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RISK FACTORS AND PREVENTION OF ESOPHAGEAL CANCER

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Picture: Andreae Vesalii, *The Humani Corporis Fabrica Liberi Septem*, 1543. Kindly provided by Ove Hagelin at the Hagströmer Library in Stockholm, Sweden.

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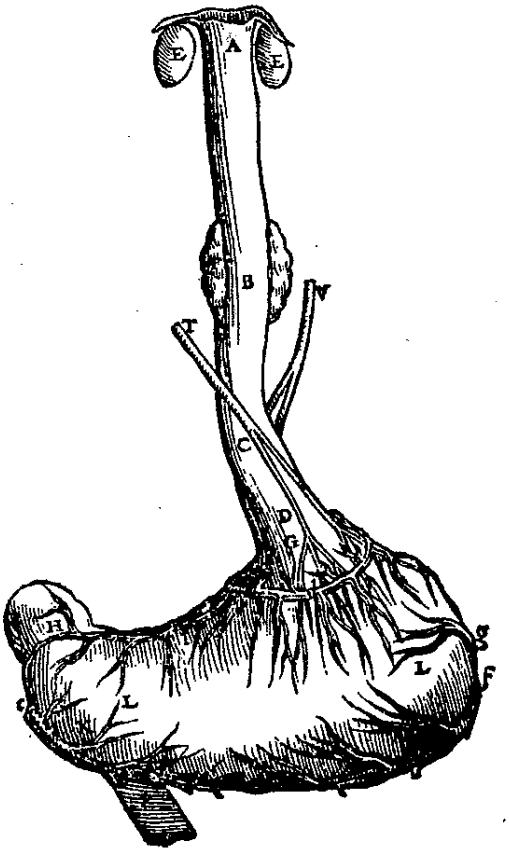

To my family

ABSTRACT

Esophageal cancer is the eighth most common cancer in the world, consisting of two major histological types: squamous cell carcinoma (dominant globally) and adenocarcinoma (rapidly increasing in incidence in the Western world during the last decades). Established risk factors for adenocarcinoma are gastroesophageal reflux symptoms, obesity and tobacco smoking, whereas squamous cell carcinoma is mainly associated with tobacco smoking and excessive alcohol intake. Esophageal cancer predominantly affects men; the gender difference in squamous cell carcinoma cases is entirely explained by the higher prevalence of risk factors in men, but the striking 7:1 sex ratio in adenocarcinoma remains unexplained. Esophageal cancer carries a very poor prognosis and despite efforts to improve survival the overall 5-year survival rate is still less than 10%, emphasizing the need for preventive factors. This thesis focuses on the etiology of esophageal cancer and the unexplained male predominance in esophageal adenocarcinoma.

The first paper investigates differences in risk factor profiles between women and men as a possible explanation for the male predominance in esophageal and cardiac adenocarcinoma. The paper was based on a nationwide population-based case-control study of all newly diagnosed cases of adenocarcinoma (n=451) and corresponding controls (n=816) in Sweden between 1994-1997. Contradictory to the hypothesis, the point odds ratios (OR) did not indicate any weaker association of the established risk factors reflux, obesity and tobacco smoking with risk of esophageal adenocarcinoma in women (4.6, 10.3, and 5.3, respectively) compared to men (3.4, 5.4, and 2.8, respectively). Protective factors such as a high intake of fruit and vegetables or infection with Helicobacter pylori showed no stronger protective effect in women. Thus, gender differences in the exposure to known risk factors do not seem to explain the male predominance in esophageal or cardia adenocarcinoma.

The second paper investigated if the higher incidence rate of esophageal adenocarcinoma in the United Kingdom compared to Sweden is explained by a higher population prevalence of established risk factors. Investigations were based on identical questionnaires filled out by a random sample of the English (n=3633) and Swedish (n=1483) populations. The prevalence of gastroesophageal reflux symptoms and obesity were significantly higher in the English population (OR 2.0, 95% CI 1.6-2.4 and OR 1.8, 95% CI 1.5-2.1), suggesting that the higher incidence of esophageal adenocarcinoma in the United Kingdom is at least partly due to the higher population prevalence of well-established risk factors.

The third paper investigates why surgical intervention of reflux does not provide protection against esophageal adenocarcinoma. All esophageal or cardia adenocarcinoma cases among antireflux operated patients in Sweden in 1965-2006 were identified and compared with matched controls from the same antireflux cohort. Recurrence of reflux after surgery was a risk factor for esophageal adenocarcinoma (OR 3.1, 95% CI 1.5-6.3), while BMI, tobacco smoking and type of antireflux surgery appeared to be of lesser importance. Recurrent reflux can explain the lack of a cancer protective effect of antireflux surgery and endoscopic surveillance might be an option for these patients.

The fourth paper investigates the association between infection with human papillomavirus (HPV) and tumor location in the esophagus. The hypothesis is based on an oral route of transmission and an association between HPV and oropharyngeal squamous cell carcinoma. Available tumor material from esophageal squamous cell carcinomas in the Stockholm County in 1999-2006 was collected and examined for presence of HPV using multiplex polymerase chain reaction (PCR) with Luminex. No increased occurrence of HPV DNA was observed in esophageal squamous cell carcinomas located in the proximal compared to a more distal part of the esophagus. The prevalence of HPV DNA (10%) was low, and the identified HPV did not seem to be biologically active, based on $p16^{INK4a}$ data.

LIST OF PUBLICATIONS

I. Löfdahl HE, Lu Y, Lagergren J.
Sex-specific risk factor profile in esophageal adenocarcinoma.
Br J Cancer 2008;99:1506-10.

II. Löfdahl HE, Lane A, Lu Y, Harvey RF, Lagergren P, Blazeby J, Lagergren J.
Increased population prevalence of reflux and obesity in the United Kingdom compared to Sweden: a potential explanation for the difference in incidence of esophageal adenocarainoma.
Eur J Gastroenterol Hepatol 2011;23:128-32.

III. Löfdahl HE, Lu Y, Lagergren P, Lagergren J.
Risk factors for oesophageal adenocarcinoma after antireflux surgery.
Manuscript submitted.

IV. Löfdahl HE, Du J, Näsman A, Andersson E, Rubio C, Lu Y, Ramqvist T, Dalianis T, Lagergren J, Dahlstrand H.
Infection with human papillomavirus (HPV) in relation to the site of squamous cell carcinoma of the esophagus
Manuscript submitted.

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LIST OF ABBREVIATIONS

BE	Barrett's Esophagus
BHP	Bristol Helicobacter Project
BMI	Body Mass Index
CagA	CytotoxinAssociated Gene A
CI	Confidence Intervals
CT	Computer Tomography
DNA	Deoxyribonucleic Acid
EBV	Epstein-Barr virus
GERD	Gastroesophageal Reflux Disease
H_2RA	Histamin-2-Receptor Antagonists
H. pylori	Helicobacter pylori
HR	High-Risk
HPV	Human Papillomavirus
IGF-1	Insulin-Like Growth Factor 1
LES	Lower Esophageal Sphincter
LCR	Long Control Region
LR	Low-Risk
MALT	Mucosa-Associated Lymphoid Tissue
NSAID	Non-Steroidal Anti-Inflammatory Drugs
OR	Odds Ratio
PET	Positron Emission Tomography
PCR	Polymerase Chain Reaction
pHR	Putative High-Risk
PPI	Proton Pump Inhibitors
RP	Reference Population study
SECC	Swedish Esophageal and Cardia Cancer study
TNM	Tumor-Node-Metastasis
UES	Upper Esophageal Sphincter
UK	United Kingdom
VacA	Vacuolating Cytotoxin Gene A
WHO	World Health Organization

INTRODUCTION

Esophageal cancer is the eighth most common cancer and the sixth most common cause of cancer mortality in the world.[1] There are two major histological types of esophageal cancer, squamous cell carcinoma and adenocarcinoma. Squamous cell carcinoma dominates globally,[2] however, the incidence of adenocarcinoma has increased rapidly during the last decades and now accounts for more than 50% of esophageal cancers in the Western world.[3 4] Established risk factors for esophageal adenocarcinoma are gastroesophageal reflux symptoms,[5] obesity[6 7] and tobacco smoking,[7] while squamous cell carcinoma is mainly associated with tobacco smoking and excessive alcohol intake.[8 9] Esophageal cancer predominantly affects men. The 3:1 male to female ratio in squamous cell carcinoma is explained by the difference in prevalence of risk factors between the genders,[10] although the striking 7:1 ratio in adenocarcinoma remains unexplained.[11]

Due to the elastic properties of the esophagus and the general aggressiveness of this type of cancer, tumors have usually proceeded to an advanced stage prior to diagnosis. More than 50% of patients have an unresectable tumor or distant metastasis at the time of diagnosis[12] and even though efforts have been made to improve treatment, the overall 5-year survival rate is still below 10%,[13] stressing the need for preventive factors.

This thesis, based on four original papers, focuses on etiological factors for esophageal cancer, including the male predominance in esophageal adenocarcinoma and aims at increasing the knowledge and reducing the incidence of esophageal cancer in the future.

BACKGROUND

The esophagus

Anatomy

The esophagus, a long flattened muscular tube and the first section of the gastrointestinal tract, begins at the pharyngoesophageal junction, approximately 18 cm from the incisors and is anatomically divided into three parts (Figure 1): the proximal- (≤ 23 cm from the incisors), the middle- (24-32 cm), and the distal esophagus (32-40 cm).[14] The esophagus can distend up to a couple of cm when food passes, although the cervical and thoracic sections of the esophagus are collapsed when in the resting state.[15]

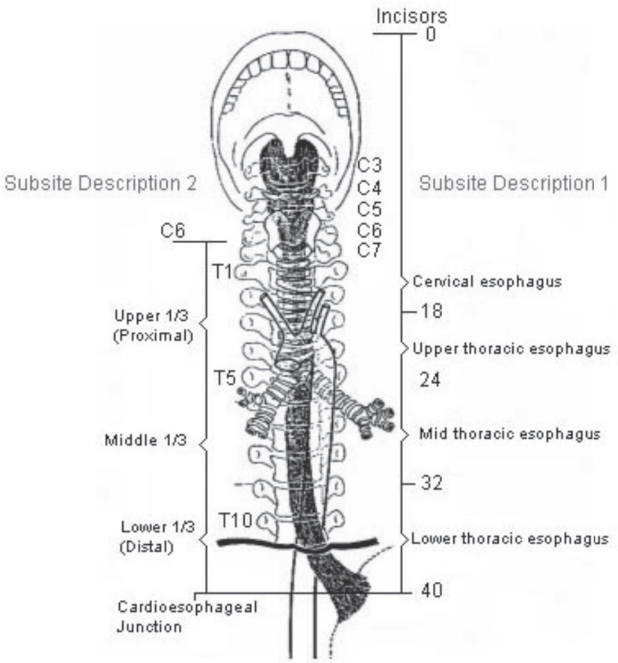

Figure 1: Location and anatomical parts of the esophagus. Reprinted in agreement with the fair use policy.

The esophagus is located within the thoracic cavity and passes anteriorly to the vertebrae, close to the trachea, pericardium and aorta, before it continues through the diaphragm and ends in the esophagogastric junction within the abdomen. The upper (UES) and lower esophageal sphincters (LES) create two high-pressure regions, limiting the esophagus and preventing gastroesophageal reflux. The LES consists of two muscle layers, an extrinsic (diaphragmatic component) and an intrinsic layer (esophageal muscle fibers). LES pressure increases or decreases in accordance with respiration and food intake.[15]

Histology

The esophageal wall consists of a thick *mucosa*, with non-keratinized squamous cell epithelium, *submucosa*, with submucosal glands and Meissner's nerve plexus, and *muscularis propria*, an outer and inner muscle layer contributing to esophageal motor functions and consisting of striated muscles in the cervical part and smooth muscles in the abdominal part of the esophagus. Within the thoracic part of the esophagus is the transition zone where no significant contraction occurs.[15] The esophagus lacks an outer layer, i.e. the serosa, and ends with the *adventitia*.

Clinical aspects

Esophageal anatomy and histology contribute to the high mortality rates in esophageal cancer. Important aspects are the esophageal ability to distend when food passes through, which enables tumors to be large before symptoms occur. In addition, the anatomic location of the esophagus results in rapid tumor spreading and difficulty in tumor extirpation. Consequently, more than 50% of patients have unresectable tumors or visible metastasis at the time of diagnosis.[12] Another aspect includes the thick mucosal layer and lack of a serosa layer which is clinical relevance in the event of surgical reconstruction, since the technique for anastomoses involves the strong mucosal layer, which is not necessarily present in other gastrointestinal organs.

Esophageal cancer

Histological types

There are two main histological types of esophageal cancer which together account for more than 90% of all esophageal cancers worldwide:[16] adenocarcinoma (a malignant neoplasm of epithelial origin which grows in a glandular pattern),[17] and squamous cell carcinoma (a malignant neoplasms of squamous cell epithelial origin).[17] Squamous cell carcinoma is dominant worldwide, responsible for more than 90% of all esophageal cancers in developing countries.[2] However, during recent decades the incidence of adenocarcinoma has risen rapidly in many industrialized populations, making adenocarcinoma responsible for approximately 50% of all esophageal tumors in the Western world.[4]

Pathogenesis

The pathogenesis of esophageal cancer is not fully understood, but it has been suggested that a series of genetic changes associated with chronic inflammation gives cells carcinogenic properties (resistance to growth inhibitory signals and apoptosis, autonomous cell proliferation, unlimited replication, angiogenesis, invasion, and metastasis), resulting in survival benefits for mutated cells which outcompetes normal cells.[18] Chronic inflammation is thought to be the causal factor of both adenocarcinoma and squamous cell carcinoma of the esophagus, although risk factors (described below) differ.[19]

More than 80% of esophageal adenocarcinomas[20] develop from Barrett's esophagus (BE),[21] defined as an endoscopic visible columnar lined epithelium in the tubular esophagus with biopsy-confirmed intestinal metaplasia.[22] However, tumors may also develop from submucosal glands or occasionally within the ectopic gastric epithelium located within the esophagus.[23] Squamous cell carcinoma develops from the normal non-keratinized squamous cell mucosa.

Tumor location and the esophagogastric junction

Tumor locations differ according to histological type: where adenocarcinoma is concerned, three quarters of the tumors are located in the distal esophagus, and in the case of squamous cell carcinoma, the tumors are more equally distributed between the middle and distal sections.[12 16] The less common cervical tumors are typically squamous cell carcinomas.[12 16]

Esophagogastric junction

The esophagogastric junction is an anatomic region located where the esophagus ends and the stomach starts identified clinically by means of endoscopy as "the upper margin of the longitudinal folds of the stomach".[24] Tumors of the esophagogastric junction are classified according to the often cited Siewert's definition from 1998 namely as tumors "that have their center within 5 cm proximal and distal of the esophagogastric junction" (Figure 2).

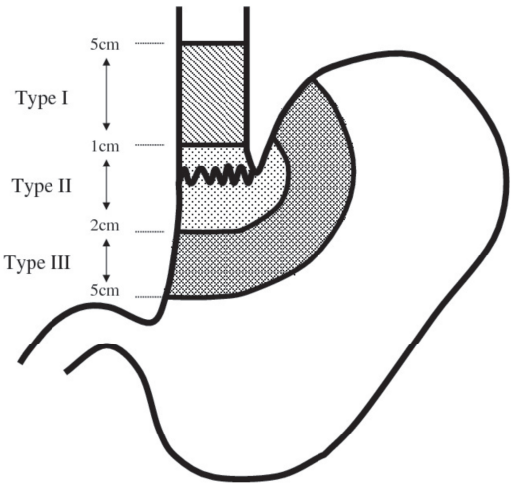

Figure 2: The Siewert's definition of the esophagogastric junction. Reprinted with permission from Springer Link.

Furthermore, tumors in the esophagogastric region are divided into type I if localized 1-5 cm proximal of the esophagogastric junction, type II if localized 1 cm proximal to 2 cm distal of the esophagogastric junction, and type III (also known as cardiac) if localized 2-5 centimeters distal of the esophagogastric junction.[20] There is a long and still ongoing controversy as to weather adenocarcinomas of the esophagogastric junction should be classified and treated as esophageal or gastric adenocarcinomas.[20 25] Tumors of the esophagogastric junction share many similarities with esophageal adenocarcinomas: both develop from the columnar cell epithelium of the gastroesophageal junction,[26] and both have a similar risk factor profile (gastroesophageal reflux symptoms[5 27] and obesity[28 29] increase the risk, whereas infection with Helicobacter pylori generates a protective effect (inverse compared to gastric cancer),[30] although the risk factors for esophagogastric adenocarcinoma are typically weaker than for esophageal adenocarcinoma). Age and sex are also equally distributed, with most tumors occurring in males over 65 years.[26 31] In this thesis, as a result of these similarities, esophagogastric adenocarcinomas were included in the investigations on etiology of esophageal adenocarcinoma in papers I and III. In paper I, the esophagogastric junction is defined as 2 centimeters proximal and 3 centimeters distal of the esophagogastric junction (this classification was used prior to Siewert's definition), whereas in paper III, the Siewert's definition is used.

Risk factors

Adenocarcinoma

Barrett's esophagus

BE is considered to be the premalignant condition for esophageal adenocarcinoma[32] and the opinion that the majority of esophageal adenocarcinoma cases arise from BE is well established.[21] BE develops from repeated mucosal injury and inflammation as a result of long-standing gastroesophageal reflux disease (GERD).[33 34] The true population prevalence of BE is difficult to establish due to the asymptomatic properties of the condition but population-based studies indicate a prevalence of 1.6-6.8%.[35] BE increases the risk of esophageal adenocarcinoma 30 to 60 times when compared to the general population.[36-38] However, only a minority of patients with BE develop esophageal adenocarcinoma with an incidence rate of between 1.2-6.3 cases per 1000 person-years and an annual risk of 0.1-0.6%.[37 38 39 40] Although BE increases the relative risk of esophageal adenocarcinoma greatly, the absolute risk of developing the disease is limited and the overall mortality in BE patients is similar to that of the general population.[41]

Gastroesophageal reflux symptoms, obesity and tobacco smoking

Gastroesophageal reflux symptoms and obesity are the two major risk factors for esophageal adenocarcinoma (further addressed below). Symptoms of gastroesophageal reflux increase the risk of esophageal adenocarcinoma 2 to 8 times [5 7 42] and the risk is further increased among patients with more severe (higher frequency/duration/severity) reflux symptoms.[5] Obesity, particularly of the abdominal type, increases the risk of esophageal adenocarcinoma between 2 and 4 times, seemingly independently of gastroesophageal reflux symptoms.[6] Tobacco smoking (addressed further below) is a moderate risk factor for esophageal adenocarcinoma and increases the risk by approximately 50%.[7 43] When gastroesophageal reflux symptoms and a high BMI are combined, the risk is increased more than expected suggesting an amplifying association, whereas no such effect has been observed when gastroesophageal reflux symptoms or obesity are combined with tobacco smoking.[7]

Other risk factors

High socioeconomic status, male gender,[44] Caucasian race [45] and achalasia[46] have also been reported to increase the risk of esophageal adenocarcinoma whereas infection with H. pylori,[47] low socioeconomic status[44] and dietary factors, such as the intake of antioxidants[48] and high intake of fruit and vegetables,[49] have shown to have a protective role against esophageal adenocarcinoma. Alcohol drinking is not considered as a risk or protective factor for esophageal adenocarcinoma, however, it has been indicated that a moderate intake of wine might decrease the risk.[50 51] Several studies support the hypothesis of aspirin and non-steroidal anti-inflammatory drugs (NSAIDs) reducing the risk of esophageal adenocarcinoma[52 53] although the results are partly conflicting. Supporting studies have shown that treatment with selective cyclooxygenase-2 (COX-2) inhibitors reduces tumor growth in vitro[54] and a large Australian study showed a 9 times reduced risk of esophageal adenocarcinoma in patients using aspirin/NSAIDs at least weekly,[55] however, other studies have shown no such preventive effect.[53 56] More research, including results from ongoing randomized clinical trials, are needed in order to clarify the role of aspirin/NSAIDs in esophageal adenocarcinoma.

Hereditary risk factors

Although familial clusters of both BE and esophageal adenocarcinoma have been reported,[57] family history of digestive cancers has not been found in population-based studies.[58 59] Dominating risk factors for esophageal adenocarcinoma are non-genetic, implying that genetic factors are of limited importance in the development of this type of cancer.

Squamous cell carcinoma

Tobacco and alcohol

Tobacco smoking and high alcohol intake are the two major risk factors for esophageal squamous cell carcinomas and responsible for more than 90% of all cases in industrialized countries.[8] The risk of cancer rises with increased quantity and duration of smoking,[8] and an intake of at least 50 grams of alcohol per day raises the risk of squamous cell carcinoma two to three times.[9] Furthermore, the risk of cancer further increases when smoking and alcohol are combined.[8 9] Genetic studies also indicate that populations with a deficiency in alcohol dehydrogenase, an enzyme catabolizing ethanol, have a greater risk of squamous cell carcinoma due to alcohol consumption, compared to populations with a normal active enzyme.[60] Smokeless tobacco, such as chewing of betel quid (a mixture of areca nut, tobacco and slaked lime, wrapped in a betel leaf) and snuff use, has also been suggested to increase the risk of esophageal squamous cell carcinoma although results are not conclusive.[61-63]

Other risk factors

Low socioeconomic status[12] and humans with a dark skinned phenotype [8 64] are other well-established risk factors for esophageal squamous cell carcinoma. Esophageal diseases, such as longstanding achalasia, may well contribute to increased risk.[65 66] Occupational exposure to metal dust such as chromium (hexavalent chromium),[67] cadmium and lead[68] also seems to increase the risk as does having a stressful occupation with high demand and low control (when measured with a demand-control model[69]). The role of infectious agents has also been investigated: Human papillomavirus (HPV) is thought to be a risk factor (further addressed below) although. H. pylori [70 71] and Epstein-Barr virus (EBV) have been ruled out as having any causative effect in esophageal squamous cell carcinoma.[72] Dietary factors contributing to esophageal squamous cell carcinoma include pickled food,[73] hot beverages especially tea[74] and manté,[75] and ingestion of lye or caustic fluids,[76] whereas a protective effect has been suggested from high intake of fruit and vegetables[49 77] and animal studies indicate that green tea might be protective, although epidemiological human studies are inconclusive.[78]

Hereditary risk factors

The etiological role of heredity in squamous cell carcinoma is not likely to be of major importance,[58] although a few families inherit the autosomal dominant skin disorder tylosis (non-epidermolytic palmoplantar keratoderma), a gene abnormality on chromosome 17q25 responsible for the development of esophageal cancer in 90% of patients by the age of 70,[79 80]

Incidence and mortality

Worldwide

Esophageal cancer is the eighth most common cancer and the sixth most common cause of cancer mortality in the world.[1] According to the GLOBACAN produced by the World Health Organization (WHO), more than 482,000 people were diagnosed with and 407,000 died from esophageal cancer in 2008 with the highest incidence and mortality rates found in developing countries (83 and 86% respectively).[1] The highest incidence of esophageal cancer worldwide is found in Zhengzhou, the capital of the Henan province in China, where 100 cases per 100,000 people are found in men and more than 50 in women.[1] High-risk regions, with incidence from 30 per 100,000 people and above, are also found in Iran, Central China, South Africa, Southern Brazil, Northern Italy and France (Normandy and Calvados).[81] Northern Europe and Northern America have, in general, a more moderate incidence rate (5.7 versus 8.3 cancer cases per 100,000 people) compared to Africa (26.8-34.5 cancer cases per 100,000 people) and South-Central Asia, including China (24.6 cancer cases per 100,000 people).[82]

The incidence of adenocarcinoma and squamous cell carcinoma varies greatly around the world. Since the rapid increase in incidence of adenocarcinoma during the last decades,[4] it now accounts for more than 50% of esophageal cancer cases in the Western world with the highest incidence found in the United Kingdom (7.0 cancer cases per 100,000 people).[45] Squamous cell carcinoma is, however, the dominating type in high-risk areas and accounts for more than 90% of esophageal cancer cases in developing countries.[2] Much of the variance in incidence between the two histological types is suggested to be due to ethnic differences, since the same incidence pattern of esophageal cancer is observed in an American study comparing humans with black (87% squamous cell carcinomas) with humans with white (55% adenocarcinomas) skinned phenotype in the United States.[3]

Incidence trends worldwide

The total worldwide incidence of esophageal cancer has been stable during the last few decades, however, the incidence of different histological types has changed greatly in the Western world. The incidence of esophageal adenocarcinoma has increased as much as a seven-fold (from 3.6 to 25.6 cancer cases per 100,000 people) during the last three decades in both North America[4] and Europe,[3 11 83] including Sweden.[84 85] The greatest increase was observed until the middle 1990s and, even though the increase has slowed, it still seems to be consistent.[4] At the same time, the incidence of squamous cell carcinoma has decreased making the overall incidence of esophageal cancer stable in Western populations.[44]

Due to misclassification of tumor location, little is known about the incidence of esophagogastric adenocarcinomas worldwide, however, the incidence seems to follow the rising trend of esophageal adenocarcinomas, albeit to a more moderate extent.[31 86 87]

Incidence in Sweden

There are approximately 440 (443 in 2009) new cases of esophageal cancer in Sweden every year.[88] Incidence rates are low compared to high-risk nations, 2.9 per 100,000 in men and 0.9 per 100,000 in women.[88] The incidence trend of esophageal cancer is similar to that in other Western countries with a rapid increase in the incidence of esophageal adenocarcinoma during the last decades.[84 85 89] The incidence is also continuing to rise although at a much slower pace, and a similar pattern is observed in gastric cardia adenocarcinoma.[89] The incidence of esophageal squamous cell carcinoma was fairly stable until the beginning of the 1990s where it has thereafter decreased,[84 85 89] resulting in an overall stable incidence of esophageal cancer in Sweden during the last decades.[85]

Sex ratio

Esophageal cancer predominantly affects men, with a male to female ratio of 1.1-3:1 for squamous cell carcinoma[11 84 90] and 3-10:1 for adenocarcinoma.[11 20 44 84 90] In Europe, the United Kingdom (UK) has a male to female ratio of 5-5.5:1 for esophageal adenocarcinoma[11 44] compared to 4:1 in Sweden.[84] Differences in gender ratio for squamous cell carcinoma are completely explained by the difference in prevalence of risk factor exposure from tobacco smoking and alcohol.[10] The male predominance in esophageal adenocarcinoma is, however, unexplained. Higher production and concentration of gastric acid,[91] higher prevalence of hiatus hernia, abnormal 24-hour pH test and defective LES in patients with symptomatic gastroesophageal reflux,[92] abdominal/android obesity[93] resulting in higher intra-abdominal and intra-gastric pressure are all more common in men and have been suggested to explain male predominance, although no conclusive results have been presented.[94] Estrogen exposure may not be a causative factor.[95 96]

Symptoms

Dysphagia and *weight loss* are the two most frequent symptoms in patients with esophageal cancer and are reported by 74% and 57% of the patients respectively.[16] Dysphagia usually occurs when the lumen diameter is less than 13 mm,[97] and weight loss is secondary to cancer cachexia, dysphagia *per se* and change in diet due to dysphagia.[97] Less frequently reported symptoms are *odynophagia* (pain when swallowing food) reflecting advanced local invasion and is reported in 17% of patients,[16] *dyspnea* due to pleural effusion, *hoarseness* and *coughing* reflecting tumor overgrowth of the laryngeal nerve, and *lymphadenopathy* reflecting metastatic disease.[12]

Due to the elastic nature of the esophagus, symptoms usually occur at a later stage and more than 50% of patients have unresectable tumors or visible metastasis at the time of diagnosis.[12] Currently, there are no effective methods for detecting esophageal cancer at an early stage.[19] Surveillance of patients with BE has been suggested, yet since the incidence of cancer is also low in these patients (0.1-0.6%),[37 38 39 40] it is difficult to identify feasible and cost effective measures of surveillance. Screening has also been suggested for patients with a profile of severe GERD and obesity, although the high prevalence of individuals with these known risk factors combined with the low incidence of esophageal cancer makes it unrealistic to implement.[98]

Diagnosis

An esophageal cancer diagnosis is usually confirmed by endoscopic visualizing of tumor mass and histological verification with biopsies. Additional investigation techniques at specialized centers include endoscopic ultrasound which accurately determines both local and regional tumor borders,[99] and a combination of computer tomography (CT) with positron emission tomography (PET) which is the best way to determine tumor spreading.[100] Tumors are staged according to the tumor-node-metastasis (TNM) classification which is based on tumor depth of wall invasion, (T0-T4), occurrence of regional lymph nodes (N0-N1) and occurrence of distant metastasis (M0-M1).[101]
Patient fitness is measured taking into account comorbidities, biological age, physical activity and, in some cases, spirometry and treadmill results.[2]

Treatment

Treatment can either be curative or palliative and is individually tailored, ideally based on a multidisciplinary team decision that is arrived at using the summary of diagnostic findings and patient fitness.[2]

Curative treatment

Curative treatment is based on surgical resection with or without neoadjuvant chemotherapy or chemoradiotherapy.[2] The preoperative treatment has been investigated in several studies, although underpowered and no treatment has been clearly superior to the others.[2] Currently the typical treatment used for the most common tumor stages considered for surgery (stage II-III) is chemotherapy followed by esophagectomy.[102]
Although surgery is the curative treatment of choice, data from case series have also indicated that curative results can be achieved with chemoradiotherapy alone, especially in older patients that are deemed not suitable for surgery.[103] The effects of such treatment and preoperative oncologic treatment can be evaluated using the combined PET and CT technique.[104]

Palliative treatment

Most patients (75%) with esophageal cancer have an advanced tumor stage or poor physical condition making them unsuitable for curative treatment.[102] The palliative treatment focuses on relieving dysphagia (preferably through self-expanding metallic stents or intraluminal brachytherapy[105]) pain therapy and feeding. Consistent for all palliative care, is that it should be performed by experienced staff in order to meet patient needs in the best possible way.[2]

Prognosis

Patients are often diagnosed at an advanced stage resulting in an overall 5-year survival rate of less than 10% in Europe.[13] Young patients with less advanced tumor stage have a better chance of surviving (20% below 50 years,[85] and 95% of patients with carcinoma in situ with no metastasis or lymphatic nodes involved survive for at least 5 years).[12] However, in reality most patients are older and diagnosed with an advanced tumor resulting in very poor chance of survival. Patients treated with palliative care have only a median survival of less than a year.[12] Weight loss more than 10% is an independent indicator for poor prognosis.[12] New data, not yet published from our group, indicates that the overall 5-year relative (disease-specific) survival in Sweden between 2000 and 2009 has improved to 15% in both esophageal and gastric cardia adenocarcinoma whereas the 5-year survival of squamous cell carcinoma remains at 10%. Tumor recurrence most often occurs within 1-year postoperatively giving 3-year survivors virtually the same prognosis as the overall population.[106]

Risk factors addressed in this thesis

Adenocarcinoma

Gastroesophageal reflux disease

Definition

GERD is defined according to the Montreal Definition and Classification of GERD as "a condition that develops when reflux of the stomach contents causes troublesome symptoms and/or complications".[107] Symptoms are defined as "heartburn or regurgitation" and troublesome is defined as "when symptoms adversely affect an individual's well being". In population-based studies the definition "mild symptoms occurring 2 or more days a week" should be used and in clinical settings the definition "patient should determine if their reflux symptoms are troublesome" should be used.[107] Esophageal complications are defined as "reflux esophagitis, hemorrhage, stricture, Barrett's esophagus and adenocarcinoma" and the Montreal definition also states that "long segment Barrett's esophagus with intestinal metaplasia is the most important identified risk factor for esophageal adenocarcinoma".[107]
The concept of GERD is very wide and it took a long time before researchers around the world agreed on a joint definition. The Montreal definition of GERD was not completed and agreed upon until 2006 making it hard to compare and compile results from prior studies, because of the different definitions used. Further complicating matters, population-based studies show that not all patients with symptomatic gastroesophageal reflux have complications[108] such as esophagitis and that not all patients with complications have gastroesophageal reflux symptoms,[108] making it even harder to study GERD because ethical considerations limit the use of large population-based endoscopic studies.
In this thesis, the term gastroesophageal reflux symptoms will be used instead of GERD in papers I-III, in response to the Montreal definitions and the design of questionnaires.

Pathogenesis

General

In a healthy individual, a physiological antireflux barrier prevents the reflux of gastric acid into the esophagus by three main mechanisms: i) the *static antireflux barrier*, created by the intrinsic part of the LES keeping a normal resting pressure (15-35 mmHg),[109] ii) the *dynamic antireflux barrier*, created by the extrinsic part of the LES/the crural diaphragm where pressure varies with breathing and iii) the *His angle* or the flap valve mechanism formed by the sharp angle between the distal esophagus and the gastric cardia within the stomach.[109] If the pressure of the inner LES is lost, reflux can still be prevented through maintained crural diaphragmatic contractions.[109]

Reflux episodes can occur through three different mechanisms:[110]

- Transient LES relaxation - simultaneous relaxation of both the static and dynamic antireflux barrier unrelated to food intake.[109]
- Transient increase of abdominal pressure.
- Spontaneous free reflux, associated with a constantly low LES pressure. No apparent decrease of LES pressure or increase of abdominal pressure occurs, although gastric reflux is developed.

Patients with esophagitis are reported to experience all three mechanisms whereas transient LES relaxation only occurs in healthy controls.[110]

Hiatus hernia

Patients with hiatus hernia, a condition in which parts of the stomach are proximally displaced above the diaphragm, are reported to have more severe gastroesophageal reflux symptoms compared to other patients.[111 112] Suggested mechanisms for increased severity include:[109 112 113]

- Impaired or prolonged acid removal from the esophagus.
- Acid, trapped in the hernia sac, is forced backwards into the esophagus when the crural diaphragm contracts.
- The diaphragm might be largely widened which will prevent the crural diaphragm from functioning properly.
- Absence of His angle, defined above.

Other factors

Obesity, especially abdominal, is known to increase gastroesophageal reflux symptoms by means of increased abdominal pressure.[114] Delayed gastric emptying, increased gastric acid secretion, delayed esophageal acid clearance and decreased tissue resistance are also important factors contributing to gastroesophageal reflux.[111] The intake of anticholinergic medications has also been suggested to increase GERD through the relaxation of LES but results are conflicting and inconclusive.[115 116]

Incidence and prevalence

The prevalence of GERD differs in different parts of the world with the highest figures in the Western world where 10-20% of the population is affected.[117 118] Population-based studies from the United States have also reported a prevalence of 10-20%, with a higher prevalence in humans with black skinned phenotype compared to those with white skinned phenotype.[117] Similar results from Europe show a prevalence of 10-18% in the UK (although as much as 41% of the population reports that they have had gastroesophageal reflux symptoms within the last six months),[119 120] 17% in Sweden, 15% in Finland and 10% in Spain.[117] GERD is also a growing problem in children and a recent report from the UK showed a high prevalence (8%) of gastroesophageal reflux symptoms, particularly in girls aged 16-17 years,[121] although symptoms do not seem to increase with age.[122]

Treatment of GERD

Medical

Histamin-2-receptor antagonists (H_2RA) and proton pump inhibitors (PPI) have, during the last few decades, been the two dominant medical treatments for gastroesophageal reflux symptoms. H_2RAs block the H_2-receptors and PPIs block the proton pumps ($H^+ K^+$ ATPase) in the parietal cells of the gastric mucosa, preventing acid release.[123] PPI has advantages over H_2RA including better symptom relief (in 80 versus 60% of patients)[124] and mucosal healing (in 78 versus 50% of patients)[125] and should be considered the "drug of choice" for gastroesophageal reflux symptoms. PPI also has relatively few short- and long-term side effects, although it should not be forgotten that PPI has been connected with an increased risk of osteoporosis,[126] pneumonia,[127] infection with Clostridium difficile[128] and interaction with Clopidogrel.[129] Medical treatment is effective in most patients although, for unknown reasons, some patients do not respond to medical treatment of GERD and other treatment methods, such as surgery, are required.

Surgical

Indications for surgical treatment are persistent clinical symptoms despite adequate medical treatment in patients with endoscopically confirmed mucosal damage due to gastroesophageal reflux or an unwillingness for lifelong medical treatment of GERD symptoms.[130 131]
The surgical technique used is almost exclusively fundoplication; the most common procedures are Nissen (posterior 360°) or the Toupét (posterior partial). Both of these fundoplication techniques are initiated with the closure of the diaphragm crura and more or less extensive mobilization of the gastric fundus which is then later pulled behind the esophagus and attached. The two techniques differ in the attachment of the gastric fundus. The Nissen fundoplication attaches the gastric fundus to "itself" creating a 360° fundic wrap around the distal esophagus.[132 133] The Toupét fundoplication attaches the gastric fundus to three different locations; the right diaphragm crus and both lateral parts of the esophagus thus creating a partial loop around the posterior esophagus.[133 134]
Studies report that more than 90%[131] of all patients are free from gastroesophageal reflux symptoms after surgery, however, side effects including dysphagia, gas, bloating, inability to belch or vomit and the recurrence of gastroesophageal reflux symptoms develop in some patients.[130 131]
Several studies have tried to conclude which surgical antireflux technique is superior to the other but there is no proven notable difference reported in gastroesophageal reflux prevention.[135] However, the incidence of side effects differs in favor of Toupét fundoplication.[133 136] Laparoscopy and laparotomy approaches[137-139] have also been compared although the results are inconclusive, indicating that the surgical result might depend more on patient selection, surgical skills and hospital methods, than approach.[140]

Two large trials conducted in Sweden have compared the outcome of patients treated with medication or antireflux surgery. Both show slightly better reflux control in the surgical group, although the incidence of side effects was also higher in this group.[141 142]

Gastroesophageal reflux and esophageal adenocarcinoma

Symptomatic gastroesophageal reflux was established as the strongest risk factor for esophageal (and gastric cardia) adenocarcinoma in 1999.[5] A Swedish population-based case-control study demonstrated an eight–fold increased risk of esophageal adenocarcinoma in patients with heartburn, regurgitation or both, occurring at least once a week. Furthermore, the risk increased with frequency, severity and duration of symptoms and, for longstanding and severe gastroesophageal reflux symptoms, the odds ratio for esophageal adenocarcinoma was 43.5.[5] Since 1999, several large and population-based studies[7 27 143] have confirmed these findings, showing a dose-response association between gastroesophageal reflux symptoms and esophageal (and gastric cardia) adenocarcinoma, where the risk of cancer increases with symptom severity.[144]

Antireflux treatment and esophageal adenocarcinoma

The association between gastroesophageal reflux symptoms and esophageal adenocarcinoma implies that the risk of cancer should be reduced with the medical or surgical treatment of such symptoms.[145] However, no such effect has been shown and current guidelines state that antireflux surgery should not be performed as a protective measure against cancer development.[27 146-149] Recurrent reflux after surgery has been suggested as an explanation for the failed protective effect of antireflux surgery, although results were inconclusive.[150] Some studies even indicate that failed antireflux surgery can further increase the risk of esophageal adenocarcinoma. [151] The medical treatment of gastroesophageal symptoms also lacks a protective effect for esophageal and cardia adenocarcinoma and studies have shown that patients with mild rather than severe symptoms treated with PPIs have a higher risk of developing both BE and esophageal adenocarcinoma.[34 152]

Overweight and obesity

General

During recent decades the Western world has experienced an "obesity epidemic" where the number of obese people has doubled since 1980.[153] In 2008, 1.5 billion people were considered overweight, 500 million were obese and the number will continue to rise.[153] The prevalence of obesity in Sweden is lower (10%) than in many other countries[154] whereas the UK has a much higher prevalence (23%).[155]
Obesity is associated with increased risk of several malignant tumors (thyroid, breast, colon, kidney, liver, gall bladder, pancreas and endometrium)[156] [157] and the association between obesity and esophageal adenocarcinoma seems to be one of the strongest, especially in men.[157] It has been suggested that the rapid increase in incidence of esophageal adenocarcinoma might be explained by the similarly rapid increase of obesity in the Western world.

Measurement

Several methods have been developed to measure overweight and obesity. Body mass index (BMI), a rather rough measurement often used in epidemiologic studies, is calculated by dividing the individual's body weight in kilograms by their height in meters squared. BMI is defined according to the WHO as underweight (<18.5), normal weight (18.5-24.9), overweight (25.0-29.9) and obesity (≥30).[153] Although BMI has many advantages, it lacks the ability to describe body composition and fat distribution. When interested in these dimensions, anthropometric measurements (waist circumference and waist hip ratio), radiologic techniques (CT scan measuring the intra-abdominal fat), biometrical impedance method (measuring fat free body mass)[158] or dual-energy X-ray absorptiometry (providing an overall body composition)[159] can be employed.

Pathogenesis

The molecular mechanism of the interaction between obesity and esophageal adenocarcinoma is not quite clear. Several studies have tried to investigate the relation between obesity and different types of cancer, including esophageal adenocarcinoma, providing a few conclusions, in brief:

- The combination of polymorphisms in the gene of insulin-like growth factor 1 (IGF-1) receptor together with obesity has been suggested to increase the risk of esophageal adenocarcinoma. A Canadian case-control study found that patients who were obese and carried the 1013G>A gene variant had a greater risk of cancer when compared with those who carried the 1013G>G gene.[160]

- Leptin, a hormone secreted from adipose tissue and increasing with rising BMI, has generally been suggested to activate epidermal growth receptors, stimulate cell proliferation and inhibit apoptosis.[161] In addition, an upregulation of receptors for leptin and adiponectin (another adipocytokine) has been observed in patients with an advanced tumor stage thus suggesting the involvement of these factors in tumor biology.[162] However, an Australian case-control study found no correlation between single nucleotide polymorphisms in the leptin receptor and esophageal adenocarcinoma,[163] and another study found an inverse relation between tumor growth and adiponectin in animals,[164] suggesting that the role of obesity in esophageal adenocarcinoma development needs further investigation.

Obesity and esophageal adenocarcinoma

The role of obesity as a risk factor for esophageal adenocarcinoma was introduced in three large population-based case-control studies in the 1990s [28 29 165] and was, during the last decade, confirmed in three large cohort studies.[166-168] Individuals with a BMI above 30 have a two- to four-fold increased risk of developing esophageal adenocarcinoma compared with individuals with a BMI below 25[166-168] and one study showed that a higher BMI, even within normal range (below 25), is correlated with an increased risk of cancer.[168]

The association between obesity and esophageal adenocarcinoma could be readily explained by the mechanism that obesity increases the intra-abdominal pressure and, thereby, the frequency of gastroesophageal reflux. However, all studies assessing gastroesophageal reflux symptoms and obesity together show an independent association[7 28 29 43 93] and individuals with both obesity and symptomatic gastroesophageal reflux have a considerably increased risk of cancer consistent with a synergetic interaction.[7] Although it has been suggested that asymptomatic gastroesophageal reflux might be more common in obese individuals than in individuals with normal weight,[169 170] obesity seems to be an independent risk factor for esophageal adenocarcinoma.[6]
When further investigating obesity, it has been discovered that increasing abdominal diameter, after adjustment for BMI, is independently associated with an increased risk of esophageal adenocarcinoma.[93] These results are striking and might contribute to the explanation of male predominance in esophageal adenocarcinoma,[89 171] since the predominantly abdominal fat distribution is typically found in men.

Tobacco smoking

Tobacco smoking is one of the main risk factors for esophageal squamous cell carcinoma,[8] however, it is considered to be only a moderate risk factor for esophageal adenocarcinoma and relatively few studies have addressed this issue.[32] Four large population-based studies have examined the relationship between esophageal adenocarcinoma and tobacco smoking with odds ratios varying from 1.4 to 2.2[7 43 172] and an increased risk was observed when combining tobacco smoking with other risk factors such as obesity.[7] Interestingly, the increased risk of adenocarcinoma when exposed to tobacco smoking is similar for esophageal and cardia adenocarcinoma (1.5 versus 1.4 and 2.2 versus 2.6).[43 172]

Helicobacter pylori

General

Helicobacter pylori (H. pylori) is a spiral-shaped, gram-negative bacterium with a polar flagella which colonizes the surface of the human gastric mucosa by breaking down gastric acid to ammonia and carbon dioxide.[173] This causes gastroduodenal inflammation which might then develop into atrophic gastritis leading to complications of vitamin B12 or iron deficiency anemia, gastric or duodenal ulcer, gastric mucosa-associated lymphoid tissue (MALT) or non-cardia gastric adenocarcinoma.[174 175] Infection with H. pylori is the strongest known risk factor for peptic (60-80% of patients are infected) and duodenum ulcer (95% of patients are infected).[176] Although H. pylori infection seems harmful, studies suggest that the bacterium has coexisted with humans since the beginning of humanity[177] indicating that there must be some advantages associated with H. pylori.

The prevalence of H. pylori infected individuals is decreasing rapidly[178] with the presence of the bacterium in only 60% of the world population.[179] The prevalence of carriers varies throughout the world and Western societies tend to have a lower prevalence than developing countries.[179] The main route of transmission is not established although infections occur mostly before the age of 10 years.[180]

Detection of H. pylori is performed through direct or indirect methods. Direct methods include endoscopy with collection of biopsy material and the disease is confirmed by means of histological examination, either directly or after growing the bacteria in culture. Indirect methods or noninvasive methods include the urea breath test and serology. The serologic test is especially useful in epidemiologic studies because of its low cost and the tests ability to detect past and present colonization.[71]

Pathogenesis

H. pylori contains two major virulent factors: vacuolating cytotoxin gene A (VacA) and cytotoxin associated gene A (CagA). VacA is a cytotoxin with several effects, including an ability to form a vacuolization in epithelial cells by forming a pore in late endosomal vesicles of the cell membrane. The pore has chloride channel activity and alternates the composition of anions within the endosome thus creating an osmotic swelling.[173] VacA can also cause host cells to activate apoptosis after pore formation in mitochondrial membranes.[181] VacA has a signal sequence (*s*) which can vary and strains of H. pylori with VacA of the s1 type are more highly associated with both ulcers and gastric cancer.[182] The CagA protein was initially discovered as a marker for H. pylori infection, as it was discovered that individuals expressing antibodies against CagA tend to have a higher incidence of peptic ulcer and gastric adenocarcinoma.[183 184] Cellular enzymes known to activate CagA are involved in carcinogenesis and CagA can stimulate the activation of growth factor receptors which then affects the structure and differentiation of epithelial cells.[185]

Helicobacter pylori and esophageal adenocarcinoma

During the last decade, a number of studies have investigated the relation between H. pylori and the risk of esophageal adenocarcinoma. Results are mainly consistent and show that individuals colonized with H. pylori, particularly those positive for the virulent CagA strain,[71] have a decreased risk of esophageal adenocarcinoma (OR 0.41, 95% CI 0.28-0.62).[47] The suggested mechanism is that H. pylori reduces the production of gastric acid resulting in reduced gastroesophageal reflux[30 186] and is further supported by the evidence that incidence of esophageal adenocarcinoma is low in parts of the world where the prevalence of H. pylori is high and vice versa.

Fruit and vegetables

Mechanism

The theoretical mechanism for high intake of fruit and vegetables as a protective factor for esophageal adenocarcinoma is based on the belief that free radicals, known to promote carcinogenesis, are produced when chronic gastroesophageal reflux causes epithelial damage.[187 188] Fruit and vegetables are the major dietary source of antioxidants[189] and are thought to bind and incapacitate the reactive oxygen spices resulting in a decreased risk of carcinogenesis.[188]

Intake of fruit and vegetables and esophageal adenocarcinoma

Several studies have indicated that a high intake of fruit and vegetables has an inverse effect on the development of both esophageal and cardia adenocarcinoma[49 190-192] although it is difficult to study dietary factors due to the wide range of fruit and vegetables, recall bias and no universal measurements. Historically, retrospective case-control studies have shown a greater protective effect than cohort studies thus suggesting that recall and selection bias are important.[190]

Recently published population-based studies showed a protective effect of high compared to low intake of fruit and vegetables for esophageal (OR 0.43, 95% CI 0.26-0.71) and cardia (OR 0.63, 95% CI .39-1.01) adenocarcinomas, although the latter was not statistically significant.[192] Furthermore, when dividing exposure into subgroups, the intake of raw vegetables reduced the risk of esophageal adenocarcinoma (OR 0.81, 95% CI 0.68-0.98) whereas brassica vegetables reduced the risk of cardia adenocarcinoma (OR 0.72, 95% CI 0.54-0-95)[191] and citrus fruits were found to be protective for both esophageal and cardia adenocarcinoma (OR 0.55 versus 0.38) with an increased effect particularly pronounced among smokers.[191]

Squamous cell carcinoma

Human papillomavirus

History

Human papillomavirus (HPV) is an enveloped closed-circular double-stranded DNA virus[193] and the main cause of cervical cancer.[194] [195] The carcinogenetic effect of the virus was first proposed in 1842 when observations showed that prostitutes had a high incidence of cervical cancer and that nuns had a complete absence of this type of cancer. [196] However, it was not until 1983, more than a century of animal and human studies later, that HPV types 16 and 18 were isolated from cervical cancer tumors and classified as human carcinogens by the International Agency for Research on Cancer in 1995.[197]

General

HPV is part of the papovaviridae family which consists, as a group of papillomaviruses and polyomaviruses.[198] Papillomaviruses infect a wide range of vertebrates but are highly species specific; they are named after the species they infect (e.g. human papillomavirus) and numbered in order of discovery. Papillomaviruses have a specific cellular tropism for squamous epithelia, and are associated with various benign lesions (warts and papillomas) as well as several invasive cancers (cervical, vulva, vagina, and oropharynx). More than 100 HPV types have been identified and categorized according to carcinogenic abilities in cervical cancer as: high-risk (HR), putative high-risk (pHR), and low-risk (LR) types (Table 1).[199]

Table 1. Classification of HPV types according to carcinogenic abilities.[199]

High risk	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82
Putative HR	26, 53, 66
Low risk	6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81

Viral particle

The viral genome consists of approximately 8,000 base pairs organized into three regions: the non-coding long control region (LCR), controlling viral transcription and the two open frame regions (ORF) consisting of the early (E) region, coding for regulatory proteins, and the late (L) region, coding for structural proteins. Below follows a brief description of all the proteins.

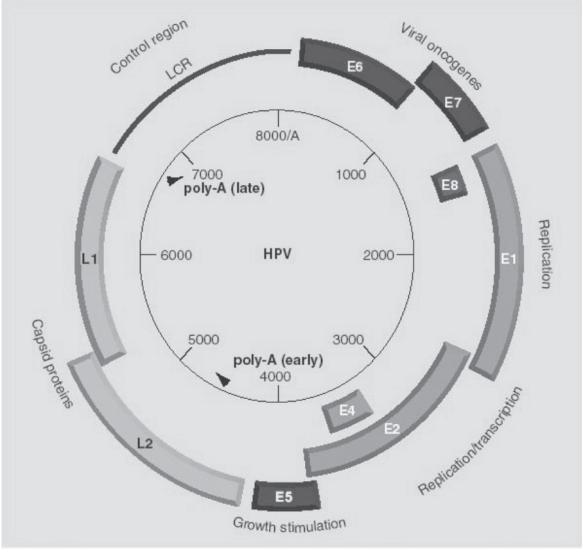

Figure 3: The organization of HPV genome. Reprinted with permission

E1 and E2 proteins are transcription factors, essential for HPV replication,[200] by forming a complex and bind to the viral origin of replication located on the LCR.[201] E1 and E2 also recruit host polymerase for viral gene transcription [202] and E2 regulates and represses the transcription of early genes by preventing host factors from binding to viral promoters.[203] E2 is often deleted in advanced cervical cancer, where the transcription of E6 and E7 proteins is deregulated.[204]

E4 protein is produced from splicing mRNA of E1.[193] The role of E4 is not yet fully understood. Speculations suggest that it has a role to create favorable conditions for viral maturation in the productive HPV infection.[193] Research has shown that E4 is common in warts, e.g. infection with HPV 1[193] and that the cytoskeleton in cell cultures can collapse due to interaction with E4 from HPV 16.[205]

E5 protein is usually absent in invasive cervical cancer but present in low-grade epithelial neoplasia, supporting the speculation of E5 playing a role early in HPV infection. The protein also seems to enrich the mitogen activity-protein pathway (MAP) resulting in an increased response to growth stimulating factors.[206]

E6 protein is one of the two oncoproteins present in the HPV particle. It consists of 160 amino acids[207] and the overexpression of E6 has been shown to facilitate oncogenesis by interfering with the host cell in several ways, briefly:

- E6 binds and degrades p53[208] inhibiting growth arrest and apoptosis after DNA damage[209] and resulting in cell proliferation and tumor development.[210]
- E6 can also, independently from p53, prevent apoptosis and contribute to cell proliferation by interacting with the pro-apoptotic bax protein and tumor necrosis factor 1 (TNFR1).[211]
- E6 immortalizes the host cell by activating transcription of human telomerase reverse transcriptase (hTERT).[212]
- E6 contributes to cellular transformation by degrading cellular proteins containing a so-called PDZ domain[213] involved in the formation of cell-to-cell adherence, cellular polarity and cell signaling. By losing these abilities, the cell transforms and becomes carcinogenic.[214 215] E6 can also contribute to anchor-independent and invasive tumor growth by degrading the PDZ domain protein called PTPN13.[216] The ability to bind proteins containing the PDZ domain is exclusive to HR HPV; LR HPV does not have the PDZ-binding motif.[217]
- E6 has also been suggested to reduce immune response by down regulation transcription of inerleukin-8[218] and modulate G protein signaling.[219]

E7 protein is the second of the two oncogenes present in the HPV particle. It consists of 100 amino acids[207] and overexpression of E7 has been shown to facilitate oncogenesis by interfering with the host cell in several ways, briefly:

- E7 induces enhanced cellular proliferation in several ways, the most famous being by interaction with retinoblastoma protein (pRb).[220] This process also contributes to overexpression of p16 through loss of feedback inhibition. P16 is considered to be a surrogate biomarker for transforming HPV infection, is correlated to degree of dysplasia[221] and found in almost all cervical cancers.[222] Overexpression of p16 is mainly seen in HR and pHR HPV types 16, 18, 31, 33, 52 and 58.[223]
- E7 enables autocrine growth in HPV positive lung tumors by upregulating expression of interleukin 6 (IL-6).[224]
- E7 contributes to the inactivation of the immune system by interacting with interferon regulatory protein 1 (IRP1) and interferon-α (IFN-α).[225]
- E7 can induce anchoring-independent cell growth by targeting the p600 protein, a member of the pRb family.[226]

L1 and L2 proteins are structural proteins forming the viral icosahedral capsid.[227] The open frame region (OFR) of the L1 gene is also used to define different HPV types. The gene sequence of the ORF has to differ at least 10% for it to be called a new subtype of HPV.[198]

HPV detection methods

Traditionally, direct probe assay (Southern blot) has been used as a "golden standard" for detecting a specific DNA sequence, i.e. HPV DNA, but due to significant time consumption, the need for large DNA samples, and the lack of advantages compared to more modern methods,[228 229] the most frequently used assays for HPV detection today are signal amplification Hybrid Capsid 2 (HC2) and polymerase chain reaction (PCR).[230]

Hybrid capture 2

Hybrid capture 2 (HC2) is a signal amplification method based on type-specific RNA probes that hybridize to a distinct DNA region. The produced RNA-DNA hybrid attaches to a micro plate coated with monoclonal antibodies and then another monoclonal antibody conjugated to alkaline phosphatase will attach to the complex. Detection of HPV occurs when chemiluminescent substrate is spliced by alkaline phosphatase, producing light measured in a luminometer as relative light units (RLU).
Sensitivity of Hybrid Capture 2 is lower than for PCR (95.6 versus 100%),[231] and HPV is detected when 500-5,000 virus genomes are present in one cell.[232]

Polymerase Chain reaction (PCR)

The PCR assay is based on amplification of a specific DNA sequence identified through primers, i.e. a chain of complementary oligonucleotides binding to the DNA sequence of interest. For detection of a wide range of HPV types, primers target the OFR of the L1 gene, a highly conserved region common in most HPV types. There are several different HPV primers; in this thesis the highly evaluated broad-spectrum BGP5+ and BGP6+ primers were used.[233]
The PCR procedure is repeated in approximately 45 cycles and starts with denaturing DNA (94°C); primers will attach in the annealing stage (40°C) and the specific DNA sequence is transcribed using the Taq polymerase (72°C).
Various methods are used for HPV genotyping, where sequencing using type specific PCR and multiplex with Luminex are two methods that are highly validated.[234] In this thesis the latter method will be used since it is a highly validated method recommended by the WHO group.[235]
The multiplex HPV genotyping with Luminex is based on hybrids of oligonucleotide probes, coupled to internally dyed polystyrene beads marked with fluorescence which attach to specific sites on the amplified HPV DNA. A Luminex reader containing two different lasers is used to measure the internal color of the HPV type specific beads and the quantity of fluorescence, measured in median fluorescence intensity.[234] There are more than 100 different beads available and the Luminex is capable of measuring more than 100 types of HPV at the same time.[234]
Multiplex HPV genotyping with Luminex has a high sensitivity and specificity compared to other methods, although its greatest advantage is that it enables accurate testing of multiple HPV types (more than 100) at the same time.[234 235]

Detection of p16INK4a by immunohistochemistry

Detection of HPV DNA in tumor material is usually not enough to demonstrate a causal relationship between the HPV and the type of cancer studied; viral/biological activity is also needed. In order to assess biological activity of HPV in tumors, $p16^{INK4a}$, a surrogate marker for HPV activity,[236 237] can be measured using immunohistochemistry. Tumor material is sectioned and stained with a monoclonal antibody against p16 and the slides are graded through microscopic examination. This method has a high sensitivity (100%), when combined with a PCR method and a low specificity (79%),[238] due to HPV independent pathways for carcinogenesis and overexpression of p16.

Human papillomavirus and cancer

General

Most HPV infections are cleared within two years after outbreak, however, a small fraction does for unknown reasons proceed from productive to transforming infection. The carcinogenic steps of HPV infection from precancerous dysplastic lesions to invasive cancer, have been studied in detail for cervical cancer. Initially, the virus implants its genome into the host cell enabling E2 production which then suppresses the expression of E6 and E7 proteins. The next step is not quite clear, although a deletion of E2 occurs resulting in an unregulated expression of E6 and E7 which then creates cellular transformation/proliferation, interference with host cell immune system and other effects mentioned above;[239 240] the cancer development then becomes a fact. HPV has been linked to several types of cancer; the text below contains a brief description.

Cervical cancer

Cervical cancer is the second most common type of cancer in women globally, with 80% of cases occurring in developing countries.[1] Numerous studies have investigated the causal relationship between HPV and cervical cancer and the current knowledge of HPV is mainly derived from this research. About 95% of cervical squamous cell carcinomas contain HPV DNA, HPV 16 being the dominating type and HPV 18 in the case of cervical adenocarcinomas.[199 241]

Oropharyngeal cancer

In 2007, HPV was classified as a risk factor for oropharyngeal squamous cell carcinoma according to the International Agency for Research on Cancer (IARC).[242 243] The causal relationship had already been suggested in 1975, when women with cervical carcinoma had a 5-6 fold elevated risk of developing a second cancer in the oral cavity.[244] Similar results were also seen in 1999, when patients with anogenital cancers were shown to have a 4.3-fold increased risk of tonsillar cancer.[245]

The frequency of HPV DNA in head and neck squamous cell carcinoma varies largely with anatomic site; [246] the highest prevalence of high risk HPV DNA is found in oropharyngeal cancer (25 to 60%).[247] When comparing patients with HPV-positive and negative oropharyngeal tumors, positive patients are characterized by their younger age at the time of diagnosis and their non-smoking status,[243] and importantly, HPV has been established as an independent prognostic factor for survival.[246 248 249 243 250] The incidence of oropharyngeal cancer has rapidly increased during the last three decades, by 3.9 times in tonsillar and 2.1 times in tongue-based squamous cell carcinoma.[1 251 252] The increased incidence might be explained by a rise in HPV infections in the oral cavity caused by a change in sexual habits, alongside an increased number of vaginal or oral sexual partners.[253]

Other HPV related cancers

HPV has also been associated with anogenital cancers; 50% of vulvar,[254] 30-65% of penile[255] and as much as 90% of vaginal cancers[197 254] contain HPV DNA, particularly of the HPV 16 type.[255] As well as in oropharyngeal tumors, HPV-positive vulvar and penile cancers tend to have a better prognosis and sexual behavior is a strong risk factor.[255] HPV has also been suggested as a causal factor for squamous cell carcinoma of the skin, although the results are contradicting and more research is needed.[247]

HPV and esophageal cancer

An association between HPV and esophageal squamous cell carcinoma was initially suggested in 1982, when morphological similarities between HPV induced lesions in the genital tract, i.e. condyloma, and esophageal squamous cell carcinoma were discovered.[256] Since then, several studies have been conducted and the results were summarized in a review article in 2002, where a range of 0 to 70% of tumors were positive for HPV infection.[257] The great diversity of results was mainly explained by the different laboratory techniques used and the different geographic regions tested. The use of a filter in situ hybridization (FISH) and in situ hybridization generated a much higher incidence of HPV compared to PCR and the incidence of HPV was higher in high risk areas for esophageal cancer.[257]
Since this review article was published, the diversity of results have continued; studies from high-risk regions in China, South Africa and Iran indicate a prevalence of 37 to 77% of HPV DNA in esophageal tumors[258-261] although some studies show completely contradicting results.[262-264] The prevalence of HPV DNA in Europe varies between 16 and 28%[265-267] and no favorable prognostic effect is found in HPV positive esophageal tumors.[267] No study has, to our knowledge, addressed tumor localization as a primary endpoint, although a Swedish study included tumor location when studying survival.[267]
To summarize, there is still confusion as to whether HPV is a causal factor for esophageal squamous cell carcinoma.

AIMS

The general aim of this thesis was to advance our understanding of the etiological factors behind esophageal cancer.

The specific aims are:

- To clarify as to whether the established etiological factors for esophageal and cardia adenocarcinoma exist to a different extent in women and men.
- To clarify as to whether the population prevalence of known risk factors for esophageal adenocarcinoma is higher in the UK as opposed to in Sweden.
- To clarify potential differences between antireflux operated patients who do or do not develop esophageal adenocarcinoma after such surgery.
- To determine as to whether HPV is more strongly associated with squamous cell carcinoma of the proximal esophagus as opposed to the distal esophagus.

MATERIALS AND METHODS

Data for this thesis has been obtained from several databases; an overview of methods used is presented in Table 2 below.

Table 2. An overview of material and methods used in this thesis.

	Paper I	**Paper II**	**Paper III**	**Paper IV**
Design	Population-based case-control study	Population-based cross-sectional study	Population-based case-control study nested within an antireflux cohort	Population-based case-control study
Data source	Swedish Esophageal and Cardia Cancer-study	Bristol Helicobacter Project-study, Reference Population-study	Patient Register Cancer Register	Cancer Register
Year	1994-1997	1996-1999, 2008	1965-2006	1999-2006
Participants	451 cases, 816 controls	3633 (English) 1483 (Swedish)	55 cases, 240 controls	20 cases, 184 controls
Study exposure	Gastroesophageal reflux symptoms, high BMI, tobacco smoking, high intake of fruit and vegetables, infection with H. Pylori	Gastroesophageal reflux symptoms, overweight/obesity, tobacco smoking	Recurrent gastroesophageal reflux, high BMI, tobacco smoking, different types of antireflux surgery	HPV infection
Outcome	Esophageal/cardia adenocarcinoma	English or Swedish outcome	Esophageal/cardia adenocarcinoma	Tumor located in the proximal part of the esophagus
Statistical analysis	Unconditional logistic regression	Logistic regression	Conditional logistic regression	Fischer exact test, logistic regression
Estimate	Odds ratio	Odds ratio	Odds ratio	Odds ratio
Matching	Age, sex	Matching for age and sex to cases of esophageal adenocarcinoma was made when sampling in the Swedish population	-	-
Confounders	Age, sex, educational level, alcohol use, gastroesophageal reflux symptoms, BMI, tobacco smoking, high intake of fruit and vegetables, H. Pylori infection	Age, sex, educational level, gastroesophageal reflux symptoms, BMI, tobacco smoking	Age, sex, recurrent gastroesophageal reflux, BMI, tobacco smoking and type of antireflux surgery	Age, sex and tumor differentiation

Data source

The Swedish Esophageal and Cardia Cancer study

The Swedish Esophageal and Cardia Cancer (SECC) study has been described in detail elsewhere.[5] In brief, the SECC-study was conducted in Sweden between December 1, 1994 and December 31, 1997 with the primary aim to identify risk factors for esophageal and cardia adenocarcinoma. All Swedish residents below the age of 80 years were eligible for participation. Patients newly diagnosed with esophageal or cardia adenocarcinoma were identified as cases by means of collaboration between all general and thoracic surgical departments, as well as departments of otorhinolaryngology, oncology and pathology throughout Sweden. Study protocol and posters were placed at all gastroenterology and endoscopy units and collaborations with six regional tumor registers were established in order to ensure that all cases of esophageal cancer were identified. A newsletter was produced and sent out regularly (every 3 months) to keep all collaborators updated about study procedures and progressions.

Tumor classification

Specific study protocols and color posters were developed in order to ensure correct tumor location and histological classification. Diagnosis was individually determined for all cases by summarizing findings from four different disciplines:

- Endoscopy: obtaining tumor location/borders and biopsies by means of endoscopic examination at least once in all cases.
- Radiology: ascertaining tumor location by means of CT scan and endoscopic ultrasound.
- Surgery: if tumors were resected or if explorative surgery was performed, tumor borders and center were carefully recorded according to a specific study protocol.
- Pathology: biopsies and surgical specimens were sectioned and histologically classified according to a specific study protocol, furthermore 97% of all tumors were re-examined by a single experienced pathologist (Anders Lindgren).

In order to distinguish between esophageal and gastric cardia tumors (data was collected before Siewert's definitions were published),[20] gastric cardia tumors were defined as located 2 cm above or 3 cm below the esophagogastric junction[268] and all patients with BE were defined as having esophageal adenocarcinoma. In ambiguous cases, a panel of investigators with experience in all four disciplines evaluated the case and agreed upon a final tumor classification.
For every case, four or five randomly selected controls were continuously obtained from the Swedish Total Population Register. Controls were frequency matched from a ten-year age and gender strata in order to mimic sex and age distributions of cases. Both cases and controls underwent face-to-face interviews as described in detail below.

Bristol Helicobacter Project

The Bristol Helicobacter Project (BHP) was initially conducted as a double-blind, placebo-controlled randomized clinical trial aiming at investigating the impact of H. pylori in patients with uninvestigated dysphagia.[269] Data was collected between July 1996 and January 1999 from a local English register, covering seven primary care centers in Bristol and surrounding areas. All residents aged 29-59 years were eligible to be contacted by mail and asked to participate in the study; non-responders were contacted by telephone after three weeks.
Study participants were asked to fill out a questionnaire (further described below in paper II), measure their height and weight and perform a urea breath test for H. pylori at their general practitioners office, after which they were randomly assigned Helicobacter eradication therapy or a placebo. The initial questionnaire is included in this thesis.

The Reference Population Study

The Reference Population (RP) study is a random sample of the adult Swedish population, collected between April and June 2008. All Swedish residents aged 40 to 59 years were eligible for the study, and were randomly selected based on age, sex and geographic distribution of esophageal adenocarcinoma cases reported in the Swedish Cancer Register in 2006. Questionnaires (identical to BHP) were mailed out to all participants and both randomization and data collected was performed by Statistics Sweden, using the nationwide and highly complete Swedish Register of the Total Population.

The Swedish Cancer Register

The Swedish Cancer Register, held by the National Board of Health and Welfare, contains virtually all cancer cases diagnosed in Sweden since 1958. Clinicians and pathologists are legally obligated to report the occurrence of new cancer cases to their regional cancer registrar, who then forwards the information to the nationwide Swedish Cancer Register.[270] Clinicians report the location of all tumors detected, regardless of biopsy specimen verification, and pathologists report histologically confirmed cancers, including those detected at autopsy.[270] The register is coded according to the International Classification of Disease for Oncology (ICD-O)[271] and patients are identified through a unique ten-digit personal identity number.[272]
The comprehensiveness of the Swedish Cancer Register is overall 96.3%,[273] and 98% respectively for esophageal and cardia cancers, although the latter condition was included in non-cardia gastric cancer only until 1970.[274] The Swedish Cancer Register is considered to have a high validity when compared to other registers in northern Europe.[273]

The Swedish Patient Register

The Swedish Patient Register, previously known as the Swedish In-Patient Register and the Hospital Discharge Register, contains diagnoses (maximum of six to eight per care episode) and surgical procedures (maximum of six to twelve per care episode) from the in-patient care of Swedish residents.[275] The register was established in 1964 and covered 60% of the Swedish population in 1969, 85% in 1983 and the entire population (100%) from 1987 onwards.[275] Diagnosis and surgical procedures from out-patient clinics have been included since 1997, although specialized out-patient care was not included until 2001.[275] The register is coded according to WHO's International Classification of Disease[271] and patients are identified through their personal identity number.[272] The completeness of the Swedish Patient Register is 98% for all hospitalizations and main diagnoses are present for 99% of all hospital admissions.[275]

Paper I – Sex-specific risk factor profile in oesophageal adenocarcinoma

Hypothesis

Due to the unexplained male predominance in esophageal and cardia adenocarcinoma (3-10:1),[11 20 44] we hypothesized that the established etiological factors are different in women and men, with risk factors being stronger in men and protective factors being stronger in women.

Design

This was a population-based case-control study comparing the distribution and strength of known risk factors for esophageal and cardia adenocarcinoma among women and men. Cases and controls, matched for age and sex, were identified from the SECC-study conducted in Sweden between December 1, 1994 and December 31, 1997.

Assessment of study exposure

Information about most study exposures (gastroesophageal reflux symptoms, height and weight, tobacco smoking and intake of fruit and vegetables) and potential confounders (alcohol consumption, socioeconomic status/educational level) were in both cases and controls collected in face-to-face interviews within five weeks after diagnosis. Interviewers were unable to be blinded as to the study outcome; however, they were unaware of the study hypothesis and trained to treat cases and controls equally. In order to avoid reverse causation, all questions were asked about study exposure and potential confounders during the last five years prior to interview. Infection with H. pylori was detected through the collection of venous blood taken from cases during their initial hospital stay and controls were asked to leave a blood sample at their local health center.

Gastroesophageal reflux was defined as recurrent heartburn or regurgitation at least once a week for more than a year, and was measured in frequency, severity and duration. A reflux symptom score was generated based on frequency (once a week=0 points, 2-6 times a week=1 point, 7-15 times a week=2 points, more than 15 times a week=3 points) and characteristics of reflux symptoms (heartburn only=1 point, regurgitation only=1 point, heartburn and regurgitation combined=1.5 points, nightly symptoms=2 points). BMI (<22.0, 22.0-24.9, >24.9) was calculated from height and weight at the age of 20, twenty years before the interview and adult extreme values (minimum and maximum weight). Tobacco smoking was defined by means of frequency, duration and number of years since smoking cessation. Smoking status was defined as current, previous (stopped smoking 2 or more years before the study) or never smoker. Pack year was calculated as the number of cigarette packets (40 cigarettes) per day multiplied by the number of smoking years. Intake of fruit and vegetables was measured with a previously evaluated food-frequency questionnaire.[276] Questions were asked about the average intake of 63 food and beverage items 20 years prior to the interview. Fruits included citrus fruits, fruit juice, apple and pears, covering 86.9% of the total fruit types consumed in Sweden (Swedish Board of Agriculture 1989). Vegetables included allium vegetable types, beets, broccoli, cabbage, cauliflower, carrots, lettuce, potatoes, spinach and tomatoes, covering 96.5% of the total vegetables consumed in Sweden (Swedish Board of Agriculture 1989). The variable

used for fruit and vegetables was constructed using quartiles from the total intake in controls; high corresponded to the 4th quartile, medium to the 2nd and 3rd quartile and low to the 1st quartile. Infection with H. pylori was detected through enzyme-linked immunosorbent assay (ELISA) (Pyloriset EIA-G; Orion Diagnostica, Espoo, Finland) with 98% sensitivity and 85% specificity[277] which measured immunoglobin (IgG) antibodies against HP-CSAs in serum (positive/negative). Antibodies against CagA (positive/negative) were detected with an immunoblot assay (Helicoblot 2.1; Genelabs Diagnostics, Singapore). All investigators were blinded to participants' case-control status.

Statistical analysis

Unconditional logistic regression stratified by sex, was used to calculate estimated odds ratios (OR) with 95% confidence intervals (CI) for risk factors in women and men. Adjustment for potential confounding factors: age (<49, 50-59, 60-69, 70-79 years), educational level (0-9, 10-12, ≥13 years) and alcohol use (yes or no), gastroesophageal reflux, BMI, tobacco smoking, intake of fruit and vegetables and H. pylori infection was performed in a multivariable model. Esophageal or cardia adenocarcinoma was the outcome and the study exposure included gastroesophageal reflux symptoms, BMI, tobacco smoking, intake of fruit and vegetables and infection with H. pylori. Separate analyses were also performed for esophageal and cardia adenocarcinoma.

Paper II – Increased prevalence of reflux and obesity in the United Kingdom compared with Sweden: a potential explanation for the difference in incidence of esophageal adenocarcinoma

Hypothesis

Due to the much higher incidence of esophageal adenocarcinoma in England compared to Sweden, we hypothesized that the English population has a higher prevalence of established risk factors for esophageal adenocarcinoma, i.e. gastroesophageal reflux symptoms, high BMI, and tobacco smoking.

Design

This was a population-based cross-sectional study comparing population prevalence of risk factors for esophageal adenocarcinoma in random samples from England and Sweden. Data was collected from the BPH and the RP study.

Assessment of study exposures

Study exposures were assessed through self-administrated questionnaires, initially developed for the BPH. Most questions were extracted from a previously validated questionnaire used in a large British study in 1996.[278] Great efforts were made to ensure that the English and Swedish

questionnaires were linguistically identical. Three native Swedes independently translated the questionnaire into Swedish and agreed on a final version. Compatibility was evaluated through a bilingual person (native English), translating the questionnaire back into English and comparing it to the original version, with very accurate results. Before the Swedish questionnaire was sent out it was tested on twenty Swedes so as to evaluate comprehensibility.
Questions included (1) demography: age, sex and educational level, and (2) risk factors for esophageal adenocarcinoma: frequency and severity of gastroesophageal reflux symptoms (heartburn and regurgitation), adult height and weight, and tobacco smoking status.
GERD was defined according to the Montreal definition[107] as heartburn and regurgitation occurring at least once a week or as troublesome for the patient,[107] and was assessed through frequency (not at all in the last three months, less than once a month, at least once a month, at least once a week, everyday) and severity (no problem at all, a mild problem, a moderate problem, a severe problem, a very severe problem), using a five point Likert scale.[279] BMI was categorized according to the WHO definitions (<18.5, 18.5-24.9, 25.0-30.0, >30.0).[153] Tobacco smoking was defined as current (smoked during the last three months), previous (not smoked for the last three months) and never (never smoked one or more cigarettes a day for one year or more).
The most important difference in data collection between the two populations was the assessment of height and weight. A nurse measured height and weight in the English sample, whereas it was self-reported in the Swedish sample.

Statistical analysis

Unconditional logistic regression was used to calculate OR with 95% CI and p-values as an effect index comparing the prevalence of gastroesophageal reflux symptoms, BMI and tobacco smoking status, in the two populations. Distribution of age, sex and educational level was different for the populations and two multivariable models were performed using the Swedish population as reference. Model one was adjusted for age (40-44, 45-49, 50-54, 55-59 years), sex and educational level (0-9, 10-12, ≥13 years) and model two was further adjusted for gastroesophageal reflux symptoms, BMI and smoking status. The primary outcome was English or Swedish origin and study exposure was gastroesophageal reflux symptoms, BMI and tobacco smoking.
Previous studies show that patients with a combination of gastroesophageal reflux symptoms and obesity have a considerably increased risk of esophageal adenocarcinoma.[7] To investigate as to whether a combination of risk factors for esophageal adenocarcinoma was more common in the English population, study exposures, gastroesophageal reflux symptoms (at least once a week and more), BMI (overweight and obesity) and tobacco smoking status (never/ever) were combined and compared in the two populations in the same way as mentioned above.

Paper III – Risk factors for esophageal adenocarcinoma after antireflux surgery

Hypothesis

Due to the previously observed absence of a cancer protective effect from antireflux surgery, we hypothesized that recurrent reflux or high exposure to other well established risk factors for esophageal or cardia adenocarcinoma are overrepresented among patients who, despite antireflux surgery, develop cancer.

Design

This was a population-based case-control study nested within a cohort of all antireflux operated patients in Sweden, between 1965 and 2006. Cases were those who developed cancer at least five years after surgery and were identified by means of linkage between the Swedish Patient Register and the Swedish Cancer Register. For every case five randomly selected controls were selected from the antireflux cohort, matched in gender, age and calendar year of antireflux surgery. Both cases and controls were operated with severe GERD as indication.

Assessment of study exposure

Medical records were collected throughout Sweden and examined by one reviewer (Hedvig Löfdahl) who was blinded as to case-control status. To avoid using medical records from the point of identification of esophageal adenocarcinoma, only records from the primary antireflux operation and up to 2 years after surgery were obtained. Collected variables included sex, age at antireflux operation (<50, 50-59, >59 years) and study exposure including recurrent gastroesophageal reflux symptoms after surgery (yes or no, including medically or surgically treated symptoms), high BMI (<25 or ≥25), tobacco smoking (never or ever) and type of antireflux surgery (Nissen, Toupét or other type).

Statistical analysis

Relative risk, addressed as OR with 95% CI was calculated using conditional logistic regression. Two multivariable models were used adjusting for sex and age of antireflux surgery in the first model and recurrent reflux, BMI, tobacco smoking, and type of operation additionally in the second model. Missing data was grouped into separate categories. The primary outcome was esophageal or cardia adenocarcinoma and study exposures included recurrence of gastroesophageal reflux symptoms after surgery, high BMI, tobacco smoking and type of antireflux surgery.

Paper IV – Infection with human papillomavirus (HPV) in relation to site of squamous cell carcinoma of the esophagus

Hypothesis

Due to the fact that previous studies showed diverse results of HPV as a risk factor for esophageal cancer and because of HPV's association with oropharyngeal cancer,[242] we hypothesized that tumor location might be connected to presence of HPV infection and that squamous cell carcinomas of the proximal esophagus are positive for HPV infection to a greater extent than tumors of the middle or distal esophagus.

Design

This was a population-based case-control study including all patients diagnosed with esophageal squamous cell carcinoma in the Stockholm County between 1999 and 2006. Cases and controls were identified through the Swedish Cancer Register using the unique personal identity number.[272] Cases represented patients with a higher location of their tumor, whereas controls were patients with a distal tumor location.

Assessment of study exposure

Age (<60, 60-70 or >70 years) and tumor location data were obtained from medical records. The tumor site was examined by one reviewer (Hedvig Löfdahl), blinded to case-control status. Patients were classified according to the 6th edition of the ASCC tumor classification,[14] as proximal (15-24 cm from the incisors), middle (25-30 cm from the incisors), or distal tumors (30-45 cm from the incisors).

Detection of human papilloma virus

DNA extraction and HPV genotyping were performed on histologically confirmed paraffin embedded endoscopic biopsies of esophageal squamous cell carcinomas, obtained before neoplastic treatment. Only endoscopic biopsies were eligible in order to avoid detection bias, since surgical specimens from tumor resection are more commonly used on tumors located in the distal esophagus making it easier to detect HPV infection. To avoid tumor misclassification, a single pathologist re-examined and graded tumor differentiation (high, middle and low) for all biopsy specimens.

Formalin-fixated paraffin embedded biopsies was sectioned on glass (4 x 15 µm). Macro-dissection was performed to make sure that predominantly tumor material was present in the analysis, and to minimize the risk of contamination, blanks were added after every 5th sample and treated in the same way as real samples during slicing and macro-dissection.
A High Pure RNA Paraffin Kit (Roche's) without DNAse was used for DNA extraction. PCR was performed by adding 10 µl of DNA sample and 40 µl of reaction mixture, containing broad-spectrum GP5+/6+ primers (BGP5+/BGP6+) to the Qiagen Multiplex PCR Master Mix (Qiagen, Hilden, Germany).[233] Amplification was made in 40 cycles, with a temperature of

38°C for 90 seconds. HPV genotyping was conducted with Luminex[234 280] together with a Multimetrix kit (Heidelberg, Germany) analyzing 24 HPV types, including 15 HR types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82), 3 putative HR types (26, 53, 66) and 6 LR types (6, 11, 42, 43, 44, 70).[199] Hybridization of PCR products (10 µl) and Luminex beads (40 µl) together with the subsequent steps were performed using standard methods. The samples were analyzed using the Luminex 100 analyzer (BioRad Laboratories, Hercules, USA) with the cut-off limits proposed by the manufacturer.

Detection of $p16^{INK4a}$ by immunohistochemistry

Biological activity of HPV in tumors was measured using immunohistochemistry for expression of $p16^{INK4a}$, a surrogate marker for HPV activity.[236 237] Biopsies were sectioned and stained using the monoclonal antibody p16 (delusion 1:200, clone JC8, Santa Cruz Biotechnology, INC) and a standard streptavidin-biotin peroxidase method. Almost all HPV positive cases and six HPV negative controls per each case were evaluated for p16 by a single pathologist who graded samples according to nucleus staining in four categories (negative, <5% or weak nuclear staining, 5-30% and >30%) and used two categories (negative/<5% or weak staining, or >5 %) in the statistical analysis.

Statistical analysis

A Fischer exact test was used to identify predictors of HPV-positive tumors, with a p-value below 0.05 considered as statistically significant. Odds ratios (OR) with 95% confidence intervals (CI) were calculated using unconditional logistic regression to determine relative risk of different tumor locations when exposed to HPV infection. Multivariable modeling was used to adjust for potential confounders (age, gender, and tumor differentiation). Any missing data was grouped into a separate category. The primary outcome was squamous cell carcinoma located in the proximal esophagus and the study exposure was HPV infection.

RESULTS

Paper I – Sex-specific risk factor profile in oesophageal adenocarcinoma

In this study, 451 (85%) cases of esophageal or cardia adenocarcinoma and 816 (72%) controls participated in the interviews. Among the cases, 63 (14%) were women and 388 (86%) were men, resulting in a male to female ratio of 7:1. The level of education was generally lower in women compared to men and in the cases compared to the controls (data not shown).

Risk factors in women and men

All established risk factors, i.e. gastroesophageal reflux symptoms, high BMI, and tobacco smoking, were associated with an increased risk of esophageal or cardia adenocarcinoma in both men and women (Table 3).

Table 3. Risk factors for esophageal and cardia adenocarcinoma in women versus men, expressed in OR with 95% CI.

Variables	Women			Men		
	Controls N = 140 (%)	Cases N = 63 (%)	OR* (95% CI)	Controls N = 676 (%)	Cases N = 388 (%)	OR* (95% CI)
Reflux (at least weekly 5 years or more prior to interview)						
No	17 (12)	25 (40)	1.0 (reference)	118 (17)	163 (42)	1.0 (reference)
Yes	123 (88)	38 (60)	4.6 (2.0-10.5)	558 (83)	225 (58)	3.4 (2.5-4.6)
BMI (20 years before interview)						
<22	56 (40)	12 (19)	1.0 (reference)	151 (22)	45 (12)	1.0 (reference)
22.0-24.9	55 (39)	25 (40)	2.4 (0.9-6.0)	311 (46)	143 (37)	1.5 (1.0-2.3)
25.0-29.9	22 (16)	16 (25)	4.3 (1.4-13.1))	196 (29)	164 (42)	2.7 (1.8-4.1)
≥30.0	7 (5)	10 (16)	10.3 (2.6-42.3)	18 (3)	36 (9)	5.4 (2.6-10.8)
Cigarette smoking status (2 years before interview)						
Never	90 (64)	29 (46)	1.0 (reference)	233 (35)	71 (18)	1.0 (reference)
Previous	28 (20)	13 (21)	1.9 (0.7-5.1)	285 (42)	200 (52)	2.3 (1.6-3.3)
Current	22 (16)	21 (33)	5.3 (2.0-14.1)	158 (23)	117 (30)	2.8 (1.9-4.2)

* Adjustments were made for age, educational level, alcohol use, BMI, cigarette smoking, intake of fruit and vegetables, and H. pylori infection.

Presence of gastroesophageal reflux symptoms increased the risk of esophageal and cardia adenocarcinoma over four times in female cases and three times in males (OR 4.6, 95% CI 2.0-10.5 and OR 3.4, 95% CI 2.5-4.6). The risk seemed to rise with increasing severity of reflux symptoms in a dose-response manner, although women had an overall higher OR than men in all subcategories of exposure (data not shown).

Being overweight twenty years before the interview was associated with an increased risk of esophageal or cardia adenocarcinoma with an OR of 4.3 (95%, CI 1.4-13-1) in women and a 2.7 (95%, CI 1.8-4.1) in men. Obesity further increased the risk of cancer OR 10.3 (95%, CI 2.6-42.3) in women and 5.4 (95%, CI 2.6-10.8) in men. The risk also seemed to rise with increased BMI in all subcategories (at age 20 years, minimum and maximum in life) (data not shown), and once again, female cases had higher point estimates than male in all BMI subcategories.

Current tobacco smoking was associated with an increased risk of esophageal and cardia adenocarcinoma more than five times in women (OR 5.3, 95% CI 2.0-14.1) and almost three times in men (OR 2.8, 95% CI 1.9- 4.2). This risk factor also showed a seemingly dose-response relationship, since higher frequency and duration of smoking was associated with increased risk (data not shown).

To summarize, for all of the three studied risk factors, there was rather a possible increased risk of developing esophageal or cardia adenocarcinoma in women compared to men, implying that these risk factors do not explain the gender difference in incidence for these tumors.

Preventive factors

Both female and male cases were less exposed to the preventive factors of high intake of fruit and vegetables (45 compared to 37% in women and 32 compared to 25% in men) and infection with H. pylori, with the endotoxin CagA (19 compared to 16% in women and 24 compared to 12% in men), when compared to female and male controls (Table 4). An increased risk of esophageal or cardia adenocarcinoma was observed in men with low intake of fruit and vegetables (OR 1.6, 95% CI 1.1-2.2) but not in women (OR 0.9, 95% CI 0.3-2.4), although this was not statistically significant. The protective effect of *H. pylori*-infection seen in men (OR 0.5, 95% CI 0.3-0.8) was not found to be the same in women (OR 1.6, 95% CI 0.5-5.4).

Table 4. Preventive factors for esophageal and cardia adenocarcinoma in women versus men, expressed in OR with 95% CI.

Variables	Women			Men		
	Controls N = 140 (%)	Cases N = 63 (%)	OR* (95% CI)	Controls N = 676 (%)	Cases N = 388 (%)	OR* (95% CI)
Intake of fruit and vegetables (20 years before interview)						
Highest	63 (45)	23 (37)	1.0 (reference)	213 (32)	95 (25)	1.0 (reference)
Medium	50 (36)	27 (43)	1.1 (0.5-2.5)	275 (41)	140 (36)	1.0 (0.7-1.4)
Lowest	27 (19)	13 (21)	0.9 (0.3-2.4)	188 (28)	153 (39)	1.6 (1.1-2.2)
H. pylori status and its virulence factor CagA status						
Negative HP and CagA	34 (24)	10 (16)	1.0 (reference)	161 (24)	90 (23)	1.0 (reference)
Mixed HP or CagA	25 (18)	7 (11)	1.3 (0.4-4.7	92 (13)	68 (18)	1.2 (0.8-1.9)
Positive HP and CagA	26 (19)	10 (16)	1.6 (0.5-5.4)	161 (24)	45 (12)	0.5 (0.3-0.8)
Missing	55 (39)	36 (57)	2.8 (1.0-7.4)	262 (39)	185 (48)	1.1 (0.8-1.5)

* Adjustments were made for age, educational level, alcohol use, BMI, cigarette smoking, intake of fruit and vegetables, and H. pylori infection.

Stratified analysis for esophageal and cardia adenocarcinoma

Additional analyses were conducted to determine point estimates for esophageal and cardia adenocarcinoma separately (Table 5). There were few female cases in some categories but the ORs for gastroesophageal reflux symptoms were particularly increased in esophageal adenocarcinoma for both women and men.

Table 5. Risk for esophageal and cardia adenocarcinoma separately, presented as OR with 95% CI.

Variables	Males		Females	
	Esophageal	Cardia	Esophageal	Cardia
	OR 95% CI	OR 95% CI	OR 95% CI	OR 95% CI
Gastroesophageal reflux symptoms				
No	Reference	Reference	Reference	Reference
Yes	6.6 (4.4-9.9)*	2.0 (1.3-2.8)*	20.4 (4.4-94.2)*	1.4 (0.5-4.1)*

*Adjustments were made for age, educational level, gastroesophageal reflux symptoms, BMI, tobacco smoking, intake of fruit and vegetables and HP.

Paper II – Increased prevalence of reflux and obesity in the United Kingdom compared with Sweden: a potential explanation for the difference in incidence of esophageal adenocarcinoma

In study II, an English population of 17,310 individuals in the age group 40-59 years was invited to participate, and 7,426 (43%) participants completed questionnaires and height and weight measurements. From these, 3,633 were randomly selected for analysis. In the Swedish population, 2,372 individuals in the age group 40-59 years were invited to participate, and 1,483 (62%) participants completed the questionnaire.
The Swedish participants were randomly selected to mimic sex and age distribution of esophageal adenocarcinoma patients, resulting in differences in the two populations regarding the distribution of age and gender (Figure 4).

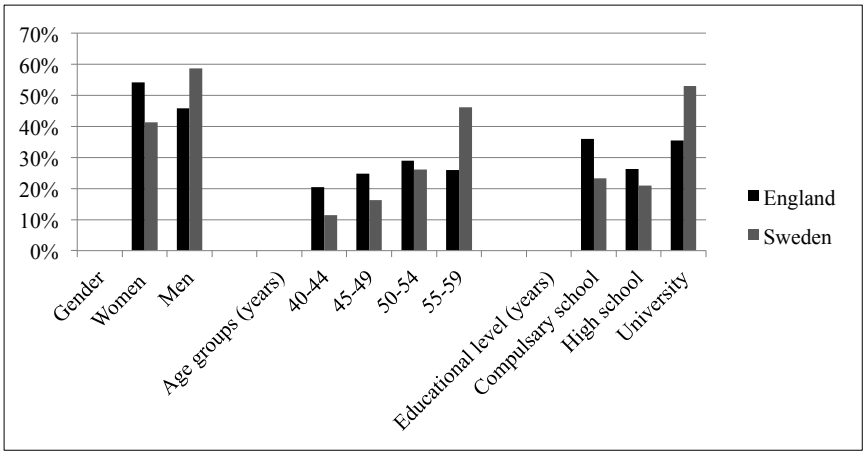

Figure 4: Distribution (ratios) of gender, age and education in the English and Swedish sample populations.

The English population was younger, had a higher female percentage and less education than in the Swedish population, although these factors were adjusted for in the logistic regression analysis.

Prevalence of risk factors for esophageal adenocarcinoma

Occurrence of gastroesophageal reflux symptoms was more common in the English population; 16.7% versus 8.8% (Table 6), and differences between the populations were further confirmed by an adjusted OR of 2.0 (95% CI 1.6-2.4) when using the Swedish population as a reference. The prevalence of a higher frequency and severity of gastroesophageal reflux was also more common in the English population, as shown by ORs of 2.2 (95% CI 1.6-3.0) for reflux symptoms once a week and 1.5 (95% CI 1.2-1.9) for a moderate problem of reflux symptoms (data not shown).

The prevalence of overweight and obesity was higher in the English population. More than 65% of the participants from England had a BMI above 24.9 compared to 57% in the Swedish population. Mean BMI was also higher in the English population 28.3 (standard deviation ±0.3) versus 26.1 (standard deviation ±0.1) in the Swedish (data not shown). Also adjusted OR of 1.8 (95% CI 1.5-2.1) for a BMI above 30 (obesity) was higher in the English population.
The prevalence of tobacco smoking was similar in the two populations; the OR for prevalence of current smoking was 1.0 (95% CI 0.9-1.2) and the prevalence of former smoking was lower in the English population (OR 0.8, 95% CI 0.7-0.9).

Table 6. Prevalence of risk factors for esophageal adenocarcinoma in samples of the English and Swedish population.

Variable	English sample	Swedish sample	OR* (95% CI)	p value
	Number (%)	Number (%)		
Gastroesophageal reflux symptoms				
No	2,835 (78.0)	1,290 (87.0)	Reference	
Yes	607 (16.7)	130 (8.8)	2.0 (1.6-2.4)	0.000
BMI				
<18.5	15 (0.4)	13 (0.9)	0.6 (0.3-1.3)	0.184
18.5-24.9	1,237 (34.1)	631 (42.6)	Reference	
25-30	1,565 (43.1)	595 (40.1)	1.5 (1.3-1.8)	0.000
>30	816 (22.5)	244 (16.5)	1.8 (1.5-2.1)	0.000
Tobacco smoking status				
Never	1,879 (51.7)	722 (48.7)	Reference	
Current smoker	787 (21.7)	260 (17.5)	1.0 (0.9-1.2)	0.762
Former smoker	921 (25.4)	466 (31.4)	0.8 (0.7-0.9)	0.001
Gastroesophageal reflux symptoms, overweight/obesity and tobacco smoking	254 (7.0)	51 (3.4)	2.6 (1.9-3.7)**	0.000
Gastroesophageal reflux symptoms and overweight/obesity	210 (5.8)	39 (2.6)	3.1 (2.1-4.6)**	0.000
Overweight/obesity and tobacco smoking	839 (23.1)	362 (24.4)	1.4 (1.1-1.7)**	0.010
Gastroesophageal reflux symptoms and tobacco smoking	62 (1.7)	23 (1.6)	1.4 (0.8-2.4)**	0.198

* Adjusted for gender, age, education, BMI, and smoking status. Evaluated variable was excluded from the multivariable model.

** Adjusted for gender, age and education.

Percentages not adding to 100% are due to missing data.

Combined effects of risk factors

Participants with a combination of gastroesophageal reflux, BMI ≥ 25 and tobacco smoking were almost three times (OR 2.6, 95% CI 1.9-3.7) more common in the English population and participants with a combination of the two major risk factors for esophageal adenocarcinoma (gastroesophageal reflux symptoms and a BMI ≥ 25) were more than three times (OR 3.1, 95% CI 2.1-4-6) as common in the English sample compared to the Swedish (Table 6).

Paper III – Risk factors for esophageal adenocarcinoma after antireflux surgery

Of the 14,102 patients included in the antireflux cohort, 64 (0.45%) developed esophageal or cardia adenocarcinoma and 320 matched controls were randomly selected. Of these 384 patients, 35 (8 cases and 27 controls) were excluded due to having had antireflux surgery for an indication other than GERD. Furthermore, 10 (1 case and 9 controls) participants were lost to follow-up as a result of a private hospital destroying medical records 10 years after the treatment session, resulting in a total of 55 cases and 240 controls included in this study. As a result of matching, cases and controls were similar in distribution of sex and age of antireflux surgery (data not shown).

Risk of esophageal adenocarcinoma

Recurrent reflux was 3 times more common in cases compared to controls (adjusted OR 3.1, 95% CI 1.5-6.3, Table 7).

Table 7. Risk of esophageal or cardia adenocarcinoma after antireflux surgery, expressed as OR with 95% CI.

Variable	Cases	Controls	Adjusted model*
	Number (%)	Number (%)	OR 95% CI
Recurrent reflux			
No	33 (60.0)	190 (79.2)	Reference
Yes	19 (34.6)	43 (17.9)	3.1 (1.5-6.3)
BMI			
< 25	13 (23.6)	69 (28.8)	Reference
≥ 25	34 (61.8)	122 (50.8)	1.6 (0.8-3.5)
Tobacco smoking			
Never	23 (41.8)	125 (52.1)	Reference
Previous/current	26 (47.3)	102 (42.5)	1.4 (0.7-2.8)
Type of antireflux surgery			
Partial fundoplication	17 (30.9)	53 (22.1)	Reference
360 degree fundoplication	30 (54.6)	130 (54.2)	0.6 (0.3-1.3)
Other	6 (10.9)	52 (21.7)	0.2 (0.1-0.7)

*Matching was made for age, gender, and calendar year of antireflux surgery, and adjustments were made for age, gender, BMI, tobacco smoking, recurrent reflux and type of antireflux surgery.

Percentages not adding up to 100% are due to missing data.

High BMI (>25) and ever smoking tobacco also seemed to increase the risk of esophageal or cardia adenocarcinoma (OR 1.6, 95% CI 0.8-3.5 and OR 1.4, 95% CI 0.7-2.8, respectively), although the results were statistically non-significant. A lower risk of cancer was suggested after Nissen (360 degrees) fundoplication compared to Toupét (partial) fundoplication, yet there was no statistically significant difference (OR 0.6, 95% CI 0.3-1.3).

Cancer developed at least 5 years after surgery

Analyses restricted to cases developing esophageal or cardia adenocarcinoma at least 5 years after the antireflux surgery revealed similar results as those of the entire sample (Table 8).

Table 8. Risk of esophageal adenocarcinoma at least five years after antireflux surgery, expressed as OR with 95% CI.

Variable	Cases	Controls	Adjusted model*
	Number (%)	Number (%)	OR 95% CI
Recurrent reflux			
No	19 (57.6)	112 (77.8)	Reference
Yes	12 (36.4)	28 (19.4)	2.9 (1.2-7.3)
BMI			
< 25	5 (15.2)	37 (25.7)	Reference
≥ 25	21 (63.6)	76 (52.8)	2.4 (0.8-7.4)
Tobacco smoking			
Never	15 (45.5)	76 (52.8)	Reference
Ever	12 (36.4)	61 (42.36)	0.9 (0.3-2.4)
Type of antireflux surgery			
Partial fundoplication	9 (27.3)	33 (22.9)	Reference
360 degree fundoplication	17 (51.5)	78 (54.2)	0.7 (0.3-2.1)
Other	5 (15.2)	30 (20.8)	0.4 (0.1-1.5)

*Matching was made for age, sex, and calendar year of antireflux surgery, and adjustments were made for sex, age, BMI, tobacco smoking, recurrent reflux and type of antireflux surgery.
Percentages not adding up to 100% are due to missing data.

Paper IV – Infection with human papillomavirus (HPV) in relation to site of squamous cell carcinoma of the esophagus

During 1999 and 2006, 348 new cases of esophageal squamous cell carcinoma were reported in the Stockholm County. Of these, 67 (19%) were excluded because of tumor misclassification (n=3, 1%), diagnosis based on autopsy results only (n=11, 3%), or failure to identify any endoscopic biopsy (n=53, 15%). Among the 281 (81%) eligible patients, 77 (27%) were classified as non-participants due to absence (n=26, 9%) or lack of tumor material (n=51,18%). In the end, 204 patients (73%) remained for inclusion in the study. Sex and age distributions were similar between the groups, although tumor location differed due to missing data (Table 9).

Table 9. Differences between included and excluded patients.

Variable	Eligible patients		Excluded patients		
	Participants	Non-participants	Misclassification of tumor	Missing, not found	Detected at autopsy
	Number (%)	Number (%)	Number (%)	Number (%)	Number (%)
Total	204 (59)	77 (22)	3 (1)	53 (15)	11 (3)
Gender					
Men	127 (62)	44 (57)	3 (100)	38 (72)	9 (82)
Women	77 (38)	33 (43)	0 (0)	15 (28)	2 (18)
Age at diagnosis (years)					
< 60	37 (18)	16 (21)	2 (67)	3 (6)	2 (18)
60-70	76 (37)	26 (34)	0 (0)	22 (42)	4 (36)
> 70	91 (45)	35 (45)	1 (33)	28 (53)	5 (45)
Tumor location					
Proximal	53 (26)	17 (22)	1 (33)	4 (8)	1 (9)
Middle/distal	147 (72)	56 (73)	1 (33)	29 (55)	4 (36)
Unspecified/missing	4 (2)	4 (5)	1 (33)	20 (38)	6 (55)

Among the 204 included patients, 20 (10%) were positive for HPV DNA; 18 (90%) of these tumors harbored HR HPV types, 1 (5%) pHR and 1 (5%) LR types (Table 10).

Table 10. Human papillomavirus (HPV) types present in esophageal squamous cell carcinoma.

HPV type	Number (%)
High-risk	
HPV 16	13 (65)
HPV 33	1 (5)
HPV 45	1 (5)
HPV 51	2 (10)
HPV 52*	1 (5)
HPV 73	1 (5)
HPV 82*	1 (5)
Putative high-risk	
HPV 66	1 (5)
Low risk	
HPV 42	1 (5)

*Double infection with HPV 16

The proportion of HPV positive tumors was higher in men than women (11% versus 8%) and highest in younger subjects. The proportion of HPV positive tumors was reduced with decreasing tumor differentiation. The prevalence of p16 expression was 24% in HPV-positive tumors and 16% in HPV-negative tumors, although these results were not statistically significant.

Association between HPV and tumor site

The majority of the HPV-positive tumors (55%) were located in the middle third of the esophagus and the HPV-negative tumors were more frequently found in the distal esophagus (42%), although no statistically significant differences were observed (data not shown).

Patients with HPV positive tumors were no more likely to have an proximal tumor than one in a middle or distal location (adjusted OR 0.6, 95% CI 0.2-1.9, Table 11). When comparing the proximal or middle versus distal tumor site the OR was possibly increased (OR 2.1, 95% CI 0.7-6.3), although results were not statistically significant.

Table 11. Risk of different sites for esophageal squamous cell carcinoma when exposed to HPV, expressed as OR with 95% CI.

Variable	Proximal	Middle/distal	Crude model	Adjusted model*
	Number (%)	Number (%)	OR 95% CI*	OR 95% CI[#]
Total	53 (27)	147 (74)		
HPV status				
Negative	49 (92)	131 (89)	Reference	Reference
Positive	4 (8)	16 (11)	0.7 (0.2-2.1)	0.6 (0.2-1.9)

* Adjustments made for sex, age and tumor differentiation.

DISCUSSION

Methodological considerations

General

Epidemiology consists of two main study types: *experimental* and *observational*. In clinical settings, *experimental studies* refer to clinical trials, ideally randomized, where observed study participants are assigned to one of several exposures according to the researchers' predetermined conditions.[281] *Observational studies* describe the natural occurrence of exposures and outcomes which might introduce systematic errors.
There are several differences between the main study types. To summarize, large randomized experimental studies are considered to have the highest validity, due to randomization contributing to the decreased occurrence of systematic errors and are ranked highest in the hierarchy of studies in evidenced based medicine (EBM).[282] However, some experts argue that the internal validity of individual studies should determine the hierarchy rather than the study design.[283] Experimental studies interfere with the natural course of events and are not feasible or ethically possible for certain research hypotheses, especially not when investigating etiological factors and observational studies are required.
This thesis investigated etiological factors for esophageal cancer using observational study design exclusively.

Study design

The studies included in this thesis are based on various epidemiological designs, some of which are relatively uncommon. Cohort-, case-control- and cross sectional studies are all examples of observational studies. A cohort study measures the frequency of a disease in exposed and unexposed participants; a case-control study assesses exposure measurements in participants with a specific disease and compares them to individuals without the disease, and a cross sectional study samples information on both the exposure and the disease at the same point in time.

Cohort study

Cohort studies have been described as "*the archetype of all epidemiological studies*".[284] They consist of a group of individuals with specific characteristics (exposed or unexposed) followed over time to measure the disease occurrence within the group at the end of follow-up. A cohort study usually consists of two groups (exposed and unexposed) and compares their disease rates.
The main advantages of cohort studies include obtaining complete information on the study exposure, temporal measurement of the exposure and disease, reduced risk of selection bias and recall bias when using a prospective design, and the ability to study multiple outcomes. The

main drawbacks of cohort studies are their inefficiency and high costs, usually due to long induction time and large quantity of individuals followed.

Case-control study

In a case-control study, individuals with a specific disease (cases) are identified and compared to individuals from the same source population without the disease under study (controls). The control group aims to mirror the exposure characteristics occurring in the source population and controls should be chosen after careful consideration in order to reduce selection bias. The exposure is usually measured retrospectively which might introduce recall bias.
Case-control studies are, in general, more efficient than prospective cohort studies. The use of sampling instead of following a cohort for several years is both cost- and time efficient, making the case-control study design suitable for esophageal cancer with its low incidence and long induction time. Disadvantages of the case-control design include the risk of selection bias when choosing controls, recall bias when collecting data of study exposure retrospectively and confounding.

The case-control study design was used in papers I, III, and IV in this thesis. Paper I was a prospective population-based case-control study covering new cases of esophageal and cardia adenocarcinoma in the Swedish population. The exposures and outcome were measured retrospectively. Controls were randomly selected from the Swedish Register of the Total Population through frequency matching within a ten-year age and gender strata in order to mimic the distribution of the cases.
Paper III was a population-based nested case-control study within a cohort of all antireflux operated patients in Sweden. Study exposures were obtained through examination of medical records from the primary antireflux surgery and up to two years after. Outcome was assessed through the linkage of personal identity numbers to the Swedish Cancer Register.
Paper IV was a population-based case-control study conducted within the Stockholm County. Exposure was assessed through laboratory measures and outcome was assessed through the examination of medical records.

Cross-sectional study

In contrast with longitudinal studies, in which information is collected at more than one time point, cross-sectional studies sample information from the source population at only one point in time; a cross-sectional study examining prevalence is known as a *prevalence study*. The advantages of cross-sectional studies are that they are generally easy and inexpensive to implement. The disadvantages include the inability to establish temporal relations, i.e. difficulty in assessing time order of exposure and outcome resulting in an increased risk of reverse causality.

A cross-sectional design was used in paper II, when population prevalence of known risk factors for esophageal adenocarcinoma was compared in the English and Swedish populations. The study only investigated differences in population prevalence of known risk factors.

Population-based

A study representative of an entire population is said to be population-based. In order to define a case-control study as population-based, all cases, or a random sample (representative of all cases) of cases and their corresponding randomly selected controls from a predetermined population for a specific time period, have to be included. In reality, 100% completeness is rarely possible but a high participation rate is important. The greatest advantage with the population-based study design is the minimal risk of selection bias. Sweden has the advantage of virtually free public health care in combination with large and complete population registers. Cancer cases are typically managed in the public health care system and registered in the Swedish Cancer Register making this country highly suitable for population-based cancer research. Individuals are identified through their unique personal identity number, and information can be linked between several population registers.
All studies included in this thesis were more or less population-based, depending on which level of non-participation rate is acceptable when using this definition. Nevertheless, papers I and III covered the entire Swedish population and paper IV included the entire population of the Stockholm County. Paper II covered two populations including the entire Swedish and defined parts of the English population.

Validity

The main objectives of epidemiological studies are to determine a valid and precise estimate of the studied hypothesis. There are two major sources of error that can be problematic in epidemiological studies: systematic error, the opposite of validity (commonly referred to as bias), and random error, the opposite of precision (further described below).
Validity can be separated into two components: internal and external, where internal validity refers to the degree of systematic error within the study, i.e. to what extent the study measures what it aims to measure. The internal validity is of importance when evaluating study quality and high internal validity is necessary for external validity (generalizability). External validity (further described below) refers to what degree findings can be translated into other populations, i.e. the ability to apply results to populations outside the study. Internal validity is commonly classified into three groups: selection bias, information bias and confounding.

Selection bias

Selection bias is introduced if the exposure or the disease under study, influences the selection of study participants i.e. the exposure or disease patterns, differs between participants and non-participants. A historically famous example of selection bias is the “healthy worker effect”, where workers included in a study are healthier than the average population, resulting in differences between study participants and non-participants regarding exposure (workers have a more healthy lifestyle) and disease (low incidence of disease). In this thesis, population-based study designs were used in all studies as an attempt to reduce selection bias, although it cannot be ruled out.

Paper I was based on the SECC-study, which had a high participation rate (85%) among the cases, which indicates a limited risk of selection bias. The participation was, however, lower in control subjects (72%), which introduces a higher risk of selection bias. In order to evaluate whether these subjects were different from the participants, separate analyses were performed on 24 control subjects who initially refused to participate but then changed their mind after some persuasion. Analyses showed no differences between participants and the 24 control subjects regarding background data or risk factor exposure, indicating that non-participating controls were not obviously different from those who participated.

Paper II was based on a questionnaire filled out by a random sample of the English and Swedish populations. The recruitment of participants was different in the English and Swedish populations with regards to sampling and inclusion which might have consequently introduced selection bias. Selection bias might also have been introduced from non-responders, especially in the English population, where the participation rate was limited (43%). Unfortunately, no analyses were available to investigate differences between participants and non-participants in this study, and selection bias cannot be ruled out.

In paper III, cases and controls were selected from the same cohort with a good rate of participation (86% and 75%). Therefore, the risk of selection bias should be low.

Paper IV had a large proportion of non-participation which was mainly due to lack of tumor material. Non-participants always potentially introduce selection bias although, when comparing participants to non-participants (Table 8), the groups were equally distributed regarding sex, age and the available tumor location indicating that selection bias might not have been a substantial error in this study.

Information bias

Information bias, also known as misclassification, is introduced when information about study subjects is incorrectly assimilated. Misclassification of exposures or outcomes can be non-differential or differential depending on whether the level of misclassification differs between cases and controls. *Non-differential misclassification*, to some extent present in almost all studies, is unrelated to the outcome although it dilutes the estimate, i.e. moves the estimate closer towards the null value. *Differential misclassification* implies that misclassification differs between cases and controls which can then distort the estimate in either direction. Recall bias is a form of misclassification of particular concern in case-control studies when exposure is collected retrospectively, i.e. after the outcome or disease has occurred. Cases, affected by the disease, tend to recall exposure differently compared with controls, thus introducing differential misclassification.

Paper I has several potential risks of differential misclassification of the exposure. Non-blinded professional interviewers conducted the interviews, however, they were unaware of the study hypothesis and specially trained to treat cases and controls no differently. Recall bias regarding exposure of gastroesophageal reflux symptoms, which occurs in the same organ as the cancer, is also possible. However, recall bias does not seem to be of importance since information on both adenocarcinoma and squamous cell carcinoma was collected without similar results of exposure. Reverse causality, i.e. the outcome (esophageal tumor) causing (gastroesophageal reflux) symptoms which are interpreted as exposure, was also considered and avoided by all

questions focusing on a time period at least 5 years prior to diagnosis. Misclassification of disease is rather unlikely, as sizable efforts were made to ascertain the exact tumor location and type and tumor material was thoroughly re-examined by an experienced pathologist.

Self-reported questionnaires used in paper II could have introduced non-differential misclassification of the exposure, although this ought to be of limited importance. The collection of BMI data, which was measured in the English population and self-reported in the Swedish, is a greater cause for concern. Although there are reports implying that anonymous self-reported height and weight are fairly accurate,[276] it is likely that differential misclassification of exposure may have been introduced and results including BMI should be interpreted with caution. Another concern is that data was collected during different time periods, in the late 1990s in the English population and in 2008 in the Swedish population. The prevalence of the evaluated risk factors might have increased during this time,[120 285] making it possible that the true magnitude of difference between the two populations is rather greater than that observed.

Paper III was partly based on data collected from medical records, which might have introduced differential misclassification. The examiner was, however, blinded to case-control status and records were created long before the outcome of the disease which should reduce any such error. Moreover, only medical records from the point of a primary antireflux operation and up to two years after surgery were collected, in order to avoid obtaining information about esophageal cancer. This would make the misclassification of exposure similar in both cases and controls (non-differential). Separate analyses were also performed for cases that developed esophageal or cardia adenocarcinoma at least 5 years after surgery so as to exclude the risk of earlier detection of currently undiagnosed cancer, i.e. detection bias. An advantage of paper III was that both cases and controls shared the pre-operative risk factor profile, including gastroesophageal reflux, since antireflux surgery is typically performed only in patients with severe reflux. Disease misclassification is unlikely in this study since cases were identified through the valid and complete Swedish Cancer Register.

In paper IV, misclassification of the HPV-exposure might have been introduced due to high sensitivity in laboratory analyses, i.e. HPV DNA irrelevant for tumor development might have been identified, although this misclassification is likely to be non-differential since all examiners were blinded to case-control status. Furthermore, measurement of the outcome might have introduced misclassification, both when clinicians reported tumor location (individualized) and when the investigator examined medical records for tumor location, although any such bias should be limited since a single pathologist re-examined all biopsy specimens.

Confounding

Confounding can be described as the mixing of the effects of a measured exposure and other known or unknown variables.[284] Confounding is of great interest in all epidemiologic study designs, especially in observational studies where randomly assigned exposure is impossible. A confounder must be associated with both the exposure and the disease and not be an effect of the exposure (intermediator) on the outcome.

The relation between gastroesophageal reflux symptoms, obesity and the development of esophageal or cardia adenocarcinoma might illustrate an example of a possible confounder present in papers I-III. Gastroesophageal reflux symptoms (exposure) are causally linked with esophageal and cardia adenocarcinoma[5] and obesity is associated with both gastroesophageal reflux symptoms (increasing the exposure) and these tumors (a risk factor for the outcome) making it a potential confounder. Confounding can be reduced or controlled through the design, e.g. by matching or other planned selection of cases and controls, stratification and use of multiple regression analysis. The use of matching is difficult, since it might introduce bias if selection is based on factors correlated to the study exposure.[281] Matching was used in paper I, and multivariable regression analysis was conducted in all studies.
In paper IV, there was little information about potential confounders, including strong risk factors such as tobacco smoking and alcohol. However, since all included participants had the same disease, the causal exposures other than infection with HPV are unlikely to differ between cases and controls.
To summarize, even though confounding have been carefully considered in this thesis, residual confounding from the studied variables or from other known or unknown variables can never be fully excluded.

Generalizability

Generalizability is, as mentioned previously, the external validity of conclusions in a study, i.e. the ability to apply results to populations outside the study. The external validity is dependent on the internal validity, i.e. how well the study population reflects characteristics of the entire population, which is of greater importance when measuring accuracy in a study. The internal validity needs to be proven before the results can be considered and applied to other populations. Each study needs to be evaluated separately regarding generalizability.

Precision

The precision of a study is determined by the amount of random error or chance, i.e. if the study contains a sizeable random error, the precision will be poor. As opposed to systematic error, where the study design is used to reduce error, random error is decreased by increasing the size of the study population.
Precision, or the amount of random error in a study, can be tested in statistical analyses and presented as a CI or p-value. The CI is calculated from the point estimate and the selected level of significance. If set to 95%, then 95% of the point estimates from all new randomly drawn samples from the same source population will be included in the CI. The p-value is calculated in relation to the null hypothesis and is derived from the same equation as the confidence interval. It is defined as a "measure of the compatibility between the hypothesis and the data".[281] If the data is in accordance with the null hypothesis the p-value is non-significant, i.e. above 0.05. There are two types of chance errors in hypothesis testing, type I and type II.

Type I errors occur when the null hypothesis is rejected even though it is true, which means that the association between the exposure and outcome is statistically significant, even though no true association exists. The probability of making a type I error is dependent on the level of significance used, generally $\alpha=0.05$. Type I errors are a threat to all studies, particularly those in which several analyses are performed, as multiple testing would result in type I errors in 5% of the tests if $\alpha=0.05$. In this thesis, attempts were made to avoid or reduce risk of type I error by writing and following study protocols where hypotheses, categorization of variables and choice of statistical analyses were pre-determined.

Type II errors occur when the null hypothesis is not rejected when it is false, meaning that there is an association between the exposure and outcome, although it is not detected. Type II errors may arise when using an inappropriate study design, when collection of exposure and outcome data is poor, or when the statistical power is low. Power is defined as the ability of the test to correctly reject the null hypothesis and is dependent on sample size, level of significance and the size of the effect measured. It is usually set to 80%, meaning that every fifth test will not detect a true association. The statistical power was limited in evaluating cancer risk in papers I, III and IV due to the low incidence of esophageal and cardia cancer.

Finally, there is an ongoing discussion in the research community regarding quality of studies being determined by statistical significance (p-value < 0.05) rather than biological background and validity.[281] Therefore, it is important to point out that both CI and p-values assume that there are no systematic errors and that the statistical significance is of limited importance when implicating results from a study.

Findings and implications

Difference in risk factor profiles in women and men

Contradictory to the study hypothesis, paper I found no decreased strengths in the risk factors of esophageal or cardia adenocarcinoma (gastroesophageal reflux symptoms, obesity and tobacco smoking) in women compared to men. Furthermore, the protective factors (high intake of fruit and vegetables, infection with H. pylori) occurring in men were absent in women.

Paper I showed the important role of gastroesophageal reflux symptoms and obesity in the etiology of esophageal and cardia adenocarcinoma in both genders. Exposure to tobacco smoking only moderately increased the risk of cancer, whereas the protective role of high intake of fruit and vegetables and H. pylori infection was seen only in men. Paper I was in agreement with another large population-based case-control study examining risk factors for esophageal cancer in relation to gender.[7] The association between obesity and cancer development was similar in women and men (OR 1.9, 95% CI 0.9-4.1 compared to 1.7, 95% CI 1.2-2.5) although the results in women were statistically non significant. Statistical power was a main problem in paper I, resulting in limited precision. The problem arose as esophageal and cardia adenocarcinomas are rare diseases, especially in women. If the statistical power in paper I had been stronger, we might have been able to confirm that known risk factors are in fact stronger in women. However, the results indicate that the association between known risk factors and the risk of esophageal and cardia adenocarcinoma are similar in both genders, which cannot explain the male predominance in these types of cancer.

Paper I showed no difference in risk factor profiles between women and men, which is contrary to our study hypothesis and to what would be expected since men have a much higher incidence rate of esophageal and cardia adenocarcinoma (1:3-7).[11 20 44 84 90] A possible explanation is that women are exclusively protected from developing these cancers by some other yet unidentified factor. The role of estrogen as a protective factor has been suggested and evaluated without any clear results. Some studies indicate that a history of breast-feeding is associated with a decreased risk of esophageal and cardia adenocarcinoma[286 287] although other studies of estrogen show no protective effect or inconclusive results.[95 96 288] Furthermore, higher production and concentration of gastric acid,[91] higher prevalence of hiatus hernia, abnormal 24-h pH test and defective LES in patients with symptomatic gastroesophageal reflux[92] and abdominal/android obesity[93] resulting in higher intra-abdominal and intra-gastric pressure are all more common in men and have been suggested to explain the male predominance. However, more research is needed before the role of each of these factors can be established as an explanation for the male predominance.

In conclusion, paper I did not find an explanation for the male predominance in esophageal and cardia adenocarcinoma; established risk factors are similarly strong in both women and men.

Difference in population prevalence of risk factors for esophageal adenocarcinoma

Paper II suggests that the prevalence of the established risk factors for esophageal adenocarcinoma, i.e. gastroesophageal reflux symptoms and obesity, are higher in the English population compared to the Swedish which might contribute to the explanation of the significantly higher incidence of esophageal adenocarcinoma in England.

The incidence of esophageal adenocarcinoma is approximately five times higher in England than in Sweden.[45] Results from paper II are in line with the hypothesis that this difference is due to the higher prevalence of established risk factors for esophageal adenocarcinoma in the English population. Some previous research also shows that the prevalence of gastroesophageal reflux symptoms[117] and obesity[155] is higher in the English population. The frequency of tobacco smoking was found to be similar in the populations but it is considered as a weaker risk factor.[43 172] Compared with previous studies, paper II is the first to use population-based sampling with an identical questionnaire in both populations. Although BMI was measured differently and data collection was performed at two different point in time, it might not explain the differences between the populations, since anonymous self-reported values of current weight have been validated and found to be accurate measurements of the true value[276] and the prevalence of risk factors have rather increased in the English population since data was collected.[120 285] Furthermore, difference in sex and age distribution between the two samples would not explain the results since these factors were adjusted for in the logistic regression analysis.

Esophageal cancer is a form of cancer associated with late diagnosis[12] and poor prognosis[13] and the incidence of esophageal adenocarcinoma has increased very rapidly during the last three decades[3 4 11 83-85] making preventive measures highly desirable and important. Paper II provides evidence indicating that the higher incidence of this type of cancer is due to the higher population prevalence of gastroesophageal reflux symptoms and obesity. This raises the question that if we can reduce these factors, will it also reduce the incidence of this cancer form? As yet neither medical nor surgical treatment against gastroesophageal reflux has been shown to have a cancer protective effect (further discussed below in paper III)[27 34 145-149 152] and regarding obesity, paper II adds yet another reason for aiming to keep BMI within a normal range.

In conclusion, the prevalence of the exposures reflux and obesity are at least several times more common in the English population thus supporting our hypothesis that the higher incidence of esophageal adenocarcinoma in the English population can be due to the higher presence of these risk factors.

Risk of cancer after antireflux surgery

Paper III provides some evidence that recurrent reflux is more common in antireflux operated patients who subsequently develop esophageal or cardia adenocarcinoma compared to those who do not develop this cancer form. However, other established risk factors for this cancer, i.e. obesity and tobacco smoking and type of antireflux surgery do not seem to have any influence on development of esophageal or cardia adenocarcinoma.

The antireflux cohort used in this study has previously revealed no cancer protective effect from antireflux surgery[145] and 0.45% (64/14,102) of patients, which is equivalent to the proportion of patients with BE (0.1-0.6%),[37-40] did go on to develop cancer. The purpose of paper III was to investigate risk factors that can identify patients that will or will not develop esophageal or cardia adenocarcinoma after antireflux surgery. The results indicate that recurrence of reflux is a key exposure and results are in line with some animal studies, suggesting that subtotal reflux control with continued acid pulses is likely to increase proliferation and decrease differentiation in Barrett's epithelium.[289 290] Furthermore, some clinical studies suggest that recurrent reflux after surgery might explain the failed protective effect of antireflux surgery.[149 150] It is also known that antireflux surgery is not guaranteed to control GERD. A substantial number of patients suffer from recurrent GERD and need antireflux medication after such surgery.[291]
Other risk factors such as overweight and tobacco smoking seem to be of less importance after antireflux surgery although paper III has limited statistical power. A surgical technique of "other" choice (mostly plication of crural crus or vagotomy, no fundoplication) seems to decrease the risk of cancer, which is not too surprising since the removal of the vagal nerve will decrease the level of gastric acid secreted, i.e. reducing the gastroesophageal reflux. However, the operation has many side effects making it unsuitable for most patients.

With regards to prevention, gastroesophageal reflux is the strongest risk factor for esophageal adenocarcinoma,[5] no cancer protective effect has been observed from either medical or surgical treatment of this condition and, as a result, antireflux surgery is no longer recommended as cancer protective treatment and should not be performed with this purpose in mind.[27 34 145-149 152] Our study provides results that might explain the lack of cancer protective effect after surgery and suggests that separate analyses might be relevant for patients with and without recurrent reflux when examining the cancer preventive results of surgery.

In conclusion, paper III indicates that recurrence of reflux is a strong risk factor for the development of esophageal adenocarcinoma after antireflux surgery. This finding could at least partly explain the lack of cancer preventive effect of antireflux surgery. Endoscopic surveillance might be considered in patients who develop recurrent GERD after antireflux surgery.

HPV infection and tumor location

Paper IV does not provide any support for an increased rate of HPV infection in patients with a proximal location of squamous cell carcinoma in the esophagus compared with a more distal site. The study also indicates a limited presence of HPV in esophageal squamous cell carcinoma in a Swedish population and does not support a carcinogenetic role of HPV in tumors based on $p16^{INK4a}$ data.

Infection with HPV is the strongest risk factor for cervical cancer[1] and has recently also been accepted as a causal factor for oropharyngeal cancer.[242 243] The relation between esophageal squamous cell carcinoma and HPV infection is unclear and results vary from total absence to more than 70% of tumors being positive for HPV.[258-267] This situation is reminiscence of the results in head-neck squamous cell carcinoma before researchers realized that HPV was connected to oropharyngeal cancers and not all tumors in the oral cavity. For this reason, and because of the suspected oral route of viral transmission, we hypothesized that tumor location is associated with presence of HPV infection. However, results from paper IV did not support this hypothesis. In addition, the presence of HPV in general was low (10%), and since no correlation with p16 was found, it is questionable as to whether the presence of the virus actually causes esophageal squamous cell carcinoma. Compared to other European studies, paper IV found a slightly lower prevalence of HPV DNA in squamous cell tumors.[265-267] Possible explanations might be geographic differences or use of a smaller DNA sample, although this might be of limited relevance since a highly sensitive laboratory method was used.[234] Other concerns include selection bias introduced by patients excluded from certain hospitals and known risk factors (tobacco smoking and alcohol abuse) not being directly measured. However, the design comparing patients with esophageal squamous cell carcinoma counteracts confounding by these risk factors. Another methodological advantage was the use of endoscopic biopsies in all participants since it avoided introduction of bias when detecting HPV in different tumor sites.

The highest prevalence of HPV DNA was found in the middle part of the esophagus. This might merely be an effect of chance or speculatively this might be a true finding. The middle part of the esophagus, the transition zone (between striated muscles in the proximal part and smooth in the distal part of the esophagus) holds no significant contraction amplitude when measuring peristaltic contractions[292] and this region, together with the proximal part of the esophagus, is collapsed during resting state which might allow virus particles to implant more easily, although this is highly speculative.
To our knowledge, this is the first study comparing the association between HPV infection and the development of esophageal squamous cell carcinoma in different locations as the primary endpoint.

In conclusion, paper IV revealed no support for the hypothesis that HPV infection would be more likely to cause proximal than distal esophageal tumors.

CONCLUSIONS

- Differences in risk factor profiles of known risk factors between women and men do not seem to explain the male predominance in esophageal and cardia adenocarcinoma.

- Prevalence of the risk factors gastroesophageal reflux symptoms and being overweight seem to be more common in the English population compared to the Swedish and this may in turn contribute to the higher incidence of esophageal adenocarcinoma in England.

- Recurrence of reflux after antireflux surgery is a risk factor for subsequent development of esophageal adenocarcinoma and might explain the lack of a cancer preventive effect of antireflux surgery. Endoscopic surveillance may be considered in patients who develop recurrent GERD after antireflux surgery

- There is no apparent increased occurrence of HPV DNA in esophageal squamous cell carcinomas located in the proximal compared to the more distal parts of the esophagus.

FUTURE STUDIES

The results from this thesis give rise to some additional questions that need to be resolved in future studies.

The male predominance in esophageal adenocarcinoma remains a mystery. Paper I suggested that differences in risk factor profile cannot explain the prevailing gender difference. Further studies are needed, including investigations of the role of sex hormones.

Due to the poor prognosis associated with esophageal cancer, there is an urgent need for preventive measures. Paper II suggests that a higher population prevalence of known risk factors increases the incidence of esophageal adenocarcinoma. Preventive measures against GERD and obesity would appear to be required. Paper III, however, suggests that antireflux surgery might not be a good cancer preventive option for patients with severe GERD although further studies are needed in order to be sure of these results.

Paper IV dismissed HPV as a risk factor associated with a proximal location of esophageal squamous cell carcinoma although more studies are needed. Future research needs to determine a universal laboratory technique for detection of HPV to make comparison of results between studies possible.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Bakgrund

Matstrupscancer är den åttonde vanligaste cancerformen och den sjätte vanligaste dödsorsaken till följd av cancer i världen. Det finns två huvudtyper av matstrupscancer, skivepitelcancer (en malign tumör som uppstår i matstrupens normala ytskikt) och adenocarcinom (en malign tumör som uppstår när matstrupens normala ytskikt ersatts av ett körtelformat ytskickt). Skivepitelcancer dominerar globalt, men under de senaste decennierna har förekomsten av adenocarcinom ökat snabbt och utgör numera ungefär hälften av alla matstrupscancrar i västvärlden. Riskfaktorer för skivepitelcancer är främst rökning och högt alkoholintag, medan högt intag av frukt och grönsaker har skyddande effekt. För adenocarcinom är gastroesofagal refluxsjukdom (reflux) den dominerande riskfaktorn tillsammans med fetma och manligt kön, medan rökning enbart medför en något ökad risk. Högt intag av frukt och grönsaker samt infektion med bakterien Helicobacter pylori (H. pylori) har visat sig ha skyddande effekt.
På grund av matstrupens höga elasticitet ger tumörer i detta organ som regel inte symtom förrän sent i förloppet, vilket bidrar till matstrupscancerns ytterst dåliga prognos. Minst hälften av alla patienter har en inoperabel tumörsjukdom eller metastaser (”dottertumörer”) vid diagnostillfället. Trots försök att utveckla och förbättra behandlingen är det fortfarande bara cirka 10% av patienterna i Europa som överlever mer än 5 år. Förebyggande åtgärder är därför av yttersta vikt.

I avhandlingen ingår följande delarbeten:

Arbete I: Könsspecifik riskfaktorprofil vid adenocarcinom i matstrupen.
Arbete II: Kan ökad förekomst av reflux och fetma i England jämfört med Sverige förklara varför England har en mycket högre förekomst av adenocarcinom i matstrupen?
Arbete III: Riskfaktorer för adenocarcinom i matstrupen efter antirefluxoperation.
Arbete IV: Infektion med humant papillomavirus i relation till tumörlokalisation av skivepitelcancer i matstrupen.

Arbete I: Matstrupscancer drabbar huvudsakligen män. Förekomsten av skivepitelcancer är dock lika mellan könen när skillnader i rök- och alkoholvanor korrigerats för. Det finns däremot ingen förklaring till att adenocarcinom är betydligt vanligare bland män, med en könsfördelning på hela 7:1. Vår hypotes var att mansdominansen i adenocarcinom till viss del kan förklaras av att riskfaktorprofilen skiljer sig mellan kvinnor och män.
Arbete I testade hypotesen genom att studera alla kvinnliga och manliga fall av adenocarcinom i Sverige mellan 1 december 1994 och 31 december 1997. Cancerfallen jämfördes med slumpmässigt utvalda kontrollpersoner ur den svenska befolkningen. Samtliga deltagare intervjuades av professionella intervjuare från Statistiska Centralbyrån angående riskfaktorer för adenocarcinom i matstrupen (reflux, fetma, rökning, samt intag av frukt och grönsaker). Blodprov samlades även in för att se om deltagarna var infekterade med bakterien H. pylori.
Resultaten var i stort sett raka motsatsen till vår hypotes. Riskfaktorerna reflux, fetma och rökning var lika starkt associerade med matstrupscancer hos både kvinnor och män, samtidigt som de skyddande faktorerna (högt intag av frukt- och grönsaker, samt infektion orsakad av H. Pylori) visade liten eller ingen skyddande effekt hos kvinnor. Eftersom sambandet mellan

etablerade riskfaktorer och adenocarcinom i matstrupen var lika för båda könen, talar resultaten för att kvinnor har någon typ av okänd skyddsmekanism som motverkar utveckling av denna typ av cancer.

Arbete II: Det finns stora och oförklarade skillnader i förekomst (incidens) av adenocarcinom i matstrupen mellan olika västerländska populationer. Storbritannien har den högsta incidensen i världen, cirka 5 gånger högre än i Sverige. Kunskap om orsakerna till denna skillnad skulle kunna förklara vilka faktorer som ligger bakom de senaste decenniernas snabba incidensökning i västvärlden, och därmed öppna nya dörrar för förebyggande åtgärder.
Arbete II jämförde förekomsten av de väletablerade riskfaktorerna för adenocarcinom i matstrupen (reflux, fetma och rökning) mellan den engelska och svenska befolkningen. Ett slumpmässigt urval av befolkningarna i England och Sverige i åldrarna 40-59 år, besvarade en enkät innehållandes detaljerade frågor om dessa riskfaktorer.
Resultaten visade att förekomsten av reflux och fetma var betydligt högre i den engelska befolkningen jämför med den svenska, något som skulle kunna förklara den högre incidensen av adenocarcinom i England.

Arbete III: Reflux, den dominerande riskfaktorn för adenocarcinom i matstrupen, är en folksjukdom som drabbar cirka 10-20% av den vuxna västerländska befolkningen. Reflux behandlas oftast medicinskt, men kan även i svåra fall behandlas med kirurgi, vilket mekaniskt motverkar att magsyra når matstrupen. Något överraskande finns i dagsläget inga belägg för att varken medicinsk eller kirurgisk refluxbehandling leder till minskad risk för utveckling av adenocarcinom i matstrupen. Tänkbara orsaker till att kirurgisk behandling inte har en förebyggande verkan är att operationen inte är tillräckligt effektiv eller att andra riskfaktorer för adenocarcinom (fetma och rökning) spelar in och är vanligare bland de patienter som utvecklar cancer efter operation.
Arbete III undersökte faktorer som kan skilja patienter som utvecklar adenocarcinom i matstrupen efter antirefluxkirurgi från dem som inte gör det. I studien identifierades samtliga patienter som behandlats med antirefluxkirurgi och utvecklat adenocarcinom mellan 1965 och 2006. För varje fall valdes 4-5 kontrollpersoner slumpmässigt från de patienter som behandlats med antirefluxkirurgi samtidigt som fallen, men inte utvecklat cancer. Journaler granskades för både fall och kontroller, med avseende på återkomst av reflux efter operationen, samt fetma och rökning.
Resultaten visade att de patienter som utvecklade matstrupscancer hade betydligt större benägenhet för återkommande reflux efter operationen, något som skulle kunna förklara varför man inte observerat någon skyddande effekt av antirefluxkirurgi. Arbete III indikerar att patienter med återkommande reflux efter antirefluxkirurgi bör övervakas regelbundet och att framtida studier som undersöker den potentiellt preventiva effekten av antirefluxkirurgi bör separera patienter som har och inte har fullgott refluxskydd efter operationen.

Arbete IV: Infektion med humant papillomavirus (HPV), kanske mest känd som den starkast bidragande orsaken till livmoderhalscancer, är även en betydande riskfaktor för skivepitelcancer i svalget. HPV skulle också kunna vara en riskfaktor för skivepitelcancer i matstrupen, men tidigare studier visar tvetydiga resultat och förekomsten av HPV DNA i matstrupstumörer varierar mellan total avsaknad av viruset till närvaro i mer än 70%. Förklaringen till de varierande resultaten kan vara att förekomsten av HPV är olika hög i olika delar av världen, samt att man använt olika laboratorietekniker för att påvisa viruset. En annan möjlig förklaring är att förekomsten av viruset varierar med matstrupstumörens lokalisation. Eftersom man tidigare observerat ett samband mellan HPV och svalgcancer blev vår hypotes att

högt belägen skivepitelcancer i matstrupen är starkare förknippat med förekomst av HPV än tumörer belägna i den nedre delen.
Arbete IV undersökte förekomsten av HPV DNA i förhållande till lokalisation av skivepitelcancer i matstrupen för nya fall i Stockholmsregionen mellan 1999 och 2006. Tumörens lokalisation fastställdes genom journalgranskning och HPV-förekomst detekterades med en PCR-metod. Resultaten visade att tumörerlokalisation inte är associerat med HPV.

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