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EPIDEMIOLOGICAL STUDIES ON GASTROESOPHAGEAL REFLUX DISEASE AND ESOPHAGEAL CANCER

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Epidemiological studies on gastroesophageal reflux disease and esophageal cancer

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By

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To my beloved family

ABSTRACT

Esophageal cancer is the seventh most common cancer and a lethal malignancy causing a considerably high number of deaths around the world. Despite intensive studies on risk factors associated with esophageal cancer, there is currently no promising method for prevention, early detection, or screening of esophageal cancer. Consequently, most patients are diagnosed at an advanced stage, therefore associated with a poor prognosis. On the other hand, the main–stream treatment for esophageal cancer patients is surgery, which might be associated with complex complications or infections, and the 5–year postoperative survival remains low.

Study I investigated sex disparities in postoperative survival after curative surgical treatment in esophageal cancer patients. A total of 1301 esophageal cancer patients who underwent curative surgery between 2006 and 2017 in Sweden were studied. Female patients showed a lower excess mortality rate than male patients, in both subtypes of esophageal adenocarcinoma (EAC) and esophageal squamous cell carcinoma (ESCC). Moreover, the sex difference was more profound in those with early clinical stages, in patients who received neoadjuvant treatment, and without postoperative complications.

Study II used data from the Barrett's and Esophageal Adenocarcinoma Consortium to examine the association between 15 antibodies against *Helicobacter pylori* (*H. pylori*), gastric atrophy and EAC. Seropositivities of all the measured antibodies were associated with lower risks of EAC. Particularly, the inverse association between *H. pylori* and EAC was not mediated by gastric atrophy, body mass index (BMI), gastroesophageal reflux disease (GERD), or the combination of these factors.

Study III explored the association between dental health, which was characterized by a group of dental diseases and remaining teeth number, and esophageal cancer. The study was carried out among 5 million individuals who visited the Swedish dental health care providers and received reimbursement between 2009 and 2016, in the Swedish Dental Health Register (DHR). Specifically, root canal infection at baseline was associated with 41% increased risk for EAC, while periodontitis was associated with an elevated risk for EAC and ESCC. Fewer remaining teeth at baseline was also observed to increase the risks for EAC and ESCC in a dose–response manner. Moreover, these findings were further corroborated when multiple visits during follow–up were included in the models.

Study IV studied the association between atrophic gastritis (AG) and GERD in 12,533 twins. AG, which was measured by serum biomarkers, was shown to be associated with a reduced risk for the occurrence of symptomatic GERD. Additionally, the results were stable when different cut–off values were used to define the AG status. The results were also showed to be independent of familiar factors and genetic factors shared between twins.

In conclusion, sex differentiated treatment should be considered to improve postoperative survival in esophageal cancer patients. *H. pylori* infection is associated with a reduced risk for EAC, dental health measured by specific diseases and remaining teeth number are potential predictive factors for esophageal cancer, and AG is associated with a lower risk for GERD, which is a risk factor for EAC. These studies shed light on etiological factors for esophageal cancer and GERD, and may help the prevention of the occurrence and improve the survival for esophageal cancer patients.

LIST OF SCIENTIFIC PAPERS

I. **Zhang J**, Bellocco R, Ye W, Johansson J, Nilsson M, Lindblad M. Effect of sex on survival after resection of oesophageal cancer: nationwide cohort study. *BJS open*. 2022 Jun;6(3):zrac035.

II. **Zhang J**, Bellocco R, Jia Y, Nasrollahzadeh D, Zagai U, Pawlita M, Waterboer T, Wu AH, Bernstein L, Chow WH, Gammon MD, Risch HA, Cook MB, Vaughan TL, Ye W. The role of gastric atrophy in the association between *Helicobacter pylori* infection and esophageal adenocarcinoma. *(Manuscript)*

III. **Zhang J**, Bellocco R, Sandborgh-Englund G, Yu J, Sällberg Chen M, Ye W. Poor oral health and esophageal cancer risk: a nationwide cohort study. *Cancer Epidemiology, Biomarkers & Prevention*. 2022 Apr 27:OF1-8.

IV. **Zhang J**, Bellocco R, Franzén J, Zagai U, Magnusson PK, Ye W. Atrophic gastritis is inversely associated with gastroesophageal reflux disease in a twin register based study. *United European Gastroenterology Journal*. 2022 Jun 22.

Contents

List of abbreviations

AG	Atrophic gastritis
aSIR	Age–standardized incidence rate
BE	Barrett's esophagus
BMI	Body mass index
DHR	Dental health register
DZ	Dizygotic
EAC	Esophageal adenocarcinoma
ELISA	Enzyme linked immunosorbent assays
EMRR	Excess mortality rate ratio
ESCC	Esophageal squamous cell carcinoma
H. pylori	*Helicobacter pylori*
GERD	Gastroesophageal reflux disease
HR	Hazard ratio
LISA	Longitudinal integrated database for health insurance and labor market studies
MRR	Mortality rate ratio
MZ	Monozygotic
NREV	Swedish National Register for Esophageal and Gastric Cancer
OR	Odds ratio
PGI	Pepsinogen I
PGII	Pepsinogen II
PPIs	Proton pump inhibitors
SALT	Screening Across the Lifespan Twin Study
SCR	Swedish Cancer Register
SIR	Standardized incidence ratio
STR	Swedish Twin Register

Chapter 1

INTRODUCTION

Esophageal cancer is a low prevalent yet devastating disease. It is the seventh most common cancer and the sixth leading cause of cancer related deaths. For a lack of effective prevention strategy and early detection technology, most patients of esophageal cancer are diagnosed at an advanced stage, when there is no curative treatment. Even for patients with the hope of recovery, surgery remains to be the main treatment regimen, which is a complex operation and might lead to potential postoperative complications. Therefore, the survival for esophageal patients remains poor. New methods for early detection and treatment are needed for the improvement of esophageal cancer patients' survival. Although the incidence of most cancers has decreased in the past decades, the incidence of esophageal cancer has shown an increasing trend in developed countries. This is partly due to the elongation of human living time. It is foreseeable that esophageal cancer will cause great disease burden paralleled with the global aging trend.

An inverse association between *Helicobacter pylori* (*H. pylori*) infection and esophageal adenocarcinoma (EAC) was observed, however its mechanism remains unclear, as well as the interplay between atrophic gastritis (AG), gastroesophageal reflux disease (GERD), and other factors associated with EAC. Although a wide range of risk factors have been established to be linked with the risk for esophageal cancer, they did not perform well in identification and prediction of high risk people in the population. The etiology of esophageal cancer remains largely unknown.

This thesis described the sex–specific postoperative survival trend for esophageal cancer, and studied etiological factors related with esophageal cancer and GERD. Specifically, the association between *H. pylori* and EAC, the association between oral health and esophageal cancer, and the association between AG and GERD.

Chapter 2

BACKGROUND

2.1 Esophageal cancer

2.1.1 Overall descriptive epidemiology

The incidence of esophageal cancer is relatively lower than other cancers, but is causing a great worldwide disease burden. Data from GLOBAN2020 shows the incidence of esophageal cancer ranks the seventh and causes 604,100 new cases (9.3 per 100,000 person–years in males and 3.6 per 100,000 person–years in females) (1). There is a substantial geographical difference for age–standardized incidence rates (aSIRs) around the world. The highest rates are in Eastern Asia (12.3 per 100,000 people), and the lowest aSIRs are observed in Central America (0.93 per 100,000 person–years). Moreover, the geographical variation also exists in Europe, with the highest aSIRs in Northern Europe (5.3 per 100,000 person–years) and the lowest aSIRs in Southern Europe (1.8 per 100,000 person–years) (figure 2.1).

Esophageal cancer is causing 544,076 (5.6 per 100,000 person–years) deaths each year around the world, and ranks the sixth leading cause of death among all cancers (1). In accordance with aSIRs, the highest age–standardized mortality rates (aSMRs) are also observed in Eastern Asia (10.7 per 100,000 person–years) and the lowest aSMRs are also reported in Central America (0.9 per 100,000 person–years). Likewise, in Europe, the highest aSMRs are reported in Northern Europe (4.2 per 100,000 person–years) and the lowest aSMRs in Southern Europe (1.5 per 100,000 person–years) (figure 2.2).

In Sweden, the aSIRs exhibited different patterns for males and females. The aSIR constantly increased for males from 1962 until 2014. The upward trend slightly decreased from 1984 to 1994, followed by another escalation after 1994, and a seemingly flatten–out curve was observed after year 2000. On the other hand, the incidence in females dropped from 1964 to 1979, and remained relatively stable afterward. The aSMRs for males and females resembled the trends of aSIRs for males and females, separately (figure 2.3).

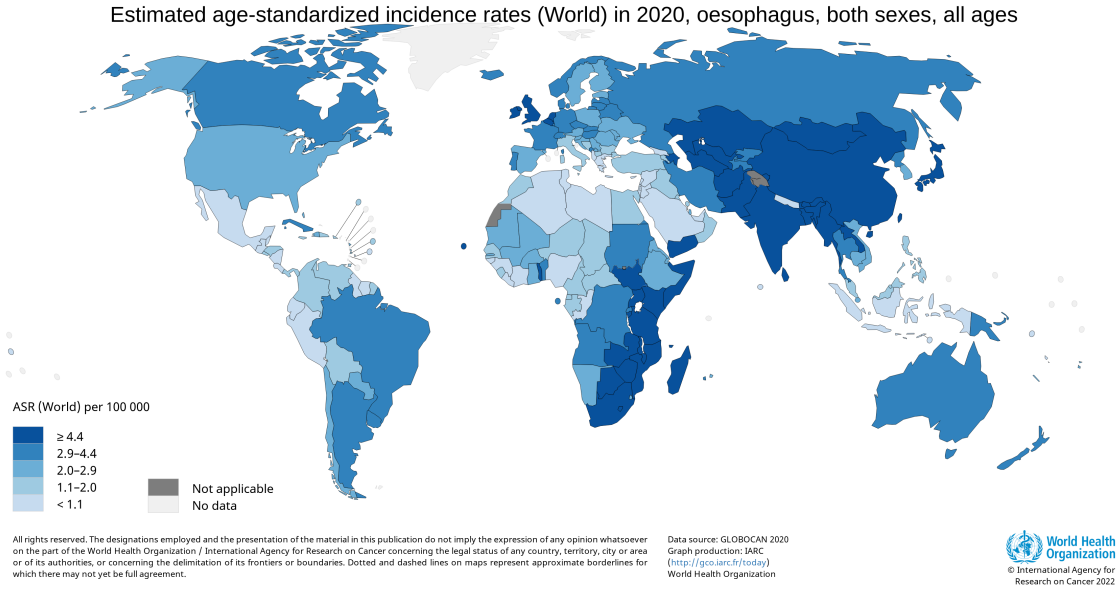

Figure 2.1: Age–standardized esophageal cancer incidence rates per 100,000 person–years, worldwide, 2020. Rates are age-standardized to the World population. Source: GLOBOCAN 2020 (IARC).

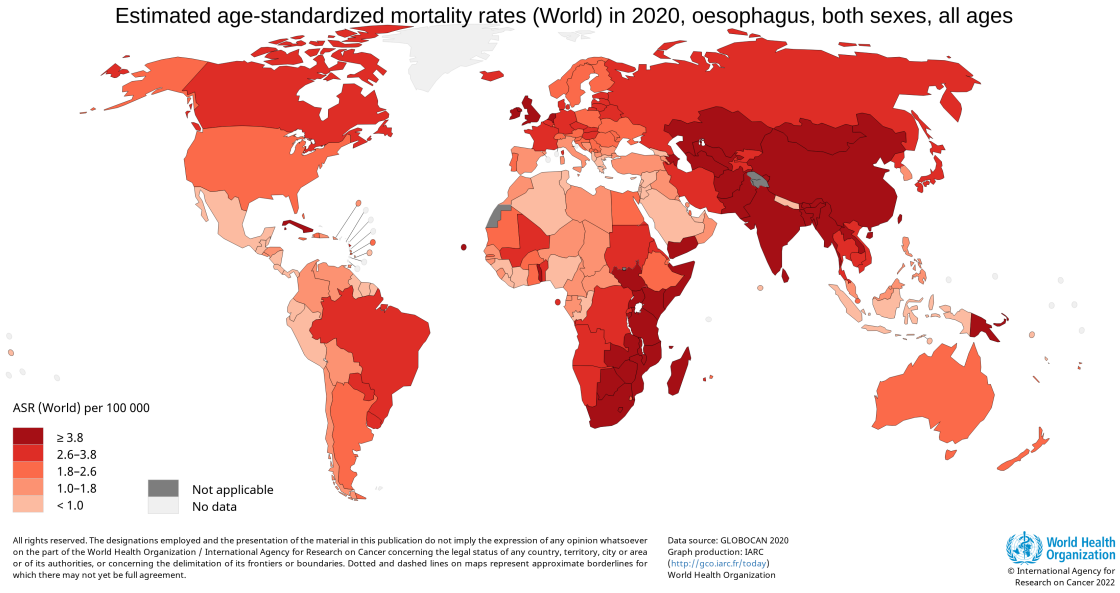

Figure 2.2: Age–standardized esophageal cancer mortality rates per 100,000 person–years, worldwide, 2020. Rates are age-standardized to the World population. Source: GLOBOCAN 2020 (IARC).

2.1.2 Histopathological subtype characteristics

Two main histopathological subtypes of esophageal cancer are esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC). They are different in many aspects, thus are investigated and treated differently. Multiple environmental factors have been suggested to act differently for ESCC and EAC.

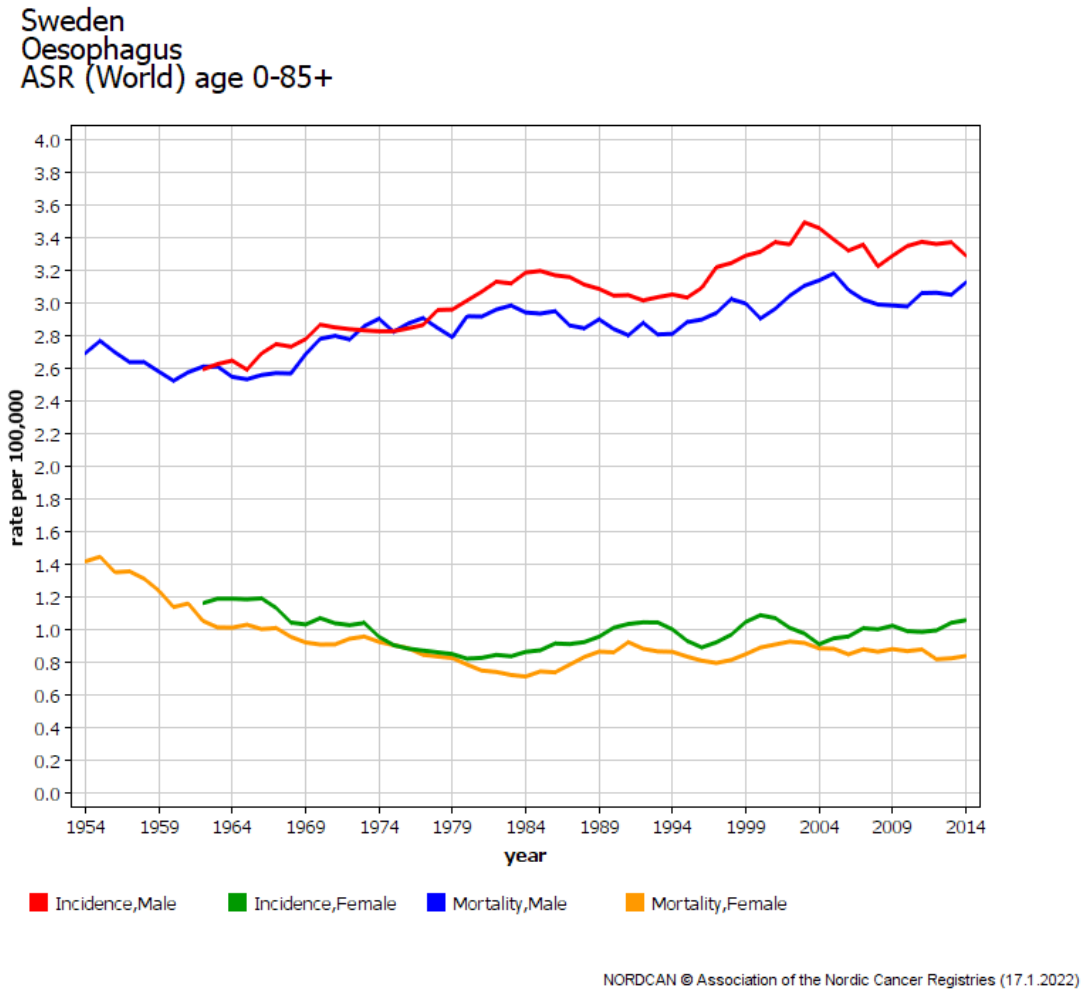

Figure 2.3: Age–standardized esophageal cancer incidence and mortality rates per 100,000 person–years, Sweden, 1954–2014. Rates are age-standardized to the World population. Source: NORDCAN.

Descriptive epidemiology for ESCC

ESCC comprises the main subtype of esophageal cancer in less developed countries, with a relatively high incidence rate and mortality rate. The global incidence rate of ESCC was 5.3 per 100,000 person–years with an estimation of 482,000 affected people in 2018. China was estimated to have 277,000 new cases, contributing 57% of all cases in the globe. Countries in Eastern Asia (63.1%), South–Central Asia (17.6%), and sub–Saharan Africa (4.6%) were estimated with the most incident cases (2). A series of countries starting from eastern Turkey and northern Iran to eastern Asian, including northern and central China have noted an exceptionally high incidence, thus they are named as “Asian esophageal cancer belt” in previous studies (3). The global incidence of ESCC is decreasing in the past 40 years for men, whereas

the time trend for women is inconsistent across different countries. The change in the incidence and mortality rate may be explained by a change of distributions of risk factors associated with ESCC, as well as advancements in early diagnosis and treatment (4).

Descriptive epidemiology for EAC

The global incidence of EAC was estimated to be 0.9 per 100 000 person–years from the statistics in 2018. Countries in Eastern Asia (33.7%), Northern America (17.5%), and Northern Europe (9.6%) contributed most incident cases in the globe (2). The incidence rate of EAC has been increasing since late 1980s, at a relatively high speed, including European countries of UK, Denmark, France, Italy, Netherlands, Spain and Slovakia. Based on data available, the incidence is expected to keep rising at least to 2030. EAC will persist to be a burden for global public health system, especially for some high–income countries (5). Males present to be with higher risk of EAC, with a global average male–to–female ratio of 4 to 5, ranging from 1.03 in Africa to 7.64 in North America, and 6.04 in Europe. The sex ratio is not possible to be explained by the changing exposure to environmental factors like tobacco using, alcohol consumption or *H. pylori* infection (2, 6).

Prognosis of esophageal cancer

Esophageal cancer remains an aggressive malignancy with a poor survival. However, some countries are seeing an improvement in the survival for patients of esophageal cancer in recent years. For the subtype of ESCC, a population based study in Sweden reports that the 5–year survival rate increased from 9% to 12% in the last 20 years with better survival for females (HR=0.86) (7). In Korea, the 5–year survival rate increased from 12.1% to 34.6% (1993-2013) (8). In the Netherlands, the 5–year survival rate improved from 9% to 27% in the period of 2005-2014 (9).

For the subtype of EAC, it is estimated that the survival rate is improving in the past 30 years in the United States. 5–year survival rate increased from 10.9% to 20.1% without apparent sex difference (adjusted HR=1.03) from 1984 to 2013 (10). A population based study in Sweden reports the 5–year survival rate increased from 12% to 15% from 1990 to 2013 without evident sex difference (7). The 5–year survival rate improved from 12% to 36% in the Netherlands from 2005 to 2014 (9). Notably, tumor stage, defined by depth of tumor, nodal involvement and distant metastases, is the best predictor for the survival of esophageal cancer (11). Improvement in the accuracy of staging, introduction of neoadjuvant therapies, centralization of complex surgery and improvement of early diagnosis are thought to be reasons for the improvement of survival in those countries.

2.1.3 Factors related with esophageal cancer

Alcohol consumption

Alcohol consumption is well documented as one of the main risk factors for ESCC, but not for EAC. Alcohol consumption has a dose–dependent relationship with ESCC risk: the risk of ESCC can be as much as 5–fold higher in heavy drinkers, compared to non–drinkers (12, 13), while the risk for ESCC after quitting alcohol drinking dropped 60% after 15 years of cessation (14). The mechanism for the excess risk in association with alcohol is mostly attributed to the oxidation process of ethanol in the liver, thus it is the concentration, rather than the type of alcoholic beverages contributing to its toxicity, but some antitoxic elements in wines could make it less harmful (15). Ethanol is metabolized to acetaldehyde, which is recognized as a carcinogen for the upper digestive tract (16). An imbalanced microbial community in the digestive tract, genetic polymorphisms in genes encoding alcohol metabolism enzymes, and DNA methylation are also involved in this association (17).

Smoking

Tobacco smoking is also one of the strongest independent risk factors for ESCC and a weak risk factor for EAC. Around half of the burden for ESCC is attributable to smoking (18). For the subtype of ESCC, current smokers and former smokers have an elevated risk with risk ratios of 3.13 and 1.68, compared to non–smokers, with a dose effect connecting to the smoking intensity and duration (19). Smoking cessation is associated with a reduced risk for ESCC, but not in EAC, and longer duration of cessation time could further reduce the cancer risk, with 64% of risk reduction for ESCC after 20 years of smoking cessation. The benefit of quitting smoking is stronger in western populations (20). From results of meta–analysis, ever–smokers and current smokers have pooled risk ratios of 1.76 and 2.32 for EAC, compared to non–smokers, and longer duration of smoking is associated with a higher risk for EAC (21). There is a synergetic effect between alcohol drinking and tobacco smoking observed in different studies. From results of a meta–analysis performed for ESCC, alcohol consumption or tobacco smoking alone is associated with 20% to 30% excess risk for ESCC, but alcohol drinking together with smoking could substantially elevate the risk for ESCC to 3–fold or higher (22–25).

Obesity

Obesity has long been investigated to be associated with the occurrence of esophageal cancer, but the risk patterns are different for ESCC and EAC. There are two main measurements for obesity providing different insights for this association: body mass index (BMI) which reflects more of the overall obesity and waist–to–hip ratio, waist circumference or abdominal diameter, which reflect more about the central obesity.

For EAC, higher BMI is consistently linked with a higher risk, with a pooled odds ratio (OR) of 2.2, and the association is more profound than other cancers (26–28). In a study using Mendelian randomization study design, risk for EAC increases by 16% with an increase of

one unit of BMI, providing the strongest evidence for the positive association between BMI and EAC (29). Some more recent studies suggest central obesity to be an independent risk factor for EAC. Increased waist circumference, abdominal diameter, and increase in waist–to–hip ratio are positively associated with the risk for EAC, separately. A pooled OR for central adiposity is estimated to be 2.51 (26, 30–32). After adjusting for BMI, the association between various measurements of central obesity and EAC does not diminish, whereas the association between BMI and EAC disappears after controlling for indications of central obesity, suggesting an independent or even more important role of central obesity, rather than overall obesity, in the etiology for EAC (30).

The association between obesity, regardless of being measured by BMI or central obesity indicators, and ESCC remains controversial. There are some studies reporting no association between abdominal diameter and ESCC (32), whereas an inverse association between BMI and ESCC was observed in some studies (33–35). The association between obesity and ESCC is yet to be validated in larger studies with multiple ethnics. Explanation for this potential inverse association is poorly understood, and one possible hypothesis is due to residual confounding effects of alcohol consumption or smoking (35).

H. pylori

A growing body of studies are reporting a strong inverse association between *H. pylori* infection and EAC, while the association with ESCC may be dependent on population. *H. pylori* is a Gram–negative microaerophilic bacterium, with a special ability to reside in the extremely high acid environment in the stomach. The global prevalence was estimated to be around 40% (36). A global declining trend of the prevalence is observed from different countries, as the consequence of the development of hygiene and urbanization. Although *H. pylori* is one of the most common bacteria infected in the population, it does not necessarily lead to disease for everyone. Multiple host factors, such as psychological status, host genetic polymorphisms, diet and nutritional status, in addition to *H. pylori* strains are associated with clinical outcomes of *H. pylori* infection (37–39).

The latest pooled analysis confirms an inverse association between *H. pylori* infection and EAC, with 45% of risk reduction. *H. pylori* is not associated with ESCC in most studies. In an earlier meta–analysis, *H. pylori* virulent strain of cytotoxin-associated gene A (CagA) is reported to be related with EAC (OR=0.64), but the association between CagA and ESCC is positive in non–Asian population (OR=1.41) whereas negative in Asian population (OR=0.74) (40). These studies suggest *H. pylori* may interact with ESCC only in some specific regions.

H. pylori could lead to harm for the esophagus through several pathological mechanisms. Colonization of *H. pylori* in the esophagus could cause physical damage to the epithelium and dysbiosis in esophageal microbiota, thus lead to chronic inflammation in mucosa. And subsequently induced production of inflammatory cytokines, activation of certain pathways, will further cause carcinogenic changes like DNA damage, genomic instability and alteration of DNA transcription factors (41). It is also hypothesized that *H. pylori* could lead to gastric

atrophy, reduce the acid exposure in the esophagus, and subsequently reduce the risk for gastroesophageal reflux disease (GERD), Barrett's esophagus (BE) and EAC. However, this hypothesis is being challenged that *H. pylori* seems not related with the occurrence of GERD with controversial results from epidemiological studies, thus further studies are needed for the understanding of the association between *H. pylori* and EAC (42).

Proton pump inhibitors

Proton pump inhibitors (PPIs) are suspected to be associated with an increased risk for both ESCC and EAC, yet a protective role in the progression from BE to EAC, but conclusive results have not been obtained from previous studies. Results from a large, population based cohort study shows that long–term maintenance use of PPIs is an independent risk factor for EAC (SIR=3.93) and ESCC (SIR=2.77), compared to general population, even in the absence of diseases related with esophageal cancer, and the risk increases with the duration of drug using (43, 44).

PPIs seem to play a protective role for the prevention of EAC in patients with BE, which is the precancerous disease of EAC. A study with the largest number of participants (case: 300, control: 798) shows 41% lower risk for EAC in patients of BE who are more likely to use PPIs. Results from randomized study also shows high–dose of PPIs could prevent the occurrence of EAC from the metaplasia change in patients of BE, and the preventive effect is strengthened in combination with the use of low–dose aspirin (45). However, results from another meta–analysis show no association between PPIs and EAC in BE patients (46).

Nonsteroidal anti—inflammatory drugs

Nonsteroidal anti–inflammatory drugs (NSAIDs) has been reported to be related with a reduced probability for ESCC and EAC from some case–control studies (47). Following studies with larger sample size, multiple–center design and better control of confounders further corroborate this finding (48–50). Case–control study with the most cases from pooled studies present a 32% risk reduction for EAC in NSAIDs users, with dose–response effects in higher frequency, longer duration of drug using. Evidence from meta–analysis presents odds ratio of 0.58 for ESCC and 0.84 for EAC, respectively. Aspirin alone is estimated to be with 33% and 39% of average risk reduction for ESCC and EAC. Studies also suggest the protective effects of NSAIDs and aspirin may act through the prevention of the transformation from BE to EAC, but results are not conclusive from randomized studies (45, 51, 52).

2.2 Gastroesophageal reflux disease (GERD)

2.2.1 Disease description

GERD is one of the most common gastrointestinal conditions caused by reflux of contents of the stomach into the esophagus. It usually happens in the distal end of the esophagus but can also affect upper regions of the esophagus. It is defined by typical troublesome symptoms of heartburn and regurgitation at least once a week with or without complications such as reflux esophagitis (53). Apart from the typical symptoms, there are also a quite wide range of less common symptoms, including epigastric fullness, epigastric pain, epigastric pressure, dyspepsia, nausea, bloating and belching and diverse extraesophageal manifestations like chronic cough, asthma, laryngitis and dental erosion. Besides, there are a proportion of patients without apparent symptoms (54). Altogether these facts make the early detection and precise diagnosis of GERD very complex.

There is no one single unifying standard for the clinical diagnosis of GERD. It is usually made by a combination of alert symptoms mentioned above, response towards practical treatment of anti–acid medicines and PPIs, endoscopic examinations, as well as pH monitoring in the esophagus (55). For large–scale epidemiological studies, it is most practical to use structured questionnaires regarding the experience of typical symptoms, with frequency and duration of the symptoms, along with medication history of anti–acid medicines and PPIs. There have been studies showing the differentiation of GERD patients based on structured questionnaires has good reliability and validity (56–59). But this also brings difficulties for the comparison of results from different studies using different criteria for the identification of GERD, and studies also showed the method used to define GERD patients is a source of heterogeneity for the estimations of prevalence worldwide (60).

2.2.2 Descriptive epidemiology

Prevalence

GERD is one of the most prevalent disorders around the world with around 1.03 billion people affected by GERD, and there has been a growing trend for this disease in all age groups, especially in the younger and middle–aged population (60). Because of the complexity of the symptoms and lack of typical presentation at early stage, there are very few studies reporting incidence, but prevalence instead. Yet there are still a lack of studies estimating the prevalence of GERD in the general population, which causes a wide range of results in meta–analysis. From previous studies, there exists a considerable geographic variation. The prevalence was estimated to be $18.1-27.8\%$ in North America and $8.8-33.1\%$ in Europe, and a consistent low prevalence of $< 10\%$ was reported in East Asia (61, 62). Specifically, studies from Sweden presented a prevalence of 8.8% and 25.9% from a random sampling of the population (63, 64).

Natural course

Patients of GERD may progress to severe complications like BE, which is recognized as the most important precursor for EAC. Therefore, it is of great importance to identify people with higher likelihood of such progression and prevent it. However, there are limited prospective data available characterizing this chronicle process and the complicated associations between these three conditions. There are two main types of GERD, namely erosive reflux disease (ERD) and non-erosive reflux disease (NERD). On one hand, some studies suggested that NERD, ERD, and BE are conditions with distinctions regarding epidemiological, pathophysiology, medication responses, and disease presentations, whereas have similar acid exposure and biological overlaps (65, 66). On the other hand, there is also evidence suggesting a progressive trend from NERD to BE (67). In a multi–center prospective study with 6215 symptomatic GERD patients treated in accordance with guideline, after years of follow–up, 25% of NERD patients progressed to some level of ERD, and after 5 years of routine treatment, BE was later detected in 5.9%, 12.1% and 19.7% of NERD, low–grade ERD and high–grade ERD patients, and 16.5% of NERD patients progressed to some severe stages. The probability to be finally affected of EAC from this pathway can be extrapolated to be even lower if patients are followed for longer time (68, 69). At the same time, some studies presented a regressive change from ERD to NERD whereas no regressive change from BE to lower level GERD was observed (70). In a large pooled case–control study, having symptoms of GERD is associated with higher risk for EAC, and this risk increases with duration of symptoms; over twenty years is associated with 6–fold increased risk (71). Notably, for most patients, they presented only mild symptoms. When followed up with regular monitoring and adequate treatment, their esophageal mucosa could remain unchanged for a long time, thus most people could survive a life without increased risk for mortality or morbidity (72, 73). Moreover, literatures regarding the screening of chronic GERD patients for the prevention of EAC do not present a difference compared to not–screening arm (74).

2.2.3 Related factors

The etiology of GERD is poorly understood. There are several confirmed factors related with the occurrence of GERD, including aging, male sex, abdominal obesity, white race, and tobacco consumption, whereas none of them is specifically associated with GERD, therefore there is no targeted preventive strategy for GERD (75).

Age

Advanced age is associated with an increased risk for GERD whereas quantitative estimations for this association are heterogeneous in previous studies. When the age was dichotomized and pooled from different studies, the OR (95% CI) for GERD was 1.32 (1.12 − 1.54) in individuals $\geq$ 50 years, comparing to those aged $<$ 50 years (60). The ORs (95% CI) were 1.17 (1.11−1.24) and 1.20 (1.12 − 1.28) in age group 35–59 and $\geq$ 60, separately, comparing to the age group

18–34 (76). Moreover, statistics from Sweden also presented an increasing trend for GERD with age, for both sexes. The prevalence of GERD ranged from 25.8% in age group 19–30 to 33.1% in age group > 70 in males and 22.1% in age group $19-30$ to 29.8% in age group > 70 in females, and a peak for severe symptoms at age $60-70$ was also observed for males (77). Such pattern was also reported in a study from the US population that hospitalization for GERD peaked at age $65-84$, and dropped at oldest age of > 85; this pattern may suggest a cohort effect for GERD and reflects the change of environmental risk factors over time (78).

Obesity

There are accumulating data suggesting that obesity is associated with a higher risk for GERD, and this finding is consistently reported in studies from different populations. The worldwide prevalence of GERD from a meta–analysis was 6.64%, 17.20%, and 22.63% in groups with a BMI of < 18.5, $18.5-29.9$, and ≥ 30, respectively (60). In German population, the ORs (95% CI) were 1.8 $(1.5-2.2)$ for overweight and 2.6 $(2.2-3.2)$ for obese people (79). In Sweden, upper normal weight, overweight, and obese were associated with 38%, 89%, and 71% elevated risk for GERD in females, respectively, compared to normal weight individuals, after controlling for familial and genetic factors in a twin study (80). A randomized trial has demonstrated the less development of reflux symptoms followed by weight loss (81). Besides the overall obesity, which is defined by a BMI ≥ 30, as commonly used in epidemiological studies, central abdominal obesity is emerging to be a stronger risk factor for GERD, independent of BMI. Such association was observed in both western and eastern populations, suggesting an etiological role of central abdominal obesity for GERD (82–84).

Smoking

Smoking is also a strong risk factor for GERD. Evidence from Norway showed daily smokers with more than 20–year smoking history had a 70% higher risk for GERD, compared with individuals who smoked less than 1 year, and a dose–effect for GERD associated with duration and smoking quantity. Tobacco smoking cessation was shown to reduce the severe to minor reflux symptoms in normal weight people (85, 86). In Sweden, this association was also confirmed using twin study design, which showed that ever smoking and smoking more than 20 cigarettes per day elevated 18% and 37% of the risk for GERD, separately (80).

Detrimental effects of smoking can be brought to the occurrence of GERD through several pathways. Muscles at lower esophageal sphincter might be relaxed since nicotine contained in cigarettes is a relaxant to smooth muscle. At the same time, the reduction of saliva secretion rate in smokers could also lead to or worsen GERD by the reduction of the compounds in saliva that help to neutralize acid reflux, and a prolonged acid clearance time (87).

Chapter 3

RESEARCH AIMS

The overall aims of this thesis were to study the factors or characteristics related to the occurrence and prognosis of esophageal cancer and GERD. More specifically, the aims were:

Aim 1 To study the sex differences in the prognosis after esophageal cancer surgery.

Aim 2 To study the association between *H. pylori* infection and EAC.

Aim 3 To study the association between dental health related diseases and esophageal cancer.

Aim 4 To study the association between AG and GERD.

Chapter 4

MATERIALS AND METHODS

4.1 National quality register for esophageal and gastric cancer (NREV)

NREV was used in the **Study I**. It was specially designed to collect data related with all the newly diagnosed esophageal and gastric cancer patients in Sweden, with or without treatment. The register was initiated on 1 January 2006 and consists of 3 surveys. The first survey is undergone at the time of workup, containing data regarding the date of clinical visit, histopathological subtypes, tumor stage, and treatment plan. The second survey is performed for individuals who have undertaken curative or palliative intended treatments, and data related with surgical procedures or perioperative treatments are registered. The third survey is performed at one year after the tumor resection, including data of postoperative comorbidities, tumor stage according to the pathological assessment, and quality of life for the follow–up monitoring. It is estimated that the register has a total completeness of 95.5%, comparing to the cancer register, which is considered to have a completeness close to 100% (89).

4.2 Barrett's and Esophageal Adenocarcinoma Consortium (BEACON)

Data in the **Study II** was based on four studies from BEACON (http://beacon.tlvnet.net/). The consortium was set up in 2005 by a group of epidemiological studies for the aim of open scientific etiological and prevention research in the diseases of BE and EAC. Rich data regarding genetics, life–style, and environmental factors from different continents and populations were collected, carefully checked, and harmonized by a centralized coordinating center, enabling high quality epidemiological studies for EAC. Information of the datasets included in this study is shown in Table 4.1.

Table 4.1: Study specific distribution of esophageal adenocarcinoma (EAC) cases, controls and prevalence of gastric atrophy

Study	Country	Time of data collection	EAC cases/controls	Gastric atrophy N (%)
US Multi–Center Study	United States	1993–1995	67 / 222	35(12)
Los Angeles Country Multi–ethinic Study	United States	1992–1997	79 / 354	30(7)
Swedish Esophageal Cancer Study (SECC)	Sweden	1994–1997	95 / 480	62(11)
Factors Influencing the Barrett's Adenocarcinoma Relationship Study (FINBAR)	Ireland	2002–2005	194 / 242	27(6)
Total			435 / 1298	154(9)

4.3 The Swedish Dental Health Register (DHR)

Study III used data from the DHR. DHR was established in 2008, with dental care information of adults of ages older than 23. The dental care information is recorded when the treatment for a tooth at a dental care provider is submitted by the dentists or dental hygienists and approved by the Swedish Social Insurance Agency for a reimbursement (91).

The registry has name and address for the dental care providers, age and sex for the patients, date of the treatment, number of the remaining and intact teeth at the time of treatment, diagnosis for oral diseases, examination or treatment for each tooth, number and position of the treated tooth. A unique identification number for each person could link DHR with other registers for research purposes. It is estimated that 2.6 million of males and 2.9 million of females visited the dentists during 2013 to 2014 and the number of remaining teeth and intact teeth in the register has a very high correctness (91.5%) comparing to the actual dental records (92).

4.4 The Swedish Twin Register (STR)

STR was originally initiated in the late 1950s for the purpose of studying the association between smoking and alcohol consumption on the risk of cancer and cardiovascular diseases, while controlling for genetic factors. After years of improvement and multiple waves of data collection, STR has become one of the biggest twin–based datasets with 216,258 twins born between 1886 and 2015, allowing for the studies of a broad range of study objectives on diverse disorders (93, 94).

The Screening Across the Lifespan Twin Study (SALT) is a telephone interview carried out among all the twins born in 1958 or earlier. Extensive data was collected during 1998 to 2002. In the interview, structured questionnaires were used for the data of different diseases and symptoms, medication use, and linked to other medical records. A total of 44,919 twins were

enrolled. In the registry, the zygosity of twins was determined and validated by self–reported questions regarding the similarities within twins, genetic–based method, or being opposite sex for dizygotic (DZ) twins, which ensured a very high overall accuracy (93, 95, 96).

Later in the year 2004 to 2008, the TwinGene project further obtained blood specimens from participants in the SALT study who were willing to provide blood samples for further studies. In total, 12,614 blood specimens were collected in the TwinGene project (96, 94). The participants were instructed to visit the local healthcare provider for the sampling of 50 ml blood. Twins who participated in the SALT study, with serum sample available from the biobank were included in **Study IV**.

4.5 The National Registers

4.5.1 The Swedish Cancer Register (SCR)

SCR was initiated in 1958 and is managed by the Swedish National Board of Health and Welfare. It is compulsory for all the health care providers in Sweden to report the newly diagnosed cancers at clinical, morphological, and from the laboratory examinations. SCR is estimated to have almost 99% completeness of tumors confirmed of morphology, but with some loss in different tumor sites, advanced stages of tumors and elderly patients. Specifically, the register contains demographical information, personal identification number, age, sex, residence area, tumor localization, stage, date of diagnosis, and histopathological diagnosis, etc. SCR is being widely used in various epidemiological studies (98).

4.5.2 The Cause of Death Register

The Swedish Cause of Death Register contains information on underlying and contributing cause of death from 1952, covering all the population registered in Sweden. The coding for the cause of death was in line with the current version of International Statistical Classification of Diseases and Related Health Problems (ICD) codes: ICD–6 (1952–1957), ICD–7 (1958-1968), ICD–8 (1969–1986), ICD–9 (1987–1996), ICD–10 (1997–). The cause of death register is based on the death certificate issued by the responsible physician, which is shown to have overall 77% agreement with the international standards and lower in elder population than younger population (99, 100).

4.5.3 The Swedish Inpatient and Outpatient Register

The Swedish Inpatient Register was launched in 1964 and completed with a national coverage of 99% in 1987, and the Outpatient Register was included since 2001. The registers include all somatic and psychiatric hospital discharges with information of hospital, clinic, sex, age, enrollment and discharge date with negligible missing. Different versions of ICD codes were used for the recording of the diagnoses: ICD–7 (1964–1967), ICD–8 (1968–1986), ICD–9 (1987–1996), ICD–10 (1997–) (101).

4.5.4 The longitudinal integrated database for health insurance and labor market studies (LISA)

The original purpose of LISA was for health and labor market research. While by linking to several external registers, it is also widely incorporated in epidemiological studies, providing rich information on covariates that may affect the diseases of interest. Specifically, LISA contains data regarding personal identification number, demographics, the highest educational level, employment, marital status, disposable income, and migration, etc., for individuals older than 16, from the year 1990 (102).

4.6 Measurements

4.6.1 *H. pylori* infection

In **Study II**, the *H. pylori* seropositivity of 15 antibodies were tested by a multiplex serology test which was developed based on the combination of glutathione-S-transferase (GST) capture immunosorbent assay and fluorescent bead technology. The experiment was performed in the collaborative laboratory (103). Antibodies tested are listed in Table 4.2.

Quality of the experiment was monitored by insertion of 2 or 3 quality control samples into each plate in the experiment, and eventually a total of 52 control samples were placed into 25 plates. Among all the antibodies tested, the intra–class correlation coefficients of absolute agreement across different plates were calculated for each antibody among all seropositive control samples (104). The reliability of the antibodies ranged from moderate to good.

Table 4.2: *H. pylori* multiplex serology antibodies

Antibody	Full name
GroEl	Chaperonin Groel
UreA	Urease alpha subunit A
HP 231	Hypothetical protein
NapA	Neutrophil–activating protein
HP 305	Hypothetical protein
HpaA	Neuraminyllactose–binding hemagglutinin homolog
Cagδ	Cag pathogenicity island protein 3
CagA	Cytotoxin–associated antigen A
CagM	Cag pathogenicity island protein 16
HyuA	Hydantoin utilization protein A
Catalase	Catalase
VacA	Vacuolating cytotoxin A
HcpC	Conserved hypothetical secreted protein
Cad	Cinnamyl alcohol dehydrogenase ELI3–2
Omp	Outer membrane protein

4.6.2 Oral health

In **Study III**, the oral health status was defined by a series of diagnostic codes from DHR and the number of remaining teeth. The diagnoses were further classified into normal, caries, root canal infection, mild inflammation, and periodontitis. Detailed definition for each condition are summarized Table 4.3. Number of remaining teeth was categorized into groups of 0-14, 15-20, 21-24, 25-27, and 28-32.

Table 4.3: Diagnostic and procedure codes for dental health status

Category	Subgroup	Diagnostic code (TILLSTAND)
4	Periodontitis	3043: Periodontitis
4	Periodontitis	3044: Periimplantitis
3	Mild inflammation	3042: Mucositis (implants)
3	Mild inflammation	3045: Pericoronitis
3	Mild inflammation	3041: Gingivitis
3	Mild inflammation	3046: Other unspecific inflammation conditions
3	Mild inflammation	3072, 3073: Stomatitis
2	Root canal infection	3051: Root canal infection and treatment
1	Caries	4001, 4002, 4011, 4012: Caries
0	Normal	

The table is reproduced from Zhang et al. Cancer Epidemiology, Biomarkers & Prevention 2022 (90).

4.6.3 GERD

In **Study IV**, GERD was defined by a series of 10 items in a validated questionnaire regarding GERD, as shown in Table 4.4. The information was achieved by telephone interview.

4.6.4 AG

In **Study IV**, chronic corpus AG was determined by serum biomarkers of pepsinogen I (PGI) and pepsinogen II (PGII), using the tests of Enzyme linked immunosorbent assays (ELISAs), by the commercialized ELISA kits (Biohit, Helsinki, Finland), according to the manufacturer's instructions.

Currently, there is no generally agreed cut–off value for PGI or PGII in the identification of patients with AG. Therefore, different cut–offs were used in our study to test the robustness of the results: PGI$<$ 30 ng$\times$ml^{-1}; PG1$<$ 70 ng$\times$ml^{-1}and PGI/PGII$<$ 3; PGI/PGII$<$ 3; and PGI$<$ 25 ng$\times$ml^{-1}or PGI/PGII$<$ 3. A multi–center, cross–sectional study performed in France showed that using PGI$<$ 30 ng$\times$ml^{-1}as criterion of AG was showed to have a sensitivity and specificity of 31.8%, and 98.0%, respectively (105). PG1$<$ 70 ng$\times$ml^{-1}and PGI/PGII$<$ 3 is a criterion widely used in Asian population and is assessed to have a sensitivity ranging between 66.7–84.6% and a specificity ranging between 73.5–87.1% (106). A study performed in the Swedish population presented a sensitivity and specificity of 71% and 98% for the cut–off of PGI$<$ 25 ng$\times$ml^{-1}or PGI/PGII$<$ 3, together with other serological biomarkers (107). When

Table 4.4: Reflux questions (English translation) used in the telephone inquiry

Questions	Options
Do you often have heartburn, often meaning more than 50 times per year?	No; Yes; Don't know; Refuse
Did you ever have a burning pain or discomfort behind the breastbone?	No; Yes; Don't know; Refuse
How old were you when you were first bothered by breastbone pain or burning?	Number of age
How often do or did you have breastbone pain or burning?	Usually less than once a week (<4 times/month); Once or more than once a week but not every day ($1-5$ times/week); Usually every day (>5 times/week); Less than one a month (<1 time/month); Don't know; Refuse
Did you ever wake up at night due to breastbone pain or burning?	No; Yes; Don't know; Refuse
Does or did your breastbone pain often go up towards the neck?	No; Yes; Don't know; Refuse
Does or did your breastbone pain or burning improve when you took antacids?	No; Yes; Don't know; Refuse
Do or did you take any of the following medicines to prevent breastbone pain or burning? (A list of all histamine H-2 receptor antagonists and proton pump inhibitors available in Sweden was then read.)	No; Yes; Don't know; Refuse
Do or did you ever have regurgitation of bitter or sour fluid coming up into the mouth or throat from the esophagus?	No; Yes; Don't know; Refuse
How often do or did you have regurgitation of bitter fluid?	Usually less than once a week (<4 times/month); Once or more than once a week but not every day ($1-5$ times/week); Usually every day (>5 times/week); Less than one a month (<1 time/month); Don't know; Refuse

The table is reproduced from Zhang et al. United European Gastroenterology Journal 2022. (97)

PGI/PGII< 3 was used as the criterion for AG, the sensitivity and specificity were estimated to be 90% and 93%, respectively (108).

The quality of the tests was monitored by a duplication of control samples provided by the kit, and external control samples generated by pooling the sera donated by 10 healthy volunteers. Statistics of control samples are shown in Table 4.5. The between–plate coefficient of variation ranged between 5–13% and the within–plate coefficient of variation ranged between 1–5%.

Table 4.5: Between– and within–plate coefficient of variation for PGI and PGII

CV	PGI kit control	PGI external control	PGII kit control	PGII external control
Between–plate CV	6%	9%	5%	13%
Within–plate CV	2%	1%	5%	5%

PGI, pepsinogen I; PGII, pepsinogen II; CV, Coefficient of variation.
The table is reproduced from Zhang et al. United European Gastroenterology Journal 2022. (97)

4.7 Statistical analysis

4.7.1 Relative survival analysis

In **Study I**, the survival outcomes were measured by mortality rate and excess mortality rate. Excess mortality rate is defined as the difference between observed mortality rate of the study cohort and the expected mortality of the background population where the individuals of study come from. In this study, the background mortality rate was obtained from the Human Mortality Database of Sweden (http://www.mortality.org). Excess mortality rate is used as the cancer–specific mortality when an accurate classification of cause of death cannot be achieved, which is often the case for the identification of the cause of death after complex surgical treatment (109). Sex difference was therefore defined by mortality rate ratio (MRR) and excess mortality rate ratio (EMRR) after time of surgery. A flexible parametric survival model was used to study the sex differences during the follow–up time. In the model, time after surgery was set as underlying timescale. Restricted cubic splines were introduced to better study associations in dose–response models. Moreover, knots of the splines for each model were determined by the statistics of Akaike information criterion, Bayesian information criterion and likelihood ratio test. Therefore, the mortality rates or excess mortality rates do not have to be proportional between males and females. In the study, the subtypes of EAC and ESCC were modeled separately (110, 111). In all the models, the potential confounding factors were age at time of surgery, Charlson co–morbidity index, the American Society of Anesthesiologists score, clinical stage of cancer, marital status, education, neoadjuvant treatment, and hospital volume.

4.7.2 Logistic regression

In **Study II**, the unconditional multivariate logistic regression model was used to study the association between *H. pylori* seropositivity and EAC, in respective groups, depending on the gastric atrophy status (yes, no). The associations between all the antibodies and EAC were summarized as ORs with the corresponding CIs. The exposure of *H. pylori* infection was also defined by the total number of positive antibodies: < 4, ≥ 4 (also grouped into $4-7$ and ≥ 8). The potential modification effect of gastric atrophy for the link between *H. pylori* infection and EAC was tested by introducing an interaction term of gastric atrophy and *H. pylori*, and a log likelihood ratio test was then performed to compare the fitness of models with and without

interactions.

4.7.3 Mediation analysis

In **Study II**, a mediation analysis was applied to study the potential pathways from the exposure of *H. pylori* to the outcome of EAC, as shown in the directed acyclic graph 4.1. In brief, gastric atrophy, BMI, and GERD were considered as potential mediators, both individually and jointly. A 4–way decomposition method for the total effect of *H. pylori* was first used for this mediation analysis. Specifically, total effect was decomposed into natural direct effect, which is defined as the counterfactual effect of exposure with mediator set at whatever value it would have taken at the reference value of the exposure and the natural indirect effect, which is defined to be the counterfactual effect of mediator, with all individuals fixed to be exposed. An inverse odds ratio weight approach was then applied for the joint mediation analysis of multiple mediators (gastric atrophy, BMI, and GERD) simultaneously.

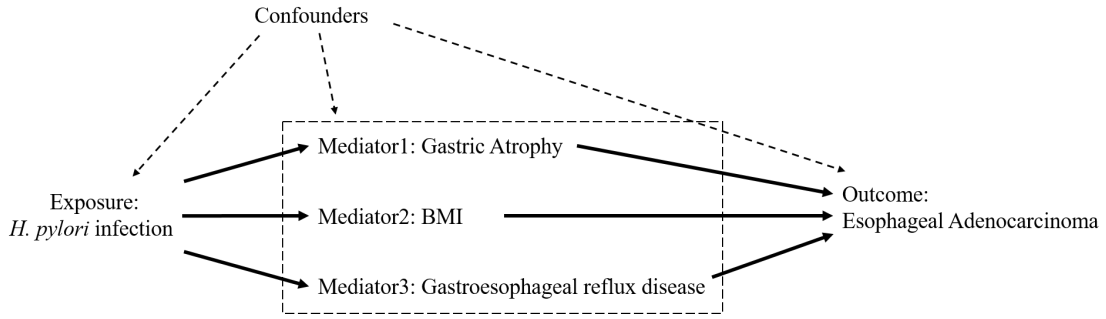

Figure 4.1: Infection of *H. pylori* is exposure of interest, and esophageal adenocarcinoma (EAC) is the outcome of interest. Body mass index (BMI), gastric atrophy and gastroesophageal reflux disease (GERD) are the mediators in the pathway from *H. pylori* to EAC. Potential confounders between exposure, mediator, and outcome were considered.

4.7.4 Cox regression

In **Study III**, the outcome was time from the first dental care visiting until diagnosis of esophageal cancer, with regard to its histopathological subtypes of EAC or ESCC, death, migration, or December 31, 2016, whichever occurred first. Hazard ratios (HRs) associated with the categorized dental inflammation groups and remaining teeth for esophageal cancer were estimated by Cox regression models. The proportional hazards assumption for each variable in the models was tested by Schoenfeld residual tests (112). Attained age was set as the underlying timescale to implicitly control for aging effect. Moreover, the dental inflammation and the number of remaining teeth were studied in the same model simultaneously to mutually control for the confounding effects. The interaction effects between dental diseases and remaining teeth were tested by comparing the models with and without the multiplicative interaction term. Furthermore, given that a substantial portion of individuals attended the dental clinics multiple times and the change of their dental health conditions during follow–up time could also poten-

tially contribute to the outcome of esophageal cancer, a time–dependent Cox regression model was used to assess the effects of dental diseases and remaining teeth in the time–varying way (113). In this model, the severest diagnosis of dental diseases in a year and the least number of remaining teeth were considered as the exposure of the year. In addition, with the prior knowledge that the progression from normal dental condition to periodontitis is non–reversible because of the chronic destruction of the tooth structure and supporting tissues, and we were aware that this change might not be recorded in the dental visits for non–treatment purposes, we disregarded records with the less severe inflammation or increased remaining teeth during follow–up, compared with the previous records. All the models controlled for potential confounders, including age at entry, sex, education, family income, smoking related diseases, alcohol related diseases, and obesity. And GERD was also controlled for in the model for EAC subtype.

4.7.5 Regression models in the twin study

In **Study IV**, GERD patients were compared with the whole study group as external controls, within monozygotic (MZ) co–twins and within DZ co–twins, respectively. ORs with the corresponding CIs were used to quantify the association between AG and GERD.

In the comparison of GERD patients with external controls, generalized estimation equation (GEE) model was used to account for the correlation within twin pairs, regardless of zygosity. In the comparison within MZ or DZ twin pairs, only twins with one person with GERD and the other without were included in the analysis. Conditional logistic regression model was used when the outcome of interest was GERD (yes, no), and a fixed effect model was used when the outcome was studied as three–way variable (no GERD, less frequent GERD, and frequent GERD). In these means of analyses, family constant factors, and genetic factors, which is 50% shared by DZ twins and 100% shared by MZ twins, were implicitly controlled for in the models (114, 115). We also controlled for potential confounders including birth year, sex, education, BMI, coffee consumption, physical exercises, smoking, and alcohol consumption in all the models.

4.8 Ethical considerations

All the studies were based on data collected from human being, thus it is always an important mission to ensure anonymity of each participant. The general aim of the studies in this thesis is to broaden the knowledge of causes, development, and survival of esophageal cancer, to improve the prevention, diagnosis and prognosis of the patients, with data from human subjects. Based on the principles of the World Medical Association Declaration of Helsinki, it is the duty of the researcher to ensure the rights and interests of all study subjects, to protect the dignity, integrity, right of self–determination, and confidentiality of sensitive information of participants (116). Given the differences in study design, ethical framework, and legal regulation, ethical issues were carefully considered in different studies, separately.

For **Study I**, the data was administered into the quality register of patients who received clinical therapy. Before registering personal information into the dataset, each patient was informed of the aim of the study, information about the security of their personal data, possible usage of their data, and given the right to delete their data from the system at any time. And to attenuate their worry they may be taken less care of if they do not denote their personal data for the study, it was mentioned in the consent that joining the study is totally voluntary, and had nothing to do with the treatment they may receive. Since their data would be stored and handled by data management authority, they were also informed of the contact information of data management authority for any further question and requests of their data. As data users, similarly, we requested anonymized data from data management authority, with permission from the steering group.

The **study II** was based on collaborative data and biological samples from Europe and the United States. The ethical approvals were provided by each research center, separately. Specially, an ethical certificate regarding the current study was issued by the main PI of the international Barrett's and Esophageal Adenocarcinoma Consortium.

Study I and **Study III** were performed with Swedish national population–based registries. According to the General Data Protection Regulation (GDPR), which was newly updated in 2018, sensitive data was redefined in a stricter way. Any data with the potential to identify a "natural person" is sensitive and needs to be carefully treated, and in many cases, cannot be directly used. Therefore, techniques should be applied to avoid such linkage to a "natural person"(117). In the Swedish registries, a study specific unique serial number was assigned to each person by the National Board of Health and Welfare to mask the connection between data and the real person. Therefore, the data cannot be linked to any "natural person". And data from different studies cannot be linked without permission.

Study IV used data and blood samples from the STR. To ensure the confidentiality of the participants, the data was used cautiously. Only the most necessary variables needed to answer the research question were granted by the data management team. The study protocol and ethical amendment were approved before the study was carried out.

Chapter 5

RESULTS

5.1 Study I

5.1.1 Population characteristics

In this study, excess mortality rates were compared between males and females in patients after undergoing surgical treatments. In total, there were 1098 male and 203 female patients of EAC subtype and 199 male and 106 female patients of ESCC subtype were analyzed in the study. Sex specific characteristics are summarized in Table 5.1.

Table 5.1: Sex–specific main characteristics in groups of esophageal adenocarcinoma (EAC) and esophageal squamous cell carcinoma (ESCC)

Characteristics	EAC		ESCC	
	Men	Women	Men	Women
N	1098	203	199	106
Operation age, year (SD)	66.1 (9.0)	65.6 (10.8)	66.7 (8.8)	64.3 (10.4)
Education >12 years, N (%)	221 (20.5)	53 (26.6)	45 (23.2)	30 (29.4)
Married, N (%)	659 (60.0)	89 (43.8)	117 (58.8)	60 (56.6)
Anaesthetist score 3, N (%)	156 (14.5)	32 (16.1)	39 (19.7)	13 (12.4)
Clinical stage III, N (%)	387 (35.2)	50 (24.6)	83 (41.9)	25 (23.6)
Neoadjuvant Chemoradiotherapy, N (%)	429 (41.0)	61 (31.6)	105 (54.1)	49 (48.0)
Charlson co–morbidity index≥3, N (%)	167 (15.2)	22 (10.8)	32 (16.1)	15 (14.2)
High hospital volume, N (%)	423 (38.7)	80 (39.4)	98 (49.5)	49 (46.2)
Postoperative complications, N (%)	442 (40.3)	79 (38.9)	98 (49.2)	50 (47.2)

Abbreviations: EAC, esophageal adenocarcinoma; ESCC, esophageal squamous cell carcinoma.
The table is reproduced from Zhang et al. BJS Open 2022. (88)

5.1.2 Time trend of sex difference

The results demonstrated that the time trend of EMRRs were slightly different between EAC and ESCC patients. For EAC patients, the EMRR was not different between male and female patients at 1 year after surgery. Around 25% risk reduction for female patients was observed at 10 year and overall follow–up duration, but the results did not reach statistical significance

after controlling for a full range of potential confounders in the models. For patients classified as ESCC subtype, no survival beneficial was observed for female patients at 1 year after surgery. However, at 5 year, 10 year and the whole follow–up time, female patients had almost 50% less excess mortality rate, comparing to male patients(Table 5.2).

Moreover, the time trend of sex–specific mortality rates and excess mortality rates after surgery were also illustrated continuously over time for EAC and ESCC patients, separately. Similarly, there was no sex difference at short time after surgery; the peak of mortality happened at 1 year after surgery, for both sexes, and decreased afterward (Figure 5.1). Notably, the sex-specific excess mortality rates after surgery between male and female patients were compared using both ratio and difference scale (Figure 5.2). When ratio scale was used, no sex difference was observed for EAC patients along the follow–up time, whereas 50% lower risk for excess mortality was observed for females of ESCC patients. When difference scale was used, the peak of the difference between excess mortality rates was at 1 year after surgery and dropped fast afterward.

Table 5.2: Excess mortality rate ratio (EMRR) at different follow-up time in each group

Esophageal adenocarcinoma						
	Death/Person–years		Crude model[a]		Adjusted model[b]	
	Male	Female	EMRR	*P*	EMRR	*P*
1 year	270/961	40/181	0.78 (0.54, 1.13)	0.189	0.82 (0.55, 1.21)	0.312
5 years	324/1691	53/373	0.77 (0.59, 1.01)	0.058	0.77 (0.58, 1.03)	0.080
10 years	40/570	6/113	0.75 (0.58, 0.98)	0.032	0.76 (0.58, 1.01)	0.057
Over-all	634/3249	99/671	0.75 (0.58, 0.98)	0.032	0.76 (0.58, 1.01)	0.056
Esophageal squamous cell carcinoma						
	Death/Person–years		Crude model[a]		Adjusted model[b]	
	Male	Female	EMRR	*P*	EMRR	*P*
1 year	55/168	20/97	0.66 (0.38, 1.15)	0.146	0.98 (0.49, 1.99)	0.963
5 years	69/269	24/203	0.52 (0.34, 0.78)	0.002	0.50 (0.30, 0.82)	0.006
10 years	11/88	4/83	0.53 (0.35, 0.79)	0.002	0.52 (0.32, 0.84)	0.007
Over-all	135/530	48/387	0.53 (0.35, 0.79)	0.002	0.52 (0.32, 0.84)	0.007

[a] Adjusted for age of surgery.
[b] Adjusted for age of surgery, comorbidity, ASA level, clinical stage, neoadjuvant treatment, marital status, education level, and hospital volume.
The table is reproduced from Zhang et al. BJS Open 2022. (88)

5.1.3 Grouped study of sex difference

Stratified analysis was performed based on clinical tumor stage, neoadjuvant therapy, and postoperative complications (Table 5.3). A better survival was observed in women of tumor stage I, who had neoadjuvant therapy and did not have postoperative complications. The pattern was alike in both subtypes of EAC and ESCC, while the female versus male sex difference was higher in ESCC patients than that in EAC patients.

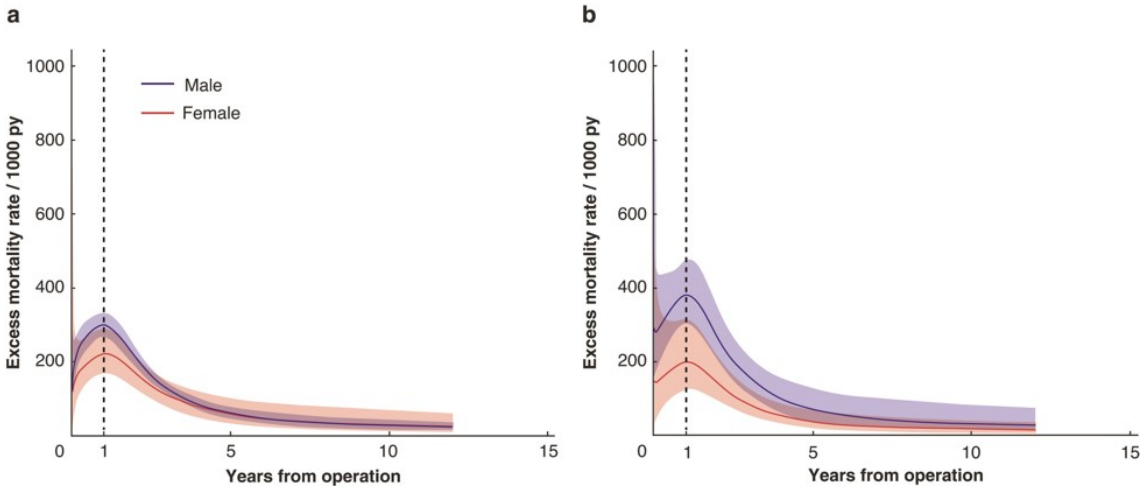

Figure 5.1: Sex–specific excess mortality rates per 1000 person-years with corresponding 95% confidence intervals in each group, adjusting for age, co-morbidity, ASA level, clinical stage, neoadjuvant treatment, marital status, education level, and hospital volume. Esophageal adenocarcinoma (a), esophageal squamous cell carcinoma (b). The figure is reproduced from Zhang et al. BJS Open 2022. (88)

Table 5.3: Female versus male excess mortality rate ratio (EMRR) in esophageal adenocarcinoma (EAC) and esophageal squamous cell carcinoma (ESCC) patients, stratified by cancer stage, neoadjuvant treatment, and postoperative complications.

	EAC EMRR (95% CI)[a]	ESCC EMRR (95% CI)[a]
Clinical stages		
Cancer stage 0 - I	0.59 (0.36, 0.97)	0.29 (0.11, 0.75)
Cancer stage II	0.88 (0.52, 1.50)	0.66 (0.26, 1.64)
Cancer stage III - IV	0.80 (0.51, 1.26)	0.62 (0.25, 1.56)
Perioperative neoadjuvant treatment		
No neoadjuvant treatment	1.05 (0.69, 1.59)	0.69 (0.35, 1.37)
Neoadjuvant treatment	0.62 (0.42, 0.92)	0.31 (0.13, 0.71)
Postoperative complications		
No postoperative complications	0.64 (0.42, 0.96)	0.28 (0.13, 0.63)
Any postoperative complications	0.91 (0.60, 1.38)	0.49 (0.25, 0.98)

[a] Adjusted for age of surgery, comorbidity, ASA level, clinical stage, neoadjuvant treatment, marital status, education level, andhospital volume.
The table is reproduced from Zhang et al. BJS Open 2022. (88)

5.2 Study II

5.2.1 Population characteristics

In this study, a total of 435 EAC patients and 1298 controls were included for the analysis, and 154 of them were defined to have gastric atrophy. Their population characteristics are summarized in Table 5.4.

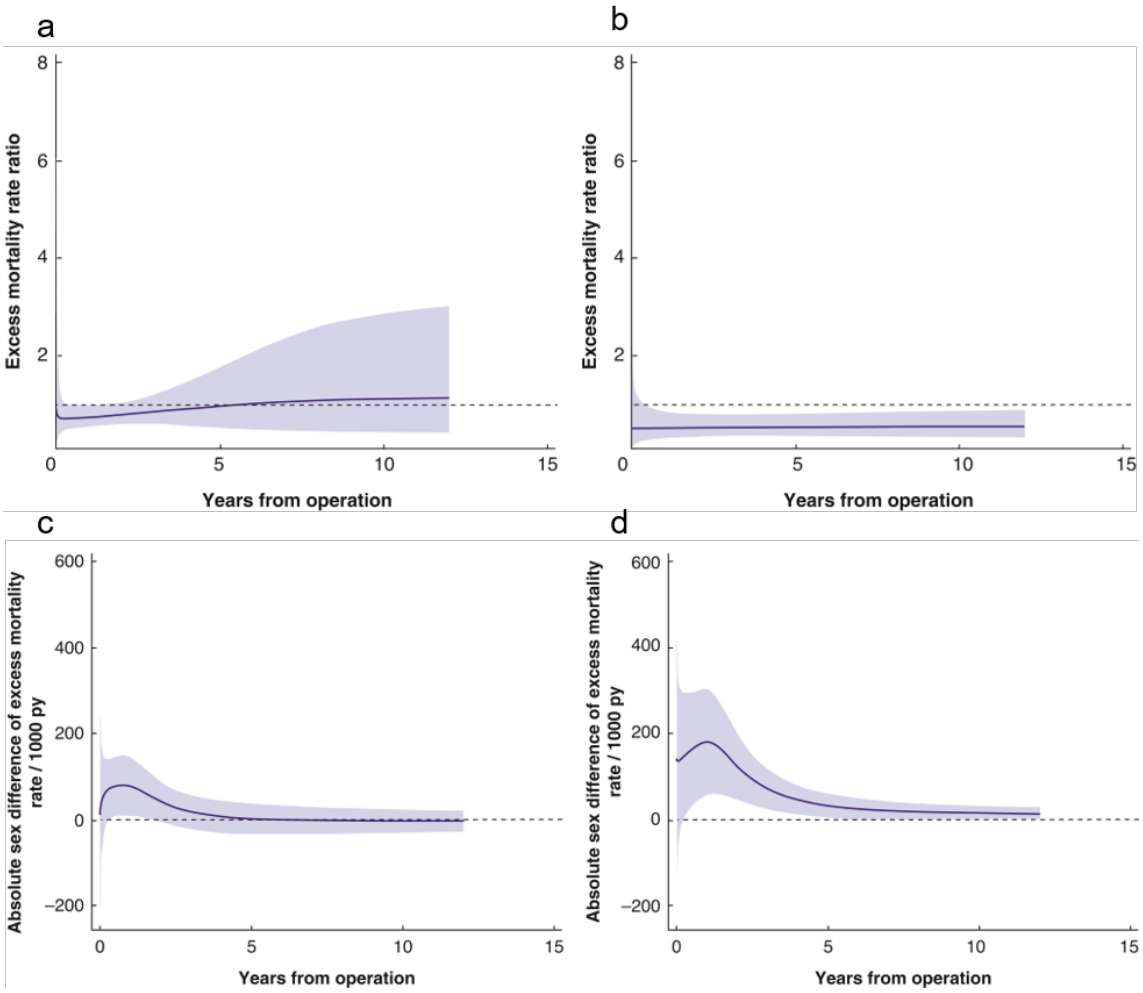

Figure 5.2: Female versus male excess mortality rate ratios (a, b) and absolute difference of excess mortality rates (c, d) with corresponding 95% confidence intervals in each group, adjusting for age, comorbidity, ASA level, clinical stage, neoadjuvant treatment, marital status, education level, and hospital volume. Esophageal adenocarcinoma (a, c), esophageal squamous cell carcinoma (b, d). The figure is reproduced from Zhang et al. BJS Open 2022. (88)

5.2.2 *H. pylori* and EAC

The association between each of the 15 antibodies and EAC is shown in Figure 5.3. All the antibodies presented a reduced point estimation, after adjusting for covariates. And the risk reduction still persisted after stratification by gastric atrophy status. For most antibodies, ORs in the gastric atrophy positive groups were lower than gastric atrophy negative groups whereas the interaction effect was only significant for antibody NapA and gastric atrophy.

When the population was stratified by the total count of positive antibodies as < 4 and ≥ 4, around 40% risk reduction was observed in individuals with ≥ 4 positive antibodies, and the risk reduction was more profound in gastric atrophy patients (OR=0.19, 95% CI: $0.10-0.36$). When the positive antibodies counts of ≥ 4 was further divided into subgroups of $4-7$ and ≥ 8, the ≥ 8 subgroup presented the lowest OR in overall, gastric atrophy positive, and gastric

Table 5.4: Characteristics of adenocarcinoma cases and control group, stratified by status of gastric atrophy

Characteristics	**Overall**		**Gastric atrophy** (+)		**Gastric atrophy** (−)	
	Case	Control	Case	Control	Case	Control
N	435	1298	43	111	392	1187
Age (70-79), N(%)	149 (34)	434 (33)	13 (30)	54 (49)	136 (35)	380 (32)
Sex (male), N(%)	379 (87)	1052 (81)	35 (81)	92 (83)	344 (88)	960 (81)
Education (lower than high school), N(%)	214 (50)	527 (41)	22 (51)	59 (53)	192 (49)	468 (40)
Race (white), N(%)	416 (96)	1150 (88)	38 (89)	99 (89)	378 (96)	1051 (89)
Current smoker, N(%)	127 (30)	253 (20)	14 (33)	17 (16)	113 (29)	236 (20)
Ever drinking alcohol, N(%)	330 (77)	1082 (83)	32 (74)	99 (89)	298 (77)	983 (83)
GERD, N(%)	208 (49)	215 (17)	17 (40)	23 (21)	191 (49)	192 (16)
High fruit consumption, N(%)	117 (27)	430 (33)	12 (28)	35 (32)	105 (27)	395 (34)
High vegetable consumption, N(%)	128 (30)	430 (33)	11 (25)	32 (29)	117 (30)	398 (34)
Overweight or obese, N(%)	298 (69)	607 (47)	32 (74)	48 (43)	266 (68)	559 (47)

atrophy negative group. The interaction between *H. pylori* counts and gastric atrophy was statistically significant (Table5.5).

5.2.3 Test of potential mediation effects

To explore the potential mediation effects of gastric atrophy, GERD, and BMI, a parametric method and a non–parametric method were used to test the mediation effects separately and jointly (Table5.6). The results showed the effect of *H. pylori* was not mediated by gastric atrophy, GERD, nor BMI.

Table 5.5: Association between total number of positive antibodies and esophageal adenocarcinoma (EAC), with and without stratification by gastric atrophy

Number of *H. pylori* positive antibodies	**Overall** N=1733		**Gastric atrophy** (+) N=154		**Gastric atrophy** (−) N=1579	
	EAC N(%)	OR (95% CI)[a]	EAC N(%)	OR (95% CI)[a]	EAC N(%)	OR (95% CI)[a]
< 4	283(65.06)	1(Ref)	29(67.44)	1(Ref)	254(64.80)	1(Ref)
≥ 4	152(34.94)	0.59(0.50, 0.70)	14(32.56)	0.19(0.10, 0.36)	138(35.20)	0.65(0.55, 0.77)
$4-7$	96(22.07)	0.84(0.67, 1.06)	7(16.28)	0.27(0.07, 1.03)	89(22.70)	0.95(0.79, 1.13)
≥ 8	56(12.87)	0.40(0.33, 0.48)	7(16.28)	0.13(0.06, 0.31)	49(12.50)	0.42(0.35, 0.50)

[a] Adjusted for age, sex, education level, race, body mass index, cigarette smoking, alcohol consumption, fruit consumption, vegetable consumption and symptoms of gastroesophageal reflux.

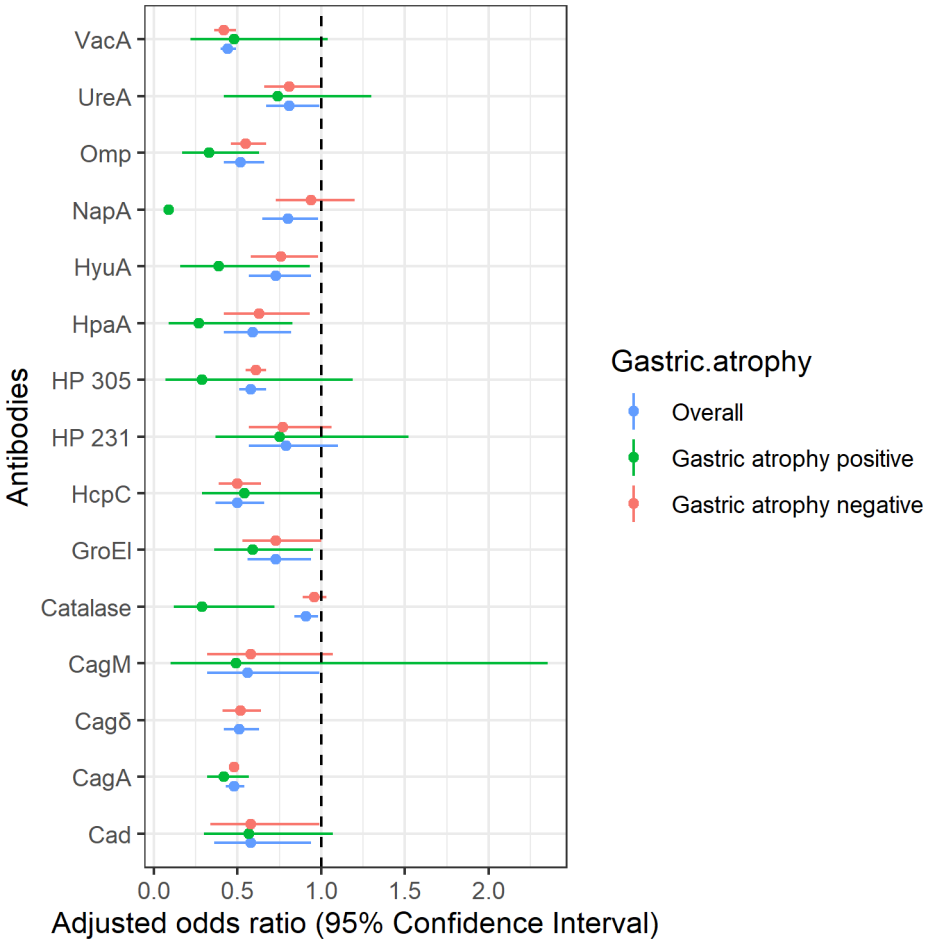

Figure 5.3: The association between each antibody against *H. pylori* and esophageal adenocarcinoma, with and without stratification by status of gastric atrophy. All the models adjusted for age, sex, education level, race, body mass index, cigarette smoking, alcohol consumption, fruit consumption, vegetable consumption, and symptoms of gastroesophageal reflux.

5.3 Study III

5.3.1 Population characteristics

In this study, a total of 5,889,537 individuals were enrolled in the study, with a mean follow–up time of 6.4 years. Among them, 2380 esophageal cancer patients were ascertained, with 1412 EAC patients and 848 ESCC patients. Most of them had a healthy dental condition (48.8%) and no tooth loss (62.9%) (Table 5.7). Cancer risk associated with different dental diseases were reported in total esophageal cancer, subtype of EAC, and subtype of ESCC, respectively.

5.3.2 Dental health conditions and esophageal cancer—time constant model

Figure 5.4 demonstrates that periodontitis was associated with an increased risk for total esophageal cancer, comparing to the healthy population. This association was found in total

Table 5.6: Mediation analysis: effect of accumulated number of antibodies

Mediator: Gastric atrophy		
Effect	Med4way	IORW
TE	−0.38 (−0.55, −0.21)	−0.38 (−0.55, −0.22)
NDE	−0.37 (−0.55, −0.19)	−0.38 (−0.55, −0.20)
NIE	−0.01 (−0.04, 0.01)	−0.01 (−0.05, 0.02)
Mediator: BMI		
Effect	Med4way	IORW
TE	−0.38 (−0.54, −0.22)	−0.38 (−0.55, −0.22)
NDE	−0.41 (−0.57, −0.25)	−0.40 (−0.56, −0.25)
NIE	0.03 (0.00, 0.07)	0.03 (−0.01, 0.08)
Mediator: GERD		
Effect	Med4way	IORW
TE	−0.37 (−0.54, −0.19)	−0.38 (−0.54, −0.23)
NDE	−0.39 (−0.59, −0.20)	−0.37 (−0.53, −0.20)
NIE	0.03 (−0.03, 0.08)	−0.03 (−0.09, 0.04)
Joint mediation		
Effect	Med4way[a]	IORW
TE	—	−0.38 (−0.54, −0.23)
NDE	—	−0.37 (−0.54, −0.21)
NIE	—	−0.02 (−0.10, 0.07)

[a] Med4way is not applicable for multiple mediator analysis.

esophageal cancer, EAC and ESCC groups. Root canal infection was associated with a higher risk only for the outcome of EAC (Fig 5.4, a). Higher number of lost teeth at the time of first visit was associated with a higher risk for total esophageal cancer, EAC, and ESCC. Moreover, the effects showed significant dose–response effect (Fig 5.4, b).

5.3.3 Dental health conditions and esophageal cancer—time dependent model

We also studied the dental health effects in a time–dependent manner, taking into account that people visited the dental clinics multiple times and their dental health condition may change during follow–up time (Figure 5.5). The results showed periodontitis was still associated with higher risk for total esophageal cancer, EAC and ESCC. Mild inflammation was associated with higher risk for total esophageal cancer and EAC, root canal infection was associated with higher risk for EAC. Likewise, more teeth loss at the time of first dental visit increased the risk for total esophageal cancer, EAC, and ESCC, in a dose–response manner.

Table 5.7: Baseline characteristics by dental health status and remaining number of teeth in the cohort identified from the Swedish Dental Health Register, 2009–2016.

Dental health status[a]	**Healthy**	**Caries**	**Root canal infection**	**Mild inflammation**	**Periodontitis**
Total (N, %)	2,414,936 (47.9)	847,074 (16.8)	203,384 (4.0)	963,421 (19.1)	613,482 (12.2)
Follow–up years (mean ± SD)	6.5 ± 1.9	6.5 ± 1.8	6.2 ± 2.1	6.4 ± 2.0	6.6 ± 1.9
Age at baseline (mean ± SD)	46.5 ± 18.9	47.7 ± 18.3	50.6 ± 16.5	48.3 ± 18.0	57.9 ± 15.5
Male (N, %)	1,110,501 (46.0)	425,794 (50.3)	109,531 (53.9)	481,085 (49.9)	316,199 (51.5)
Education (≥ 13 years, N, %)	798,662 (33.1)	258,088 (30.5)	48,381 (23.8)	336,467 (34.9)	157,813 (25.7)
Missing (N, %)	47,006 (1.9)	16,530 (2.0)	4,794 (2.4)	21,431 (2.2)	21,845 (3.6)
Family income (High, N, %)	932,009 (38.6)	283,205 (33.4)	58,249 (28.6)	372,778 (38.7)	207,666 (33.9)
Smoking–related diseases (N,%)	29,503 (1.2)	11,540 (1.4)	4,010 (2.0)	11,205 (1.2)	14,562 (2.4)
Alcohol–related disorders (N, %)	47,062 (1.9)	20,956 (2.5)	9,868 (4.9)	19,586 (2.0)	20,356 (3.3)
Obesity (N, %)	26,974 (1.1)	14,647 (1.7)	5,901 (2.9)	11,542 (1.2)	9,367 (1.5)
GERD (N, %)[b]	72,609 (3.0)	28,687 (3.4)	8,484 (4.2)	28,905 (3.0)	24,320 (4.0)
Remaining number of teeth	**28+**	**25-27**	**21-24**	**15-20**	**1-14**
Total (N, %)	3,169,727 (62.9)	950,783 (18.9)	478,088 (9.5)	256,840 (5.1)	186,858 (3.7)
Follow–up years (mean ± SD)	6.3 ± 2.0	6.8 ± 1.6	6.8 ± 1.7	6.5 ± 2.0	6.0 ± 2.2
Age at baseline (mean ± SD)	40.9 ± 15.1	55.2 ± 15.9	64.0 ± 15.3	72.1 ± 11.0	73.5 ± 12.3
Male (N, %)	1,610,443 (50.8)	427,758 (45.0)	207,099 (43.3)	112,294 (43.7)	85,514 (45.8)
Education (≥ 13 years, N, %)	1,179,770 (37.2)	276,801 (29.1)	94,058 (19.7)	30,815 (12.0)	17,966 (9.6)
Missing (N, %)	43,314 (1.4)	32,446 (3.4)	19,890 (4.2)	9,812 (3.8)	6,144 (3.3)
Family income (High, N, %)	1,229,313 (38.8)	361,573 (38.0)	156,051 (32.6)	66,519 (25.9)	40,451 (21.6)
Smoking-related diseases (N, %)	21,081 (0.7)	13,225 (1.4)	12,607 (2.6)	11,789 (4.6)	12,118 (6.5)
Alcohol-related disorders (N, %)	67,972 (2.1)	21,077 (2.2)	12,412 (2.6)	8,283 (3.2)	8,084 (4.3)
Obesity (N, %)	38,826 (1.2)	13,611 (1.4)	7,870 (1.6)	4,649 (1.8)	3,475 (1.9)
GERD (N, %)[b]	67,141 (2.1)	38,692 (4.1)	26,625 (5.6)	17,463 (6.8)	13,084 (7.0)

[a] Dental health status was defined by the diagnosis at baseline: Healthy, caries, root canal infection, mild inflammation (stomatitis, mucositis (implants), pericoronitis, gingivitis, other unspecific inflammation conditions), and periodontitis (periodontitis and periimplantitis).

[b] GERD, Gastroesophageal reflux disease.

The table is reproduced from Zhang et al. Cancer Epidemiology, Biomarkers & Prevention 2022 (90).

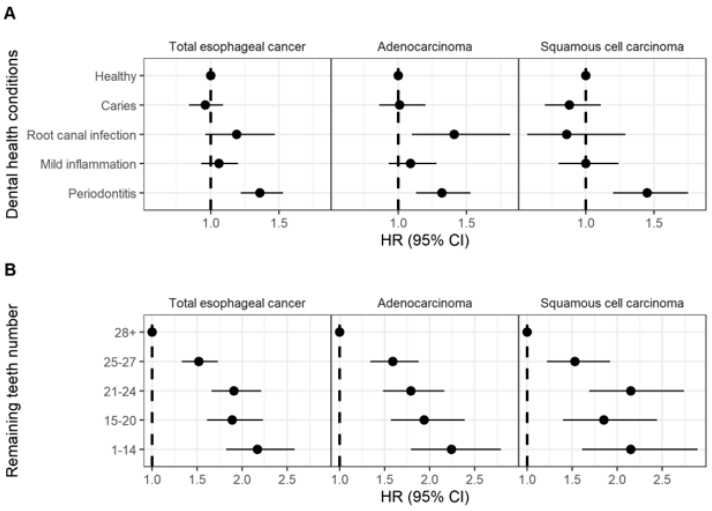

Figure 5.4: Hazard ratios (HRs) for esophageal cancer and its histopathological subtypes according to dental health in a cohort identified from the Swedish Dental Health Register, 2009–2016. The figure is reproduced from Zhang et al. Cancer Epidemiology, Biomarkers & Prevention 2022 (90).

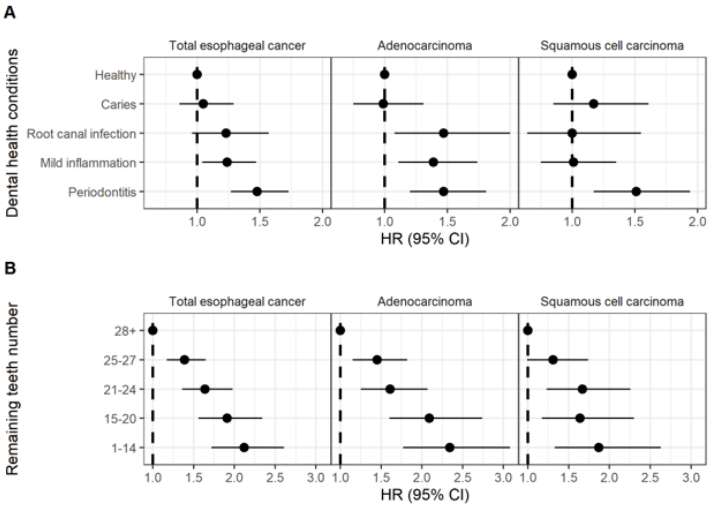

Figure 5.5: Time–varying hazard ratios (HRs) for esophageal cancer and its histopathological subtypes according to dental health in a cohort identified from the Swedish Dental Health Register, 2009-2016. The figure is reproduced from Zhang et al. Cancer Epidemiology, Biomarkers & Prevention 2022 (90).

5.4 Study IV

5.4.1 Population characteristics

In this study, among 12,533 participants from the twin registry, 4729 (37.7%) people with less frequent GERD symptoms and 2338 (18.7%) people with frequent GERD symptoms were ascertained. Individuals with GERD symptoms were more likely to have higher BMI, to be smokers, to have the habit of alcohol consumption, and lighter physical activities. When different cut–offs were used for the identification of AG, the prevalence of AG ranged from 6.1% to

11.8% in the GERD free group and ranged from 3.2% to 7.3% in the GERD group (Table 5.8). There were 1002 MZ twins and 3188 DZ twins with one person having GERD symptoms while the other not (Table 5.9).

Table 5.8: Characteristics of 12,533 twin study participants with and without symptomatic gastroesophageal reflux disease (GERD)

Characteristics	No GERD n (%)	With GERD n (%)		
		Total	Less frequent	Frequent
Total	5466	7067	4729	2338
Education (0–9 years)	1,632 (29.9)	2,077 (29.4)	1,322 (28.0)	755 (32.3)
Body mass index (≥ 30)	313 (5.7)	624 (8.8)	373 (7.9)	251 (10.7)
Coffee (≥ 6 cups/day)	978 (17.9)	1,261 (17.8)	860 (18.2)	401 (17.2)
Is or has been a smoker	2,261 (41.4)	3,426 (48.5)	2,230 (47.2)	1,196 (51.2)
Moderate alcohol consumption[a]	335 (6.1)	493 (7.0)	335 (7.1)	158 (6.8)
Light physical activity	960 (17.6)	1,461 (20.7)	945 (20.0)	516 (22.1)
Atrophic gastritis				
PGI<30				
No	5132 (93.9)	6838 (96.8)	4566 (96.6)	2272 (97.2)
Yes	334 (6.1)	229 (3.2)	163 (3.4)	66 (2.8)
PGI<70 and PGI/PGII<3				
No	5050 (92.4)	6775 (95.9)	4527 (95.7)	2248 (96.2)
Yes	416 (7.6)	292 (4.1)	202 (4.3)	90 (3.8)
PGI/PGII<3				
No	4897 (89.6)	6608 (93.5)	4422 (93.5)	2186 (93.5)
Yes	569 (10.4)	459 (6.5)	307 (6.5)	152 (6.5)
PGI<25 or PGI/PGII<3				
No	4822 (88.2)	6551 (92.7)	4383 (92.7)	2168 (92.7)
Yes	644 (11.8)	516 (7.3)	346 (7.3)	170 (7.3)

Abbreviations: GERD, gastroesophageal reflux disease; PGI, pepsinogen I; PGII,pepsinogen II.

[a] Alcohol drinking is categorized into light(≤ 1 drink per day), moderate (1 to < 4 drinks per day) and heavy(≥ 4 drinks per day);1 drink unit is defined by NIAAA National Instituteon Alcohol Abuse and Alcoholism units per day for total alcohol consumption.

The table is reproduced from Zhang et al. United European Gastroenterology Journal 2022. (97)

5.4.2 AG and GERD in twins

The AG cases were first compared with all AG free controls, regardless of twin conditions. Around halved risk for GERD was observed in AG positive individuals. The results were consistent using different cut–off values. Moreover, the risk reduction was slightly more profound in the frequent GERD (≥ 1 per week) group than in the less frequent GERD (< 1 per week) group. Furthermore, AG was inversely associated with the occurrence of GERD when the analysis was restricted in DZ twins and MZ twins, at the same level comparing to the results in the total twins (Figure 5.6). Likewise, using different cut–off values in the classification of AG cases did not produce remarkably different estimations. As a sensitivity analysis, individuals with a medication history of anti–acid medicine or proton–pump inhibitors were excluded, and the results were not substantially altered (data not shown).

Table 5.9: The distribution of symptomatic gastroesophageal reflux disease (GERD) in same-sexed dizygotic (DZ) and monozygotic (MZ) twin pairs.

	MZ pairs, n (%)	DZ pairs, n (%)
Concordant, both twins have GERD	1104 (39)	2382 (34)
Concordant, neither twin has GERD	728 (26)	1506 (21)
Discordant for GERD[a]	1002 (35)	3188 (45)
Age at interview for GERD (median; interquartile)	56; 9	57; 10
Age at onset of GERD (median; interquartile)	40; 20	40; 25

DZ, dizygotic; GERD, gastroesophageal reflux disease; MZ, monozygotic.
[a] One twin has GERD, while the other does not have GERD.
The table is reproduced from Zhang et al. United European Gastroenterology Journal 2022. (97)

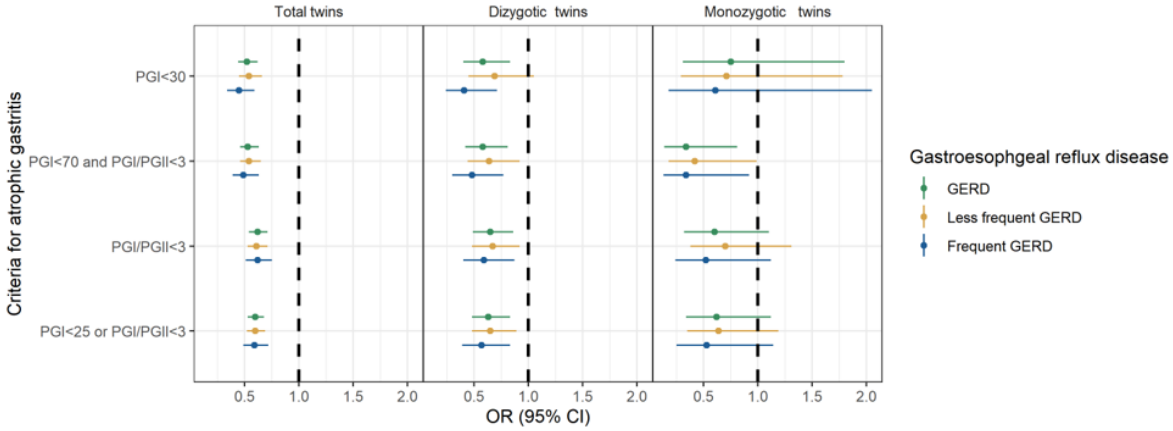

Figure 5.6: The association between atrophic gastritis and GERD in total twins, DZ twins and MZ twins. All the models adjusted for year of birth, sex, BMI, education level, coffee intake, physical activity, smoking, and alcohol consumption. The figure is reproduced from Zhang et al. United European Gastroenterology Journal 2022. (97)

Chapter 6

DISCUSSION

6.1 Interpretation of the results

6.1.1 Sex difference in the prognosis of esophageal cancer surgery

In **Study I**, we found that female patients had an overall better survival than males after curative surgical treatment. This sex difference presented to be more prominent in ESCC patients than EAC patients, and no sex disparity was observed in gastric cancer patients. When the sex difference was presented over follow–up time, it was observed to reach a peak shortly after surgery for both subtypes of EAC and ESCC, and gradually decreased thereafter. This might be associated with the recurrence of esophageal cancer, as it is estimated that most recurrence for esophageal cancer patients happens within $2-3$ years after surgery (118, 119). Previously, a study using Swedish register data also reported a better 5–year survival in female patients than male patients in ESCC patients, whereas no sex difference was reported in 5–year survival in EAC patients (120). A study in the Japanese population also reported a better overall survival in female esophageal cancer patients undergoing surgery (121). The present study suggested a sex difference in a time varying manner, in both ratio scale and difference scale.

In the stratification analysis, the sex difference was further found to be restricted in the cancer stage 0–I, in patients undergoing neoadjuvant treatment, or in patients without any postoperative complications. This suggests that these surgery related factors may play an explanatory role in the sex difference in the postoperative survival. It is known that sex differentiated pharmacokinetics and pharmacodynamics could affect chemotherapy effects regarding the exposure, clearance, efficacy, toxicity of the medicine and adverse effects related to treatments. This is mainly because males have about 20% better renal function than females, which leads to a better drug clearance, thus female patients are 15–25% more exposed to anticancer drugs, comparing to males with the same dose of drugs. Therefore, males may have less circulating concentration of drug and a poorer postoperative survival than females (122, 123). As a result, females tend to be over–treated, while males tend to be under–treated. In addition, female patients were showed to be more likely to achieve complete pathologic response towards preoperative chemoradiotherapy, and have less risk for recurrence than males (124, 125). This sex differentiated response towards preoperative neoadjuvant therapy supports the finding in

this study that the postoperative sex difference was only restricted in patients who underwent neoadjuvant treatment in EAC patients.

6.1.2 *H. pylori*, gastric atrophy and EAC

In **Study II**, we confirmed an inverse correlation between fifteen antibodies against *H. pylori* and EAC. This further supports previous studies using overall serostatus of *H. pylori*, antibody against CagA, or other indications of *H. pylori* infections (126–131), whereas there are also studies showed a null relationship between *H. pylori* infection and EAC (128, 132, 133). The null association might partly be due to underpower or potential misclassification of the cases. Nevertheless, all the meta–analysis investigating the association between *H. pylori* infection and EAC reported an inverse association between, either *H. pylori* seropositivity or CagA seropositivity, with EAC or the precursor disorder of EAC (40, 134–136). Among 15 antibodies tested, CagA seropositive and VacA seropositive strains presented the lowest risk for EAC. This agrees with previous studies (126, 127, 130, 137). CagA protein binds with host molecules to deregulate their functions. CagA could cause strong mucosal inflammation response, and trigger the oncogenic transformation of epithelial cells (138–140). At the same time, VacA toxin acts as a virulent factor contributing to the tumorigenesis of *H. pylori*. It is involved in the disruption of epithelial cells, antigen presentation, autophagy suppression, inhibition of immune cells, and helping *H. pylori* to establish a permanent infection (138). This result suggests CagA and VacA may also play a major role in the potential link between *H. pylori* infection and EAC. Additionally, this study provided data of those less studied *H. pylori* antibodies on the risk of EAC. 12 out of 13 antibodies against *H. pylori* were associated with a lower risk for EAC, besides CagA and VacA. These proteins are with different virulence and help with the oncogenic ability of *H. pylori*. It is the first time they are connected with EAC. Further studies are required to fully understand the precise mechanism of these virulence markers for EAC.

6.1.3 Oral health and esophageal cancer

Dental diagnoses and esophageal cancer

In **Study III**, it was observed that diagnosis of periodontitis at baseline was associated with an elevated risk for overall esophageal cancer; root canal infection and periodontitis at baseline were both associated with a higher risk for EAC; and periodontitis at baseline was also linked with a higher risk for ESCC, after adjusting for the number of remaining teeth and potential confounding factors. Furthermore, when the changes of oral health conditions over time were taken into consideration, multiple records of dental health conditions and groups of remaining teeth number were included into the model in a time–dependent manner. The results further supported the association between poor oral health and esophageal cancer by slightly increased point estimations for impaired dental conditions and fewer recorded number of remaining teeth.

The association between poor oral health and esophageal cancer, measured by either impaired dental health diagnoses, poor oral hygiene habit, or dental health score has been reported

in previous studies (141–153). In addition to prior findings, this study adds up evidence for this association with the largest population–based prospective study, and definite diagnoses from clinical records, which enables to further disentangle the link between oral health and esophageal cancer. Periodontitis has been shown to be a risk factor for cancers in multiple organs whereas its biological mechanism is yet to be well revealed. One possible explanation is the systematic detrimental effects and overall high inflammation status brought by poor oral hygiene, which is one of the most recognized mechanisms in cancer development (154, 155). Specifically, caries and root canal infection are related to localized and confined single tooth inflammation. Mild inflammation and periodontitis are results of generalized chronic inflammations in the soft tissues as well as in the alveolar bone, surrounding single teeth, whole dentition, or oral cavity (156). At the same time, carcinogenic viruses inappropriately stored in the periodontal pockets could also contribute to this overall hyper–inflammation status by dispersing into the saliva and transporting into the systemic circulation from the sites of periodontal diseases, and therefore causes diseases remotely (157). This partly explains the reported association between periodontal diseases, tooth loss and diseases in various, multiple organs (158–160). Meanwhile, there are also studies showing an interplay between bacteria and poor oral health for an elevated cancer risk. Notably, *Tannerella forsythia* and *Porphyromonas gingivalis*, the compositional species of one of the major complexes related to periodontal diseases are associated with higher risks for both subtypes of EAC and ESCC (161, 162). *Porphyromonas gingivalis* could act through activation of immune cells, inhibition of apoptosis procedure, and dehydrogenation of ethanol to acetaldehyde to cause carcinogenic transformation in esophageal epithelial cells (163–165). The replacement of normal microbiota by *Campylobacter* is showed to contribute to the progression of esophageal cancer (166), and the domination of *Campylobacter* in the gastrointestinal tract could lead to the generation of carcinogenic cytokines, like interleukin-18 (IL-18), in epithelial cells of the esophagus, which could promote inappropriate immune response and promote tumor cell proliferation, invasion and metastasis. The abundance of subgingival dental plaque bacteria, including multiple species, was showed to be different between esophageal cancer patients and cancer free individuals (167). But none of causal relationship could be concluded from these case–control studies, and experimental evidence is needed to confirm specific bacteria as potential biomarkers for esophageal cancer.

Remaining teeth number and esophageal cancer

Fewer remaining teeth at baseline was related with a higher risk for total esophageal cancer, EAC subtype, and ESCC subtype, comparing to people with more than 28 teeth at baseline, with a dose–response effect. This association was independent of dental conditions after controlling for a number of measured confounding factors. This finding is in line with previous studies (141–145, 148, 153, 168–172). The association was also confirmed in meta–analyses (173, 174). The association between fewer teeth number and the risk for esophageal cancer consolidated the finding that poor oral health contributes to the occurrence of esophageal cancer. However, it is noteworthy that although tooth loss has been constantly linked with a higher risk for

esophageal cancer, it is not only an indicator for oral health, but also markedly influenced by socio–economics factors, overall health condition, and accessibility to dental healthcare facility (175–177). This complex interaction between biology and social factors causes difficulties for concluding a causal relationship between remaining teeth number and esophageal cancer.

6.1.4 The association between AG and GERD

In the present study, we found an inverse association between AG and symptomatic GERD in the STR, after adjusting for a rich range of demographic and behavior confounding factors, including birth year, sex, BMI, education level, coffee consumption, physical activity, smoking, and alcohol consumption. This inverse association between AG and GERD was also reported in previous studies among independent individuals, however results were inconsistent (178–183). This inconsistency is largely due to the differences in the measurements for AG or GERD. Specifically, in a study with 302 elective patients who underwent endoscopy, fewer chronic corpus gastritis was observed in GERD group than that in the GERD–free group (178). In a study performed in 627 ambulatory pH monitoring confirmed reflux esophagitis patients, the status of AG was determined by endoscopic checking and lower rate of GERD was observed in AG patients (179). In another study with 8936 elder population, serological biomarkers PGI and PGII were used to determine the AG status and the symptom of recent heartburn was used as the proxy for GERD. The authors reported a 70% reduced risk for heartburn in obese/overweight individuals detected of AG comparing to obese/overweight individuals without AG (180). In addition to these studies performed in unrelated individuals, this study demonstrated an inverse association between AG and GERD when the analysis was restricted within DZ or MZ co–twin pairs. This implies an association independent of factors shared between the twin pairs. These shared factors include inheritable genetic factors, environmental exposures and lifestyles in the early life. Besides, since the results within MZ twins were not substantially different from the results in the overall population, this also suggests that the association between AG and GERD is more likely to be caused by factors that are related to individual lifestyles and environmental factors that are different between twin pairs. Moreover, we confirmed that the results did not vary by the cut–off values of PGI and PGII for the identification of individuals reported of AG.

Possible mechanism for the inverse association between AG and GERD remains unclear. The primary pathogenesis for GERD is the acid injury with inflammation responses of esophageal epithelial cells caused by the acidic secretion reflux into the esophagus from the stomach (184). Corpus and fundus AG could lead to reduced or eliminated secretion of hydrochloric acid, due to the loss of the oxyntic glands and parietal cells (185). Taken together, achlorhydria associated with advanced AG might prevent GERD by reducing the injury to esophageal mucosa due to reduced gastric acid. Furthermore, corpus AG is shown to be prevalent among Swedish adults (186), which supports the hypothesis that the decreased occurrence of GERD in symptomatic individuals was partly explained by corpus AG. However, there was a study showing that one fourth of body AG patients still had GERD, implying that AG caused by hypochlorhydria could not fully prevent the occurrence of GERD (187), and further studies for the underlying

mechanisms are warranted.

6.2 Methodological considerations

6.2.1 Confounding

Comprehensive consideration for potential confounding effects is a key mission in epidemiological studies trying to address the causal effects. Whereas it is one of the main limitations in many register based studies that some important confounding factors may not be well recorded and therefore distorted the effects of interest. In **Study I**, obesity is one of the main missing confounder of concern that may affect the sex differentiated postoperative survival. It was estimated that obese patients were associated with higher possibilities of surgical complications like anastomotic leak, yet they showed a prolonged overall postoperative survival time. Moreover, the obesity effect on postoperative survival is also dependent on surgical procedures, races and prognostic outcomes, which make it more complex (188, 189). In addition, obesity is associated with inaccurate cancer diagnosis, imprecise calculation of chemotherapy doses, as well as surgical complications, which also bring difficulties for the precise prediction for surgical outcomes (190). Therefore, lack of obesity information could possibly bias the estimations in **Study I** to an unexpected extent. In **Study III**, to control for confounders of smoking, alcohol consumption, BMI, and GERD, their proxies were retrieved from the Swedish National Patient Registers and included in the regression models: chronic obstructive pulmonary disease, alcohol–related disease, obesity–related diseases, and diagnoses for GERD in the analysis within EAC subgroup. However, it should be noted that people diagnosed with chronic obstructive pulmonary disease, alcohol–related disease, or obesity–related diseases were more likely to be heavy smokers, heavy alcohol drinkers, or obesity individuals, and GERD patients without apparent symptoms may not be diagnosed and missed out from the patient register, therefore residual confounding effects of these variables could still affect the estimations of the association of interests.

6.2.2 Selection bias

In **Study I** and **Study III**, selection bias could happen if the loss to follow–up is dependent on the exposure. In these studies, the outcome of death was retrieved from the Cause of Death Register and the outcome of esophageal cancer was identified from the SCR, which are national registers with almost complete coverage to the total population. There is less likely to exist a difference in the loss of follow–up. In **Study II** and **Study IV**, selection bias could occur if the controls are not a representative sample from the population that cases originate from. In **Study II**, the population–based study is less likely to suffer from this selection bias. Whereas in **Study IV**, controls in this volunteer–based study might not well represent the general population, as the response rate was suggested to be higher in elder groups ($>$ 65 years) (94).

6.2.3 Misclassification

In the study about the association between exposure and outcomes, it is essential to correctly measure exposure status, important population characteristics, and outcomes among all the participants. Misclassification, or measurement errors in exposures or outcomes could lead to bias in the estimations for the associations of study (191). Misclassification can be grouped into non–differential or differential misclassification, depending on if the measurement errors are equally or not equally distributed between exposed and unexposed groups or between groups with or without outcomes. Non–differential misclassification could cause underestimation of the association. In population register based studies, differential misclassification of exposures or outcomes are less likely to happen whereas non–differential misclassification could happen and lead to bias of the results towards null.

In **Study I**, the outcome of interest was overall mortality rate and cause–specific mortality rate. However, it has been noted that for serious treatment like surgery in esophageal cancer, it is hard to discriminate the cause of death, and it is also estimated that 25% of deaths within 1 month after diagnosis and cancer–related surgery were not attributed to dying from cancer in the United States (192). To address the potential misclassification in the cause of death after surgery, the excess mortality rate was used to measure the mortality due to cancer, which does not require identification of cause of death after surgery (109).

In **Study II**, *H. pylori* infection was measured by seropositivities of fifteen antibodies against *H. pylori*. The method was estimated to have a sensitivity of 89% and a specificity of 82% in identifying the seropositivity of *H. pylori*. Therefore, there are possibilities of misclassification in the identification of *H. pylori*. However, there is no evidence that this misclassification is different between AG status, thus the association between *H. pylori* and EAC might be underestimated in this study.

In **Study IV**, outcome of interest was GERD defined by a series of typical reflux symptoms. Misclassification for the GERD might be inherent for the lack of recognition of symptom–free individuals and people with a high tolerance to the GERD symptoms. Moreover, the group of non–acidic reflux cases cannot be identified by the questionnaire, which is independent of AG. Therefore, this non–differentiated outcome misclassification could cause an underestimation of the associations. In the study, this issue was tested by restricting the analysis within people free of any PPI or anti-acid treatment. The largely unchanged results supported the findings in this study will not be invalidated by the misclassification problem. But better–designed studies are needed for the precise estimation of the association between AG and symptomatic GERD.

Chapter 7

CONCLUSIONS

- A survival benefit was observed in female patients compared to male patients who underwent curative intent surgery for esophageal cancer. This sex difference was found to be more evident in short and middle term after surgery and diminished after a long term. Moreover, more profound sex effect was found in patients with early clinical stage, neoadjuvant treatment, and without postoperative complications. A sex–differentiated treatment is suggested to improve survival of male esophageal cancer patients.
- *H. pylori* infection measured by 15 antibodies against *H. pylori* was associated with a lower risk for EAC. Antibodies other than CagA and VacA were also involved in this inverse association. More accumulated positive antibodies were also linked with a reduced EAC risk. And this association was not mediated by gastric atrophy, BMI, GERD, or the combination of these factors.
- Impaired dental health measured by root canal infection, periodontitis and fewer remaining teeth was associated with excess risks for two main histopathological subtypes of esophageal cancer. Dental health might be a novel risk factor and potential intervention target for the prevention of esophageal cancer.
- AG was associated with a reduced risk for GERD in a co–twin setting. This association was independent of familial and genetic factors shared within twins. The association was also persistent using different cut–off values for defining AG cases.

Chapter 8

FUTURE PERSPECTIVES

After years of research efforts in esophageal cancer field, it remains to be a malignant disease without valid techniques for early detection or curative therapy. First, the incidence of esophageal cancer, especially the subtype of EAC, has been increasing in the past decades in those well–developed countries. Second, the male predominance in the incidence remains unresolved. Third, promising novel risk factors or biomarkers for early detection still need to be explored and verified. Potential chain from *H. pylori* infection, AG, GERD, EAC, as was studied in this thesis needs to be further clarified. This study showed a sex–differentiated characteristic for the survival after curative surgery for esophageal cancer. It also brought new knowledge about the association between *H. pylori*, AG, GERD, and EAC. Moreover, it highlighted the potential role of dental health for the risk of esophageal cancer.

- In **Study I**, we observed a sex–differentiated prognosis after curative esophageal cancer surgery. Future studies with detailed information about confounding variables, like BMI, smoking, alcohol consumption, will help to eliminate the impact of residual confounding for the estimations. Moreover, postoperative complications might also affect postoperative survival in a sex–differentiated manner, thus a standardized measurement, like Clavien–Dindo classification would help to study the role of postoperative complications for a sex–differentiated survival. However, the Clavien–Dindo classification was included into the NREV register from 2012–01–01, so we did not have enough data to estimate the effect of postoperative complications for sex and survival. Therefore, it is worthwhile to study the role of postoperative complications for postoperative survival when more data is collected in future studies. Following the sex–differentiated survival presented in this study, further studies are also needed to learn how sex differentiated treatment might contribute to improve the living for males after esophageal cancer surgery.

- In **Study II**, we presented comprehensive relationships between 15 antibodies against *H. pylori* and EAC, besides the most commonly studied antibodies, CagA and VacA. Although other antibodies are less studied in previous studies, our results showed that they are also involved in the potential inverse association between *H. pylori* and EAC. Thus, additional studies based on these less studied antibodies would help to better understand the

underlying mechanism between *H. pylori* and EAC. Additionally, the interaction between gastrointestinal microbiota and *H. pylori*, in relation to AG, GERD and EAC, should be further explored. In the chronic progression of AG, the diversity and abundance of intragastric microbiome experienced complex change, which has been shown to affect the carcinogenesis of gastric cancer (193, 194). To meet this end, well–designed prospective studies are needed to capture the dynamic change of intragastric environment and to estimate their influence on esophageal cancer risk precisely.

- **Study III** from this thesis provided evidence that dental health, especially periodontitis is a potential risk factor for esophageal cancer. This needs to be further validated in different populations, as dental health is also affected by eating habit, the health reimbursement policy, socioeconomic status, etc. Validation studies in countries with different societal structure are required to establish a causal relationship between dental health and esophageal cancer. Moreover, studies based on basic science are also needed to elucidate the molecular mechanism underlying the association between dental infectious conditions and esophageal cancer, as this will be helpful for the development of targeted intervention for the prevention of esophageal cancer.

- Large–scale, well–designed longitudinal studies should be established to study the association between AG and GERD. The case–control study in this thesis retrospectively collected information of previous GERD symptoms. Prospective study could potentially reduce the probability of recall bias of medical history of PPI therapy, and the misclassification of GERD patients. A more comprehensive measurement of GERD patients is needed for GERD related studies, as nonacid reflux may cause similar symptoms as gastric acid related GERD. However, they were caused by different pathological process. Most current studies identified GERD patients based on severity and frequency of GERD symptoms measured by questionnaires for the cost–effectiveness considerations, therefore GERD patients without a sensible symptom might be missed. A precise classification of GERD patients would help to elucidate the association between AG and GERD.

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