000). Studies performed at endoscopy clinics have generally found similar tendencies (Vicari *et*

al., 1998; Grimley *et al*., 1999; Loffeld *et al*., 2000; Weston *et al*., 2000). No study has presented data showing an increased prevalence of *H. pylori* in an oesophageal adenocarcinoma population compared to a non-malignant control group.

Gastro-oesophageal reflux disease is one important risk factor for Barrett's oesophagus and oesophageal adenocarcinoma (Winters *et al.,* 1987; Lagergren *et al.,* 1999*a*). A reduction in gastro-oesophageal reflux has therefore been suggested to account for the preventive effect of *H. pylori* seropositivity, possibly by gastric atrophy. Other mechanisms may however be important, as adjustment for pepsinogen did not influence the association between *H. pylori* and oesophageal adenocarcinoma in a recent study (Ye *et al.,* 2004). Other suggested mechanisms of gastric acidity reduction are production of ammonia by *H. pylori* and dilution of gastric acid by an increased leakage of interstitial gastric fluid through an inflammatory damaged epithelial lining (Feldman *et al*., 1998).

Recently, a retrospective population-based study in Taiwan has reported an inverse association between *H. pylori* seropositivity and oesophageal squamous cell carcinoma, at an adjusted odds ratio of 0.51 (95% CI: 0.27 - 0.96) and an unadjusted odds ratio of 0.37 (95% CI: 0.22 - 0.62) (Wu *et al*., 2005). Another retrospective population-based study instead found an increased risk of 2.1 (95% CI: 1.1 - 4.0) (Ye *et al*., 2004). One further hospital-based study with 41 cases of squamous cell carcinoma found no association (Talley *et al*., 1991). The biological mechanism for a reduced risk among *H. pylori* seropositive subjects is unclear, but the Taiwanese study group found that the risk reduction was more pronounced for squamous cell carcinomas in the lower third of the oesophagus (Wu *et al*., 2005).

Clinical trials on *H. pylori* eradication should therefore take both oesophageal and squamous cell carcinoma into account, when estimating benefits of curing *H. pylori* infection.

9.2.5. Overall Risk for Gastric and Oesophageal Carcinoma

The overall risk for gastric and oesophageal carcinoma associated with *H. pylori* seropositivity was significantly increased in the Malmö Preventive Medicine cohort, *i.e*., *H. pylori* seropositive subjects had a significantly higher incidence of carcinoma in either the stomach or oesophagus than seronegative subjects. Would it be possible to generalize this result to other populations?

Gastric cancer incidence has decreased approximately by a factor of four in the last half century (The Swedish Cancer Registry, 2005). The prevalence of *H. pylori* in developed countries has at the same time decreased (Rothenbacher and Brenner, 2003; Bergenzaun *et al.,* 1996). The prevalence of *H. pylori* infection in the birth cohorts in which most gastric cancer occur today however is still about 50%. Other factors, such as better socio-economic status, better hygiene and refrigeration may have influenced gastric cancer incidence in combination with the decreasing prevalence of *H. pylori* infection. Simultaneously, the incidence of oesophageal adenocarcinoma is rising, dramatically in relative terms, not so in absolute numbers due to the still comparatively low incidence (Devesa *et al.,* 1998). In the case of oesophageal adenocarcinoma and cardia gastric adenocarcinoma, its incidence is independent of socio-economic status.

In order to enable a generalization of our result to other populations, for instance the city of Malmö ten years from now, it would be important to consider whether *H. pylori* infection interacts with other risk factors or not. Theoretically, if there were no change in the prevalence of other risk factors and we could prevent *H. pylori* infection, for example by vaccination or by breaking transmission pathways, we would expect the incidence of gastric and oesophageal carcinoma to be close to that of the *H. pylori* seronegative group of our studies. However, if there was a cofactor to *H. pylori* that was necessary for gastric but not for oesophageal carcinogenesis, and the prevalence of such a cofactor decreased, then a situation may arise where the net effect of prevention or eradication would become unfavourable.

On the other hand, our studies showed a strong association between *H. pylori* seropositivity and non-cardia gastric adenocarcinoma, but non-significant tendencies to inverse associations with oesophageal adenocarcinoma and squamous cell carcinoma. There is support in the literature for an inverse association with oesophageal adenocarcinoma (de Martel *et al*., 2005). Corresponding support for oesophageal squamous cell carcinoma is still limited. The incidence of non-cardia gastric adenocarcinoma in our studies was about twice that of oesophageal squamous cell carcinoma and about six times that of oesophageal adenocarcinoma. It would follow that the absolute number of non-cardia gastric adenocarcinoma cases associated with *H. pylori* infection would be greater than the corresponding number of oesophageal adenocarcinoma and oesophageal squamous cell carcinoma cases.

On the basis of our data it would therefore be reasonable to expect *H. pylori* prevention to reduce the overall incidence of gastric and oesophageal carcinoma.

9.2.6. Gastric Cancer Prevention by *H. pylori* Eradication

If *H. pylori* infection is eradicated instead of prevented, we would expect the incidence of gastric and oesophageal carcinoma to be somewhere in-between that of the *H. pylori* seronegative and seropositive groups of our studies, as disease processes might already have started. The literature provides only limited data on whether eradication of *H. pylori* of would reduce the risk of developing gastric cancer and no corresponding data regarding oesophageal cancer. Wong *et al.* (2004) have investigated gastric cancer and performed a prospective, randomised, placebo-controlled population-based primary prevention study in China. They included 1630 healthy carriers of *H. pylori*, mean age 42 years, and followed them from 1994 to 2002. The gastric cancer incidence was 37% lower among treated subjects, however the difference was non-significant. There occurred 7 cases of gastric cancer in the treatment group compared to 11 cases in the placebo group, P = 0.33. Because of their assumption of 30 cases during 7 years of follow-up and a 3-fold difference between treatment and placebo groups, the trial was underpowered. In a *post hoc* analysis, they found a significant reduction in gastric cancer incidence among subjects with no precancerous lesion at study entry. This result may suggest that gastric cancer prevention would benefit from early *H. pylori* eradication and that precancerous lesions may represent a point of no return. In another non-randomised study by Uemura *et al.* (1997), eradication of *H. pylori* prevented recurrence of gastric cancer in patients treated for early gastric cancer, suggesting that late eradication may still be beneficial. In their study 6 new cases of early gastric cancer occurred among 67 *H. pylori* infected subjects, whereas no recurrence occurred among 65 treated subjects during a 2-years follow-up. Take *et al.* (2005) have followed 1,120 peptic ulcer subjects treated for *H. pylori*, mean age 50 years, during a mean of 3.4 years. Gastric cancer occurred in 8 of 944 subjects cured for infection and in 4 of 176 subjects with persistent infection, P = 0.04. None of the subjects with duodenal ulcer developed gastric cancer, but 8 of the 12 cases of gastric cancer had scars of older duodenal ulcer. Interestingly, diffuse type gastric cancer, five cases, occurred only in subjects cured of infection. Uemura *et al.* (2001) followed 1526 subjects with duodenal ulcer, gastric ulcer, gastric hyperplastic polyps and non-ulcer dyspepsia for a mean time of 8.5 years, mean enrolment age 52 years, among whom gastric cancer occurred in 36 of 1246 *H. pylori* infected subjects and in none of 280 uninfected subjects. Eradication therapy was received by 253 *H. pylori* infected subjects of whom none developed gastric cancer during a mean follow-up of 4.8 years, however the reason for giving eradication therapy was not described in the study.

The role of *H. pylori* eradication in cancer prevention have still to be confirmed and further clinical trials are warranted. Clinical trials should however monitor oesophageal malignancies.

9.2.7. Tobacco and Gastric Adenocarcinoma in *H. pylori* Seropositive and Seronegative Subjects

Current tobacco smoking at the time of enrolment and *H. pylori* infection were both found to be significant risk factors for the development of gastric adenocarcinoma. Specifically, current smoking at the time of enrolment was a risk factor also among *H. pylori* infected individuals. The relative risk of current smoking was the same among uninfected and infected subjects, *i.e.* independent of infection status. Thus, the combined effect of current smoking and *H. pylori* infection on the incidence of gastric adenocarcinoma was more than additive, *i.e.* multiplicative or synergistic.

This was the first report on the joint effect of tobacco smoking and *H. pylori* infection on gastric adenocarcinoma. Our results are in line with previous findings in this area. A meta-analysis of 40 studies has estimated a risk in the order of 1.5 - 1.6 for gastric cancer among smokers compared to non-smokers (Tredaniel *et al.*, 1997). Komoto *et al.* (1998) found both *H. pylori* and tobacco smoking to be associated with gastric carcinoma (odds ratios for tobacco smoking 3.0, 95% CI: 1.6 - 5.9 and 2.2, 95% CI: 0.77 - 6.2; and for *H. pylori* 5.2, 95% CI: 2.0 - 13.2 and 8.0, 95% CI: 1.02 - 62.8 in intestinal and diffuse gastric carcinoma, respectively). Parsonnet *et al.* (1997), however, found no association between tobacco smoking and gastric cancer. In other studies the association between *H. pylori* and gastric cancer was not altered when adjusted for tobacco smoking (Lin *et al.*, 1995; Aromaa *et al.*, 1996; Webb *et al.*, 1996; Hansen *et al.*, 1999). The synergistic effect of tobacco smoking and *H. pylori* has been confirmed by Brenner *et al.* (2002).

Our findings were originally reported in gastric adenocarcinoma cases identified until Dec 31st 1992 using ELISA to detect *H. pylori* seropositivity. We were able to confirm these results in the larger case-control set identified until Dec 31st 2000, now stratified on cardia and non-cardia locations and with *H. pylori* seropositivity detected by Helicoblot 2.1. Again, current smoking was associated with a higher risk of non-cardia gastric adenocarcinoma in the *H. pylori* seropositive subgroup. Ex-smoking was, however, not a significant risk factor, but the point estimate was now in-between that of never-smokers and current smokers, supporting a dose-response relationship.

9.2.8. Interpretation of the CagA Band

The CagA band was present in almost all (91%) *H. pylori* seropositive subjects and in about half (42%) of *H. pylori* seronegative subjects, when *H. pylori* serostatus was detected by Helicoblot 2.1.

The CagA band was found to have a bimodal peak intensity distribution in *H. pylori* seropositive subjects, whereas the CagA band was of low intensity in most *H. pylori* seronegative subjects.

Helicoblot 2.1 has a sensitivity of 96%. Therefore most *H. pylori* seronegative but CagA seropositive subjects did not have an *H. pylori* infection at the time of blood sampling. Furthermore our analyses showed that younger birth cohorts had a higher prevalence of the combination CagA seropositivity and *H. pylori* seronegativity than older birth cohorts (Figure 8.6.2.2.).

Let us assume that the combination CagA seropositivity and *H. pylori* seronegativity analysed with Helicoblot 2.1 is a sign of past *H. pylori* infection. Eradication of *H. pylori* on the subjects in our material would then have occurred more often in younger birth cohorts, despite the fact that older birth cohorts probably have a higher degree of gastric atrophy, a higher prevalence of *H. pylori* infection and have had the infection for a longer time. They would therefore be expected to have a higher degree of spontaneous or intentional eradication.

Eradication of *H. pylori* was not generally established as a therapy at the time of enrolment. One might however, expect younger birth cohorts to have used more antibiotics. Improved living conditions might have increased the spontaneous eradication rate during childhood. One might speculate that CagA antibodies would be retained longer in younger subjects compared to older subjects after a resolved *H. pylori* infection. In order to be compatible with our material however, such mechanisms would require spontaneous *H. pylori* eradication to occur at the specific rate needed to keep the proportion of CagA seropositivity in *H. pylori* seronegative subjects constant in different birth cohorts (Figure 8.6.2.3.).

Alternatively, CagA seropositivity in *H. pylori* seronegative subjects detected by Helicoblot 2.1 may be a false positive reaction. Such a false positive reaction, which may be due to a non-immune protein-protein interaction or a cross-reactivity with antibodies primarily formed to a different widely distributed antigen, would be constant and independent of birth cohorts. This kind of false positive CagA band however, would only be uncovered in *H. pylori* seronegative subjects. Therefore, with decreasing *H. pylori* seropositivity, this entity would be increasing, as in our material. Furthermore the CagA seropositivity in *H. pylori* seronegative subjects is higher than reported in other studies. Fusconi *et al.* (1999) have investigated subjects highly selected to be

H. pylori negative in five tests (histology, culture, rapid urease test, urea breath test and IgG ELISA serology) and found CagA seropositivity in 8 of 80 (10%) *H. pylori* seronegative subjects.

There was no correlation between CagA intensity and freezing time in our material. Seroprevalences of high and low intensity CagA did both increase and decrease with increasing year of birth, suggesting that an effect caused by sample storage is less likely.

We therefore propose that CagA seropositivity in a majority of subjects who were *H. pylori* seronegative by Helicoblot 2.1 should be regarded as a false positive reaction.

It follows that if CagA seropositivity in Helicoblot 2.1 *H. pylori* seronegative subjects is a false positive reaction, then the same false positive reaction would occur also in Helicoblot 2.1 *H. pylori* seropositive subjects. Most *H. pylori* seronegative subjects had CagA seropositivity of low intensity (Figure 8.6.2.1.). If the proportion of CagA false-positivity to CagA seronegativity in *H. pylori* seronegative subjects is similar in *H. pylori* seropositive subjects, then about half of *H. pylori* seropositive subjects with low intensity CagA seropositivity, but only a small fraction of high intensity CagA seropositivity, would have a false positive reaction. It would therefore be reasonable to consider low intensity CagA seropositivity in Helicoblot 2.1 *H. pylori* seropositive subjects as indeterminable regarding their CagA status.

It may not be possible however to generalize the interpretation of the CagA band to subjects in which the *H. pylori* serostatus has been determined by other methods than Helicoblot 2.1. Sörberg, *et al.* (1997) have found that post-treatment CagA antibody titers decreased slower than *H. pylori* enzyme immunoassay (EIA) IgG antibody titers in an eradication study on ulcer patients with 32 months of follow-up. Ekström *et al.* (2001) have found that CagA seropositivity among *H. pylori* seronegative subjects according to ELISA was highly associated with non-cardia gastric adenocarcinoma in a case-control study. Thus there is support for the idea that CagA seropositivity in *H. pylori* seronegative subjects represents past *H. pylori* infection when *H. pylori* is determined by other methods than Helicoblot 2.1.

Park *et al.* (2002) found the sensitivity and specificity for detection of *H. pylori* infection by Helicoblot 2.1 to be 99% and 98%, respectively, when compared to a gold standard consisting of histology, culture and ELISA serology by HM-CAP (high molecular cell associated protein). The corresponding figures for Helicoblot 2.0 were 95% and 89%. The CagA band of the Helicoblot

2.1 had a sensitivity of 99%, the specificity was however only 53%. For Helicoblot 2.0 the CagA band sensitivity and specificity were both 90%. The gold standard in these analyses was an immunoblot assay that detected the CagA band in sampled and cultured *H. pylori* strains, using polyclonal antibodies from mouse immunized with recombinant CagA. Figueiredo *et al.* (2001) compared five different assays, including Helicoblot 2.0 and Helicoblot 2.1, for the serological detection of CagA in *H. pylori* infected samples. The golden standard was genotyping of *cag*A. The sensitivity in the five tests ranged from 67.9% to 85.7% (Helicoblot 2.1 CagA: 78.6%), the specificity from 54.2% (Helicoblot 2.1 CagA) to 100%. Dubious Western blot bands were however considered seronegative. Thus, there is in these studies support for a large proportion of false positive CagA band in Helicoblot 2.1, even if dubious bands are excluded.

H. pylori seropositivity by Helicoblot 2.1 alone was associated with the same magnitude of risk for non-cardia gastric adenocarcinoma as the combination of our in-house ELISA with CagA serology. The strength of this association implies a low degree of misclassification. Furthermore, no risk for non-cardia gastric adenocarcinoma was found in subjects who were CagA seropositive but *H. pylori* seronegative by Helicoblot 2.1 in our analyses. Thus, Helicoblot 2.1 in combination with used interpretation methods may have detected *H. pylori* infection relevant to gastric carcinogenesis.

Although correction factors were used in order to make the serological evaluation population representative of the Malmö Preventive Medicine cohort, the main conclusions were not affected of whether these correction factors were used or not.

9.3. Methodological Considerations

9.3.1. Study Design

Papers I - III and V were designed as case-control studies nested in the prospective Malmö Preventive Medicine cohort. This study design combines the advantages of the cohort study of collecting samples and exposure information before diagnosis of the disease with the time and statistical efficiency of the case-control study (Rothman and Greenland, 1998*a*). Selection bias is limited by drawing case and control subjects from the same cohort, recall bias on questionnaire items is limited, and laboratory measures are less affected by the disease under investigation, because questionnaires and samples have been collected before diagnosis.

The study design was similar for all four carcinomas studied, including identification of cases from the same population and during the same time period and analysis by similar regression models with the same number of adjustment parameters. The laboratory analyses by ELISA were mostly performed separately for gastric and oesophageal carcinomas, whereas the Helicoblot 2.1 was analysed simultaneously for all four carcinomas. The findings of different kinds and strengths of associations between the type of carcinoma and *H. pylori* and CagA seropositivity indicate that the associations primarily reflect the investigated diseases and not the study design (Breslow and Day, 1980).

Paper IV compared changes in seroprevalence over time in a standardised sub-sample of the Malmö Preventive Medicine cohort.

9.3.2. Sample Size

The power to detect a certain odds ratio at different sample sizes in univariate analysis is illustrated by Figure 9.3.2.1.

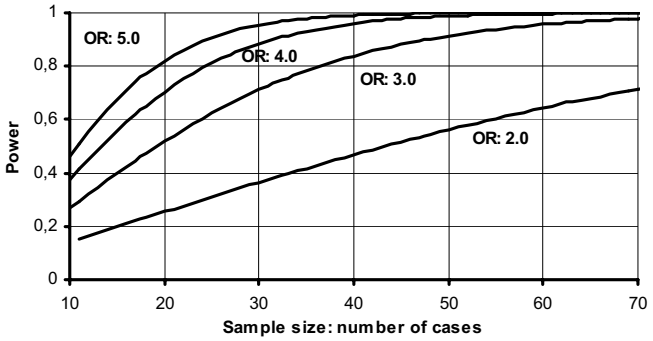

Figure 9.3.2.1. The power to detect a given odds ratio by logistic regression. Sample size is measured in number of cases, with four matched control subjects per case. Background exposure prevalence is 50% and $\alpha = 0.05$. (PS Power and Sample Size Calculations, Version 2.1.30, February 2003, Vanderbilt University School of Medicine, Department of Biostatistics, Nashville; Dupont and Plummer, 1997).

Our studies were able to detect an odds ratio of 2.2 or higher among the 67 cases of non-cardia gastric adenocarcioma, an odds ratio of 4.1 or higher among the 24 cases of cardia gastric adenocarcinoma, an odds ratio of 0.33 or lower among the 37 cases of squamous cell carcinoma

and an odds ratio of 0.10 or lower among the 12 cases of oesophageal adenocarcinoma with 80% power, on the conditions of four matched control subjects per case, *H. pylori* seropositivity present among about 50% of control subjects and a significance level of 0.05 (Dupont and Plummer, 1997).

A matched study design was used to increase the statistical efficiency (Rothman and Greenland, 1998*b*). Close matching on gender, birth date and date of enrolment within 6 months was possible.

The number of adjustment parameters used in different logistic regression models was usually 10% or less of the number of cases. The hallmark symptom of bias when stratification exceeds the limits of the data is that the odds ratio of interest deviates further from the null, as more adjustment parameters are added (Rothman and Greenland, 1998*c*). There was no regression model reported in Paper V however where the addition of an adjustment parameter lead to an unexpected deviation of the investigated odds ratio, that was not understandable from the deviation that occurred when such an adjustment parameter was added as the first and only adjustment parameter in the model. There was in general a close correspondence between adjusted and unadjusted estimates.

Exact logistic regression algorithms were used for analyses of gastric cardia adenocarcinoma, oesophageal adenocarcinoma and oesophageal squamous cell carcinoma in Paper V because of sparse data. Exact logistic regression algorithms were also used because of imbalanced data for CagA seropositivity in *H. pylori* seropositive subjects of non-cardia gastric adenocarcinoma. Exact algorithms in general have wider confidence limits than asymptotic algorithms. There was however no regression model in which the asymptotic algorithm, but not the corresponding exact algorithm, showed a significant result.

9.3.3. Selection Biases

The Malmö Preventive Medicine cohort maintained a high participant rate during the health screening period. Participants in the Malmö Preventive Medicine cohort have been shown to differ from non-participants with regard to marital status, educational level, socio-economic status, housing and being foreign-born (Berglund *et al.*, 2000). The risk ratio for total mortality among non-participants has been estimated to 2.2 (95% CI: 2.1 - 2.3) for males and 2.4 (95% CI: 2.1 - 2.8) for females. Despite these differences, there was almost no difference in gastric cancer

occurrence and only a slightly lower occurrence of oesophageal cancer among participants compared to non-participants of the Malmö Preventive cohort.

Selection bias may occur if subjects that would not normally attend a health screening choose to do so because they have early or insidious symptoms from yet undiagnosed tumour diseases. As a consequence case and control subjects would come from different subpopulations. Exclusion of cases occurring during the first two years after enrolment however did not materially change the estimated risks presented in Paper V.

Previous partial gastrectomy is a documented risk factor for gastric adenocarcinoma. Peptic ulcer surgery is a common reason for partial gastrectomy (10 of 11 cases in our material) and thus associated with *H. pylori (*Tersmette *et al*., 1990). Whether the patients had undergone a previous partial gastrectomy was only known for the cases and it was not possible to match or stratify this confounding factor for the control subjects. Cases with previous partial gastrectomy were therefore excluded. Because it was not possible to perform a corresponding exclusion of control subjects, a slight underestimation of the risk associated with *H. pylori* and CagA seropositivity may have occurred.

Malignant disease is reported to the Swedish National Cancer Registry (SNCR) and reporting is compulsory for both physicians and pathologists according to the National Board of Health and Welfare. The reporting is estimated to be close to 99% of all diagnosed cases (The Swedish Cancer Registry, 1994). In total, 191 tumour cases were identified. Tumour cases diagnosed until Dec 31st 1997 were identified through SNCR. From Jan 1st 1998 to Dec 31st 2000 tumour cases were instead identified through the Pathology Department at University Hospital Malmö. Loss to follow up during this period because of movement from the catchment area of the Pathology Department was estimated to be equivalent to five (2.6%) malignant tumour cases. Bias due to this loss to follow up would be limited.

Conditional regression analysis was performed with a varied number of control subjects in each set. This is however allowed and constitutes no source of bias (Breslow and Day, 1980).

9.3.4. Information Biases

Information biases were reduced by collecting blood samples and questionnaires prior to tumour diagnosis. Reversed causation, *i.e.* the influence of the disease on measured risk factors, may still

occur, especially in malignant diseases which generally have long sub-clinical periods. This is particularly true for *H. pylori*, in which gastric atrophy and spontaneous eradication of the bacterium may occur long before the disease process reaches the gastric adenocarcinoma (*Helicobacter* and Cancer Collaborative Group, 2001). Such spontaneous eradication may though lead to an underestimation of the risk associated with *H. pylori.*

Laboratory technicians were blinded to case-control status. The interpretation of scanned immunoblot strips was performed by the investigator without revealing case-control status until *H. pylori* serostatus was determined for all strips. There was no tendency in blood samples to declining or increasing antibody titers with increasing freezing time. The predefined cut-off level of our in-house ELISA seemed adequate in an analysis of absorbance level distribution. The sensitivity and specificity of laboratory analyses have not been considered in the risk analyses. Misclassifications due to these reasons would however be non-differential and lead to an underestimation of the risk.

It is interesting to note that even if all cases are affected in a population where the risk factor prevalence is 50%, the expected value (*i.e.* the mean of several random estimations) with a test that has a 90% sensitivity and specificity would be an odds ratio of no more than 9.0. With a test sensitivity and specificity of 95%, the expected odds ratios would be 19.0, with 98%, 49.0. The expected odds ratio is also sensitive to the prevalence of the risk factor among the population, with a test sensitivity and specificity of 95% and risk factor prevalences of 30% and 70%, the expected odds ratio would be 40.4 and 8.9, respectively.

Data on current smoking were collected as the actual status at the time of enrolment, making recall bias limited to the ex-smoking group. Among current smokers, 90.5% had been smoking for more than 10 years and it is reasonable to believe that most of them have continued with their smoking habit at least as long as they did not have any insidious symptoms from the malignancy or another disease. Similarly, at the time of enrolment, the subjects were in their middle ages and it is not likely that more than a few of the non-smokers have begun to smoke after their enrolment.

The four main categories of occupation were used as a measure of socio-economic status. Residual confounding relative to 14 subcategories available at the National Registry was estimated to be less than 3%.

10. CONCLUSIONS

- *H. pylori* seropositivity prior to tumour development is associated with a higher risk of non-cardia gastric adenocarcinoma.
- There is no evidence for an association between *H. pylori* seropositivity and cardia gastric adenocarcinoma
- CagA seropositivity is associated with a higher risk of non-cardia gastric adenocarcinoma in subjects who are *H. pylori* seropositive.
- The magnitude of the association between *H. pylori* seropositivity and non-cardia gastric adenocarcinoma may depend on the serological test used. Western blot may be a better serological method in epidemiological studies.
- The size of our material did not allow for the estimation of the associations between *H. pylori* or CagA seropositivity and oesophageal adenocarcinoma. There is however, evidence of an inverse association in the literature.
- The size of our material did not allow for the estimation of the association between *H. pylori* or CagA seropositivity and oesophageal squamous cell carcinoma. *H. pylori* seropositivity did however have a tendency to lower the risk for oesophageal squamous cell carcinoma, but there is almost no literature evidence in this area.
- Clinical trials on gastric cancer prevention by *H. pylori* eradication or vaccination should monitor oesophageal adenocarcinoma and squamous cell carcinoma.
- Tobacco smoking is associated with a higher risk of gastric adenocarcinoma in both *H. pylori* seropositive and seronegative subjects.
- Tobacco smoking is associated with a higher risk of developing oesophageal malignancies.
- The Helicoblot 2.1 CagA band has a bimodal intensity distribution. The CagA band is predominantly of a high intensity in subjects who are *H. pylori* seropositive and predominantly of a low intensity in subjects who are *H. pylori* seronegative.
- The Helicoblot 2.1 CagA band has a low specificity in *H. pylori* seronegative subjects.
- The Helicoblot 2.1 CagA band with a low intensity in *H. pylori* seropositive subjects should be regarded as indeterminable.

11. POPULÄRVETENSKAPLIG SAMMANFATTNING

Helicobacter pylori är en av de vanligaste infektionerna hos människan. *H. pylori* framodlades för första gången 1983 av Robin Warren och Barry Marshall. Bakterien ger en kronisk inflammation i magslemhinnan. *H. pylori* orsakar magsår och möjligheten att behandla sjukdomen med antibiotika har inneburit en revolution inom sjukvården. Hos flertalet sker infektionen i barndomen och den blir ofta livslång. Tidigt uppkom misstanken att *H. pylori* även skulle kunna vara orsaken till magcancer. I den här avhandlingen studeras sambandet mellan *H. pylori* och cancer i magsäck och matstrupe.

Vi har studerat risken för att få magsäck- och matstrupscancer hos de personer som deltagit i Förebyggande Medicins hälsoundersökning under åren 1974 till 1992. Avdelningen för Förebyggande Medicin i Malmö genomförde då ett hälsoundersökningsprogram för sammanlagt 32 906 personer. Samtidigt sparades blodprov. Med hjälp av Cancerregistret har vi kunnat de hitta de personer som fått magsäcks- eller matstrupscancer och jämföra med personer som inte fått dessa cancerformer. Blodproven har analyserats serologiskt för antikroppar mot *H. pylori* och mot den mer sjukdomsalstrande *cag*A-stammen av *H. pylori*. För antikroppsanalyserna har vi använt dels laboratoriets egen ELISA-metod, dels en kommersiellt tillgänglig Western blot-metod, Helicoblot 2.1.

Magsäcken består anatomiskt av kardia, fundus, korpus, antrum och pylorus. Kardia är endast en liten del av magsäcken alldeles vid matstrupens mynning. Magsäckens syraproduktion sker i fundus och korpus som motsvarar två-tredjedelar av magsäcken. Fundus är den övre delen ovan matrupens inmynning, korpus ligger mellan fundus och antrum. Antrum är den nedre tredjedelen av magsäcken som övergår i tolvfingertarmen. Pylorus är den nedre magmunnen.

Våra studier har visat att *H. pylori* ger en ökad risk för cancer i magsäcken, med undantag för kardia. Risken i vårt material för icke-kardia cancer var förhöjd 17.8 ggr (95% konfidensintervall (k.i.): 4.2 - 74.8). Vi kunde däremot inte påvisa någon risk för magcancer belägen i kardia. Vi bekräftade att tobaksrökning ger en ökad risk för magcancer. Vi kunde visa att *H. pylori* ökade risken för icke-kardia magcancer hos både rökare och icke-rökare. Risken för icke-kardia magcancer var 25.2 ggr (95% k.i.: 3.3 - 190) högre hos rökare med *H. pylori* jämfört med icke-rökare utan tecken till infektion. Vi kunde visa att *H. pylori* med den mer sjukdomsalstrande *cag*A-stammen gav en ökad risk för icke-kardia magcancer jämfört med *H. pylori* utan *cag*A.

Vårt material tillät inte några statistiskt säkerställda slutsatser om sambanden mellan *H. pylori* och de två typerna av cancer i matstrupen, körtelcancer och skivepitelcancer. Det fanns dock en tendens till en minskad risk för skivepitelcancer i matstrupen hos personer med *H. pylori*.

Med Western blot separeras de antikroppsbildande proteinerna från *H. pylori* efter storlek. Helicoblot 2.1 är en kommersiellt tillgänglig sådan analysmetod. De mer sjukdomsalstrande *cag*A-stammarna av *H. pylori* bildar proteinet CagA, som syns som ett avgränsat band i Helicoblot 2.1 analysen. Vi har utvärderat CagA-bandet bland personer i Förebyggande Medicins hälsoundersökning som inte hade cancer i magsäck eller matstrupe. Vi fann att CagA-bandets intensitet var i huvudsak antingen hög eller låg. Personer med ett CagA-band men utan andra serologiska tecken på *H. pylori* infektion hade vanligen ett CagA-band med låg intensitet. Personer med andra serologiska tecken på *H. pylori* infektion hade vanligen ett CagA-band med hög intensitet men även personer med ett CagA-band av låg intensitet förekom. Genom att studera hur förekomsten av CagA-banden av hög och låg intensitet förändrades med personernas födelseår kunde vi sluta oss till att CagA-band med Helicoblot 2.1 metoden troligtvis är en falsk reaktion hos personer utan andra tecken till *H. pylori* infektion. Vidare är troligen CagA-band av låg intensitet hos personer med andra tecken till *H. pylori* infektion lika ofta en falsk som en sann reaktion på den sjukdomsalstrande *cag*A-stammen.

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