BARRETT'S ESOPHAGUS REVISITED:

Epidemiology, Risk Stratification, and Cancer Prevention

P.J.F. DE JONGE

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Barrett's oesofagus herbezien:
epidemiologie, risico stratificatie en kanker preventie

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Voor mijn ouders.

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Chapter 1

General introduction and outline of the thesis

INTRODUCTION

Esophageal cancer is the sixth leading cause of cancer-related death worldwide.[1] In 2006, 1614 patients were newly diagnosed with this malignancy in the Netherlands.[2] Despite there being numerous improvements in both diagnostic and therapeutic techniques over the past three decades, esophageal cancer continues to have a poor prognosis, with 5-year survival rates between 10% and 13%.[3] During the last three decades, there has been a striking change in the epidemiology of cancer of the esophagus, predominantly affecting industrialized countries. Since the 1980s the incidence of esophageal adenocarcinoma (EAC) has been rising 4-10% annually, and still continuous to rise at present.[4-6] This tremendous increase in EAC incidence has led to a complete epidemiological shift such that in the United States and other industrialized countries, adenocarcinoma has replaced squamous cell carcinoma as the most common esophageal malignancy.[7] The causes for this alarming increase in EAC incidence are unclear. However, it has been postulated that this increase is due to an underlying rise of the prevalence of gastroesophageal reflux disease (GERD) and its complication Barrett's esophagus (BE) in the general population.[8, 9]

Barrett's esophagus is a well recognized premalignant condition for the development of EAC.[10, 11] It is characterized by the replacement of the esophageal squamous epithelium by metaplastic columnar epithelium as a consequence of chronic exposure to gastroesophageal reflux. This may lead to an incomplete form of intestinal metaplasia, named specialized intestinal metaplasia (SIM). BE predisposes to the development of EAC, following a multi-step cascade through stages of esophagitis, intestinal metaplasia, low-grade dysplasia (LGD), high-grade dysplasia (HGD), to invasive adenocarcinoma.[12] The excess risk of developing EAC in BE relative to the general population ranges between 30- and 60-fold.[13-15] Endoscopic screening of subjects with chronic GERD symptoms has been proposed as a method for detecting BE. Once BE has been diagnosed, surveillance endoscopy is advised at intervals based on the presence or absence and grade of dysplasia, in order to detect early stage cancers suitable for curative treatment, and ultimately to prevent deaths from EAC.[16]

SCREENING FOR BARRETT'S ESOPHAGUS

Risk factors for Barrett's esophagus

Screening for BE in the general population and in GERD patients could decrease EAC mortality and morbidity. Data from epidemiological studies on risk factors for BE and EAC provide valuable information for defining criteria to select the at-risk population. Multiple studies have found associations between male gender, white race, age>40, increased frequency and duration of GERD symptoms, and increased incidence of BE.[17] Also, familial aggregation of BE, EAC, and adenocarcinoma of the gastroesophageal junction (GEJ) has been documented in

white adults, suggesting that a positive family history should lower the threshold of screening for BE.[18, 19] Other investigators have shown that obesity is associated with an increased risk of BE, operating mostly by increasing gastric pressure, transient lower esophageal sphincter relaxations and esophageal acid exposure, as well as via additional humoral mechanisms mediated by visceral fat in particular.[20] The role of other factors in the etiology of BE and EAC, such as smoking, alcohol consumption, medication use and socioeconomic status is still rather inconclusive and needs further clarification. As long as other high risk markers for development of BE are not identified, the highest yield for BE screening will probably be in older Caucasian males with longstanding heartburn.

Challenges to screening for Barrett's esophagus

Screening for BE remains controversial because of the lack of documented impact on mortality from EAC. One of the major challenges to screening for BE is that the at-risk population is too broadly characterized. Symptoms of GERD are ubiquitous in the general population,[21] and, as has been estimated for the US population, even if screening is limited to those older than 50 years of age with at least weekly symptoms, more than 10 million endoscopies would be needed to prevent perhaps 1,500 EACs, a vanishingly small yield for an invasive screening test.[22] This strategy would soon overtax health care resources. Another diagnostic challenge concerns the large number of patients that lack reflux symptoms but have BE. Those with asymptomatic or minimally symptomatic BE will not enter into the GERD treatment process as they will not seek medical consultation. It has been estimated that clinically identified cases of BE represent just 6% of the total BE population in the general population, with undetected rates estimated at 376 per 100,000 based on autopsy studies.[23] A recent population-based study in Sweden reported a BE prevalence rate of 1.6%; forty-four percent of the BE patients lacked troublesome GERD symptoms over the past 3 months.[24] Moreover, more than 40% of patients with EAC report no antecedent symptoms of reflux and thus potentially could be missed.[25] It has also been argued that even though the incidence of EAC continues to rise, fewer than 5% of patients with the cancer are known to have BE before they develop symptoms of cancer.[26] This highlights the possibility that generalized BE screening methods may not detect BE in more than 95% of EAC cases.

As current risk factors are insufficient for identifying those patients who would benefit from screening endoscopy, new preferably non-invasive and less costly detection methods are needed to support screening. As an alternative to esophagogastroduodenoscopy (EGD), ultra-thin video endoscopes have been developed, which can easily be passed transorally or transnasally without sedation. These instruments can provide an efficient, cost-effective alternative to standard endoscopy, and can be offered as an option to conventional examination, particularly in the setting of screening.[27-29] However, use of unsedated small-caliber endoscopy is not yet accepted on a wide scale. Another newly introduced diagnostic modality is esophageal capsule endoscopy (CE), which offers a method of visualizing the esophagus

without the need for sedation, and without the discomfort and risks of EGD. Initial pilot studies demonstrated a high diagnostic yield of CE in the detection of BE.[30, 31] However, other investigators reported less optimal test characteristics, probably due to limitations of the video capsule itself and deviations from the ingestion protocol.[32, 33] Further research is necessary to evaluate the accuracy and cost-effectiveness of this detection method, before it can be introduced in a screening setting.

In conclusion, at present screening for BE in the general population cannot be recommended, given the inability to predict who has BE prior to endoscopy, the lack of evidence of efficacy, and the invasiveness and expense of endoscopy. The use of screening in selective populations at higher risk remains to be established and should be individualized.

SURVEILLANCE OF BARRETT'S ESOPHAGUS

Current surveillance guidelines

Once BE is diagnosed, patients are offered to participate in a surveillance program. Consideration for including patients in a surveillance program concern age, likelihood of survival over the next five years, patient's understanding of the process and its limitations for detection of cancer, and the willingness of the patient to adhere to the recommendations. Current recommendations for BE surveillance in the Netherlands are based on recent guidelines by the American College of Gastroenterology.[16] In general, in patients with no dysplasia, surveillance endoscopy is recommended at an interval of every three years. For patients with LGD or those with HGD, a yearly surveillance endoscopy and intensive endoscopic surveillance at every three months are advised, respectively. A systematic endoscopic biopsy protocol, generally accepted to be four quadrant biopsy specimens taken every 2 cm of Barrett's mucosa starting at the GEJ, is used to obtain a histologic diagnosis and grading of dysplasia.

Dilemmas in surveillance of Barrett's esophagus

Although surveillance endoscopy is intuitively reasonable and endorsed by many international gastroenterological societies, guidelines are inadequately evidence-based, as data in support of this strategy and proof of its efficacy are unequivocal. No randomized controlled trials have been performed on the efficacy of surveillance in terms of EAC prevention or prevention of cancer-related death in BE patients. Retrospective studies have shown that among patients diagnosed with EAC, those who were in a surveillance program were likely to have their cancer detected at an earlier stage than those not under surveillance.[34] In addition, nodal involvement is far less likely to occur in patients found to have cancer on surveillance endoscopies compared with those detected outside surveillance.[35] Subsequently, early recognition of HGD or intramucosal tumors has been associated with an improved survival from EAC.[36] On the other hand, although BE patients have a considerable increased risk of EAC as

compared to the general population, the majority of patients will not develop EAC,[26, 34, 37] and even fewer patients with BE will eventually die from EAC. In a cohort study from the Netherlands, only 5.6% of total mortality was related to EAC.[38] Another observational study that followed a cohort of 409 BE patients for 10 years showed that only four of them died secondary to EAC.[39] These findings suggests that BE surveillance programs will have only minimal effects on population health. In fact, BE patients do have an increased mortality risk, but this is largely due to increased incidence of cardiovascular and pulmonary disease.[40]

The cost-effectiveness of BE surveillance is crucially dependent on the annual cancer risk. Wide variation in this risk has been observed, with published estimates ranging between 0.2% to 2.9% per year.[41-44] These estimates were based primarily on patients referred to tertiary centers, whose cancer risk may exceed that for patients managed by non-referral centers. Furthermore, published data predominantly come from small retrospective cohort studies with relatively short follow-up, showing higher cancer incidence than may be observed in larger surveillance studies.[42] As a result of the low degree of ascertainment of BE in the general population,[24, 45] there is a lack of both large scale and long-term follow-up studies of BE patients, providing more reliable risk estimates for malignant progression. Such studies are, however, urgently needed to re-appraise the potential value of surveillance endoscopy in BE patients and to optimize the recommended follow-up intervals. While the exact rate of malignant progression in BE patients remains uncertain, it becomes even more important to take the burden of endoscopic surveillance in BE patients into account, as the majority of patients experience discomfort from EGD and are distressed beforehand.[46] As such, previous models of cost-effectiveness have most recently shown that surveillance either does more harm than good compared with no surveillance. According to a British study using an economic model, an annual cancer risk of 0.5% would mean that surveillance conferred less benefit and more costs than no surveillance at all, irrespective of the surveillance interval used.[47] This indicates that both quality of life benefit and cost-effectiveness of Barrett's surveillance are highly questionable unless surveillance can be targeted at those BE patients who are at the highest risk of cancer.

Risk stratification in patients with Barrett's esophagus

While risk factors for the development of both BE and EAC in the general population have been well investigated, it is largely unknown which patients with BE have an increased risk for malignant progression. Previous reports identified Caucasian ethnicity, older age, and male gender, increasing Barrett's segment length, large hiatal hernia size, and esophageal ulcer or stricture, to be associated with progression from BE to EAC.[39, 48-60] In addition, severe acid reflux, obesity and smoking and alcohol consumption are proposed to be risk factors for malignant progression.[10, 25, 48, 61, 62] However, some of these reports were based on observations or univariate analyses, and did not control for confounding variables. The role of profound acid suppression with proton pump inhibitors (PPI) as a protecting factor for EAC development in

patients with BE is controversial. Evidence exists that PPI therapy in BE reduces esophageal acid exposure,[63] decreases mucosal cell proliferation and increases differentiation,[64] and possibly reduces the length of Barrett's segment and dysplasia incidence.[65-67] Others, however, did not find any effect of longstanding PPI treatment on the incidence of EAC in BE, and EAC has still been reported to occur after successful medical and surgical therapies for GERD.[68]

At present, histological assessment of the degree of dysplasia in BE is the gold standard for determining the risk of progression. The presence of LGD or HGD at histology has been shown to be associated with increased risk of EAC development. However, there are a number of problems with dysplasia as a risk marker, such as the chance of sampling errors. In addition, in series of patients who had esophagectomies because of a diagnosis of HGD with no apparent tumor mass, invasive cancer has been seen in 30-40% of the resected specimens.[69] Conversely, there are reports of patients whose preoperative biopsy specimens showed intramucosal carcinoma, but resected specimens of the esophagus had no evidence of cancer.[70] Another problem is inter-observer variation in grading of dysplasia in BE. Considerable variability in the interpretation of no dysplasia or indefinite for dysplasia and LGD in BE not only exists between non-experts and experts, but also between expert pathologists.[71] Also, the interobserver agreement in differentiating HGD from intramucosal cancer is only fair.[17]

There have been a number of attempts to identify biomarkers that can predict which patients are at greatest risk of developing EAC. Ideal biomarkers are those that have variable expression associated with a pathologic process and that are detectable at an early stage in disease. More than 60 have been suggested for BE alone.[72] Genetic abnormalities within the Barrett's mucosa, such as aneuploidy and tetraploidy, have shown promise as markers of elevated cancer risk,[73, 74] but no method for the detection of these abnormalities is yet ready for clinical application. Other markers that can identify cell-cycle abnormalities are cyclin D1, which is a cell-cycle regulator, and Ki67, which is present during active, but not resting, phases of the cell cycle.[72] In principle such markers are attractive options for the detection of at-risk patients, but larger, prospective studies need to be performed before biomarkers can be widely used in the clinic.

At present, the evidence on risk factors for malignant progression in BE remains inconsistent, and as a result none of these aforementioned risk factors have been routinely included in planning BE surveillance programs, except for the presence of dysplasia. Identification of additional risk factors, preferably those that can be obtained without endoscopy, could be of use to improve surveillance recommendations.

AIM AND OUTLINE OF THIS THESIS

As outlined above, cancer of the esophagus is a highly aggressive malignancy worldwide, of which the incidence has increased tremendously during the last decades, and still con-

tinues to rise at present. Barrett's esophagus is the only recognized precursor lesion and is associated with the majority, if not all, cases with adenocarcinoma. Unfortunately, the efficacy of screening and surveillance of BE remains a strongly debated issue, as there are many unresolved epidemiological dilemmas, of which the inability to predict who has BE prior to endoscopy, and the lack of data on the natural history of BE are the major ones. Improved risk stratification could improve the effectiveness of screening and surveillance in BE patients, and achieve the ultimate goal of reducing EAC mortality.

The aim of the studies described in this thesis is to reassess the yield of screening for and surveillance of BE in the prevention of EAC, by exploring the natural course of BE, by investigating various risk factors involved in the progression of chronic GERD to BE and finally to HGD or EAC, and by examining the value of non-invasive techniques in the identification of high risk groups.

Chapter 2 describes epidemiological time trends of BE in the Netherlands over the period 1991 to 2006 and provides an age-period-cohort analysis, in order to gain more insight in possible causes of changes in BE incidence trends. In **chapter 3**, we investigate the distribution of environmental risk factors among patients with adenocarcinoma or squamous cell carcinoma of the esophagus, and among patients with adenocarcinoma from the gastric cardia. **Chapter 4** describes the result of a study on the accuracy of esophageal capsule endoscopy for the detection of esophageal mucosal disorders, using a new ingestion protocol. **Chapter 5** comprises a systematic review and meta-analysis of studies on the risk of cancer in BE, as well as on the risk of mortality due to EAC. In **chapter 6** we estimate the incidence of both HGD and EAC in a nationwide cohort of BE patients, and study potential predictors for malignant progression in BE patients. **Chapter 7** focuses on risk factors that could be used to discriminate between low-risk and high-risk BE patients for the development of EAC, using a hospital-based case-control study design. **Chapter 8** reports on the risk of colorectal cancer in patients with BE as compared to the general population, with risk analyses for different follow-up periods, in order to explore the temporal relationship between both conditions. In **chapter 9** we study the effect of PPI treatment on the immune response and oxidative damage in the distal esophagus, in patients with chronic GERD. Finally, in **chapter 10**, the findings of this thesis and their implications in the context of ongoing research are discussed, and directions for future research are highlighted.

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Chapter 2

The changing epidemiology of Barrett's esophagus in the Netherlands

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ABSTRACT

Background

The Netherlands is among the countries with both the highest and the steepest rising incidence rates of esophageal adenocarcinoma (EAC). Data on the incidence rate of its premalignant precursor, Barrett's esophagus (BE) is valuable as it allows prediction of EAC incidences in the coming decade.

Aim

To evaluate time trends in the epidemiology of Barrett's esophagus in the Netherlands.

Methods

Patients with a first-time diagnosis of BE between 1991 and 2006 were identified in the Dutch nationwide registry of histopathology (PALGA). Annual age-standardized BE incidence rates (ESRs) and age-specific incidence rates were calculated. Time trends were evaluated by age-period-cohort models.

Results

In total, 42,467 patients were newly diagnosed with BE. After correction for the annual increases in the numbers of first esophageal biopsies of 1.70% (95%CI: 1.55-1.85) in males and 0.96% (95%CI: 0.78-1.14) in females (both $p<0.001$), the net annual increase in BE incidence was 3.45% (95%CI: 3.12-3.78, $p<0.001$) in males, and in females 1.28% (95%CI: 0.89-1.68, $p<0.001$). For both genders, age-period-cohort models demonstrated a non-linear period phenomenon for BE, with a rapid increase in BE diagnoses before 1996, leveling off thereafter (both $p<0.001$). In addition, non-linear cohort phenomena for BE in both genders were found, with the greatest increase in cohorts born after 1945 (both $p<0.001$).

Conclusion

The incidence of histologically confirmed BE is strongly rising in the Dutch population, predominantly affecting males. The increasing BE incidence is a harbinger of a further rise in the number of EACs of nearly 35% in males and 13% in females within the coming decade, and emphasizes the need of strategies that have a direct impact on cancer risk in BE.

INTRODUCTION

As in other Western countries,[1, 2] the incidence of esophageal adenocarcinoma (EAC) is on the rise in the Netherlands,[3] with an increase that has been greater than for any other type of cancer, and an associated mortality rate that continues to be alarmingly high.[4] Although the cause of this increase is unknown, it is very likely to result from a similar rising incidence of its pre-malignant condition, Barrett's esophagus (BE), as it is commonly accepted that virtually all EACs are preceded by BE.[5]

Epidemiological data describing temporal trends for BE are, however, scarce. True population-based incidence rates of BE are difficult to study, as the ascertainment of BE in the general population is low.[6, 7] In a large proportion of patients, BE is asymptomatic and, even in symptomatic cases, its diagnosis depends on referral for esophagogastroduodenoscopy (EGD), and adequate recognition and histological confirmation during endoscopy.[8] Nevertheless, increases in the incidence and prevalence of BE have been reported, although it has often remained unclear whether this reflected a true increase, a greater awareness of BE, increased detection of short segment BE (SSBE), or increased use of EGD. In some studies the increase in incidence paralleled higher EGD use,[9, 10] whereas in others it was independent of EGD rates.[11, 12] Clarification of the existence of a true rise in BE incidence and of its contributing factors is important, as, if present, it will herald a continuing increase in incidence of EAC in the near future. In addition, an increasing BE incidence has significant implications for health resource utilization and costs, as most patients will be offered endoscopic surveillance according to international guidelines.

A previous study from the Netherlands, analyzing data from a nationwide registry of pathology reports (PALGA) and focusing on the time trends in the incidence of BE between 1992 and 2003, concluded that a true increase in the incidence of BE seemed likely, although the findings could in part be explained by changes in endoscopic practice.[13] In order to achieve greater certainty about the existence of a secular rise in the incidence of BE and to explore its causes, the present study has extended the PALGA BE data to 2006, and examines the constancy of the age and gender specific esophageal biopsy rates as well as performs age-period-cohort analyses.

METHODS

Histopathology database

In the Netherlands all histopathology and cytopathology reports are collected in a national archive (PALGA database), which encompasses all sixty-four pathology laboratories in the Netherlands. Since 1991 PALGA has had nationwide coverage and currently contains about 42 million excerpts from nearly 10 million patients in a total national population of 16 mil-

lion.[14] Every excerpt in the database contains encrypted patient identification, a summary of the original pathology report and diagnostic codes similar to the Systematized Nomenclature of Medicine (SNOMED) issued by the College of American Pathologists.[15] This diagnostic code contains a topological term, the type of sample, and a morphological term describing the finding, *e.g.,* 'esophagus*biopsy*intestinal metaplasia'. Details regarding the number and intra-esophageal location of biopsies are not uniformly registered. Each pathology report can, however, be traced to an individual patient, allowing follow-up of individuals, irrespective of where subsequent biopsies were taken or resections were performed. For each report, gender, date of birth, date of pathology review, summary text and diagnostic codes were made available. It was not, however, possible to access additional clinical data as the PALGA registry contains pathology reports only. The present study was based on data recorded in the PALGA database between 1991 and 2006.

Data collection

All patients registered in the database between 1991 and 2006 with an initial, histological diagnosis of BE were identified.[16] Codes that were used to classify biopsies as BE are described in the Appendix. Patients with excerpts reporting either gastric or esophageal surgery or malignancy, registered prior to, or simultaneously with the first diagnosis of BE, were excluded from the cohort.

As an estimation of the population undergoing an EGD, the total numbers of patients with a first esophageal biopsy per calendar year were also collected for the period 1991-2006.

Data analysis

Age-standardized rates of histopathologically confirmed BE were computed for the period 1991-2006, using the European Standard Population (European Standardized Rates (ESR)). To examine the age distribution of BE cases, age specific incidence rates were computed separately for each 5-year age group and provided for both genders. In order to correct for changes in frequency of EGD, the ratio of the number of new BE cases relative to the number of patients with a first esophageal biopsy was calculated. In addition, annual BE incidence changes were corrected by regression analysis for annual fluctuations in the number of patients with a first esophageal biopsy during the study period.

Time trends in the incidence of BE during the study period were evaluated by age–period–cohort models using log-linear Poisson regression analysis. For the estimation of cohort models, a mean birth year was calculated for each 5-year age class. The estimated drift parameters rendered the percentage annual change (PAC) in incidence rates, corrected for age and population size. Linear splines were used to calculate non-linear period and cohort effects. Instead of one exponential curve from the beginning to the end of the study period, we extended the model to three exponential lines connected by knots at 1996 and 2001 for period estimates and at 1930 and 1945 for those of birth cohorts. The presence of significant

non-linear period or cohort effects was estimated by performing likelihood ratio tests (comparison of scaled deviances).[17, 18]

RESULTS

Study population

In total, 42,467 patients were newly diagnosed with BE (61% male, median age 62.2 yrs), 4,351 (10.2%) of whom were classified with dysplasia. BE patients with dysplasia were significantly older (65.3 vs. 61.8 yrs, $p<0.001$), and more often male (64% vs. 61%, $p<0.001$) than those without.

Age and sex distribution of the incidence of Barrett's esophagus

The incidence of BE significantly increased with age (Figure 1). In males, the age specific BE incidence rates increased rapidly between the ages of 30 to 70 years, after which they leveled off and decreased sharply in those aged over 80 years. In females, however, the rise in incidence rates started later and reached its maximum between the age of 80 to 85 years. Logistic regression analyses demonstrated that between the ages of 20 to 70 years, the BE age-incidence curves were practically identical for both sexes, with an increase in BE incidence rate by 0.71% (95%CI: 0.62-0.80) for each additional year of age, although there was an age shift of 10.3 years between these parallel male and female age-incidence curves.

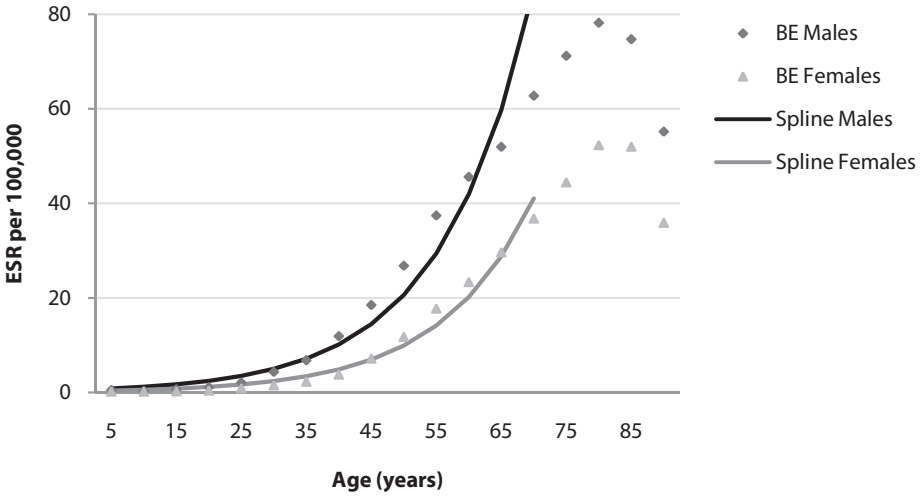

Figure 1. Age-specific incidence rates of Barrett's esophagus by gender.

Time trends in the incidence of Barrett's esophagus

Over the 16-year period 1991-2006, the ESR age-standardized incidence of BE increased by 92% (from 13 to 25 per 100,000) in males, by 45% in females (from 8.3 to 12 per 100,000), and by 73% (from 11 to 19 per 100,000) for both combined (Figure 2). The ratio of the number of detected BE cases to the number of patients with a first esophageal biopsy increased by 45% in males (from 22 to 32 per 100) and by 23% in females (from 22 to 27 per 100).

The annual increase in BE incidence was 4.07% (95%CI: 3.79-4.35, $p<0.001$) for males, but considerably lower in females (1.84%, 95%CI: 1.50-2.18, $p<0.001$). During the same period, the number of patients with a first esophageal biopsy increased annually by 1.70% (95%CI: 1.55-1.85) in males and by 0.96% (95%CI: 0.78-1.14) in females (both $p<0.001$) (Figure 3). Subsequently, after correction for the simultaneous increase in patients undergoing a first esophageal biopsy, the net annual increase in BE incidence was 3.45% (95%CI: 3.12-3.78, $p<0.001$) for males, and 1.28% (95%CI: 0.89-1.68, $p<0.001$) for females.

Age-period-cohort effects

The results of the cohort and period estimates are shown in Table 1. Both for male and female BE patients, the mean changes in the annual incidence for each of three periods (cohorts born before 1930, between 1930 and 1944, and after 1944) were differentiated by year of birth for cohort effects, and by calendar year for period effects. Significant differences between the values for the three periods indicated a non-linear cohort or period effect.

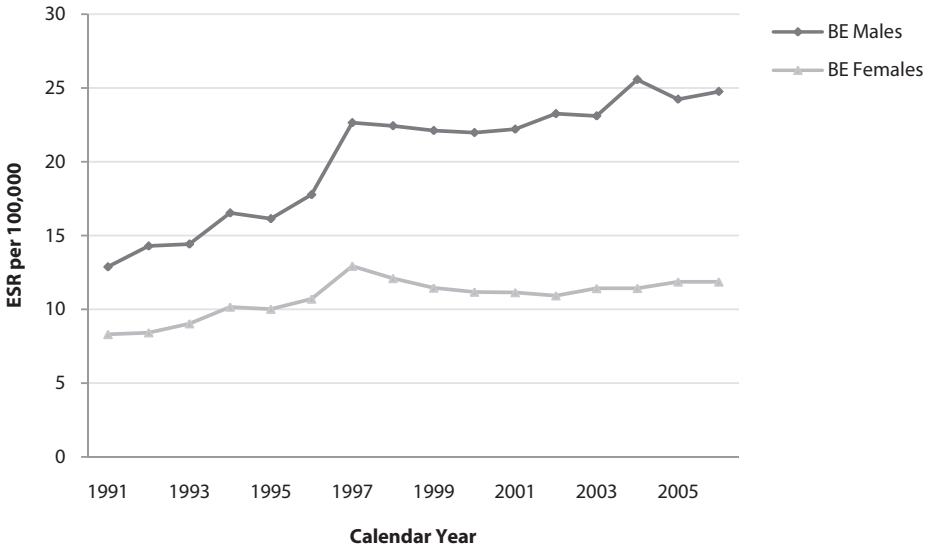

Figure 2. Annual European standardized rates for Barrett's esophagus by gender for the period 1991-2006.

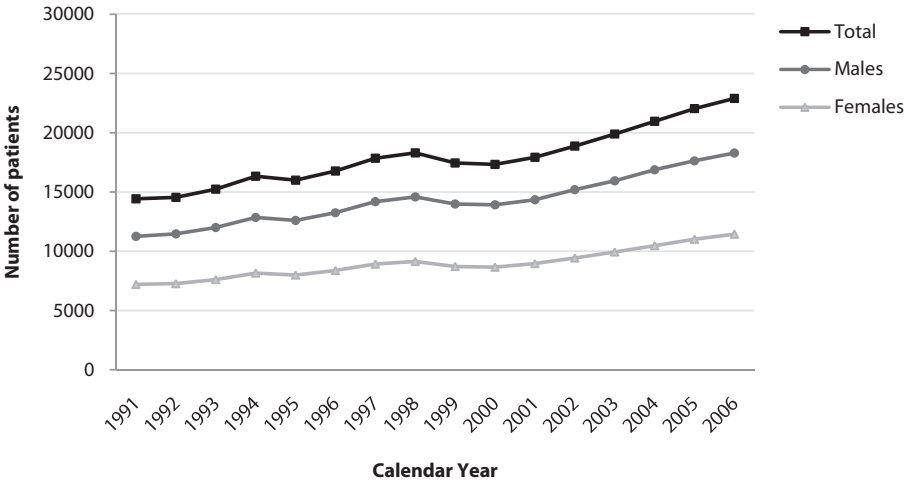

Figure 3. Number of patients with a first esophageal biopsy during the period 1991-2006.

Table 1. Period or cohort effects for Barrett's esophagus by gender 1991-2006.

	Period effect				Cohort effect			
Gender	**1991-96**	**1996-01**	**2001-06**	***p*-value**	**<1930**	**1930-44**	**>1944**	***p*-value**
Males	+9.1	+3.5	+1.5	<0.001	+1.3	+3.6	+5.3	<0.001
Females	+7.9	-0.7	+1.1	<0.001	-0.8	+1.8	+4.4	<0.001

Estimates are means of the annual changes for each of the periods, differentiated by year of birth for cohort effects and by incidence year for period effects.

In both genders a non-linear period phenomenon was observed with a rapid increase in BE diagnoses before 1996, and subsequently leveling off (both $p<0.001$) (Figure 4A and B). In females, the incidence actually decreased annually between 1996 until 2001, thereafter, the incidence increased again by 1.1% per year. There were significant cohort effects in both males and females, demonstrating a rising incidence trend in cohorts born after 1945 (both $p<0.001$) (Figure 5A and 5B).

DISCUSSION

This study shows a substantial increase in the number of new patients diagnosed annually with BE in the Netherlands during the 16-year period from 1991 to 2006. This rise was seen in both men and women, but was stronger in men. The annual increase in BE incidence significantly exceeded the annual increase in number of patients with a first esophageal biopsy. In addition, concomitant with the rise of new BE cases, birth cohort effects were demonstrated

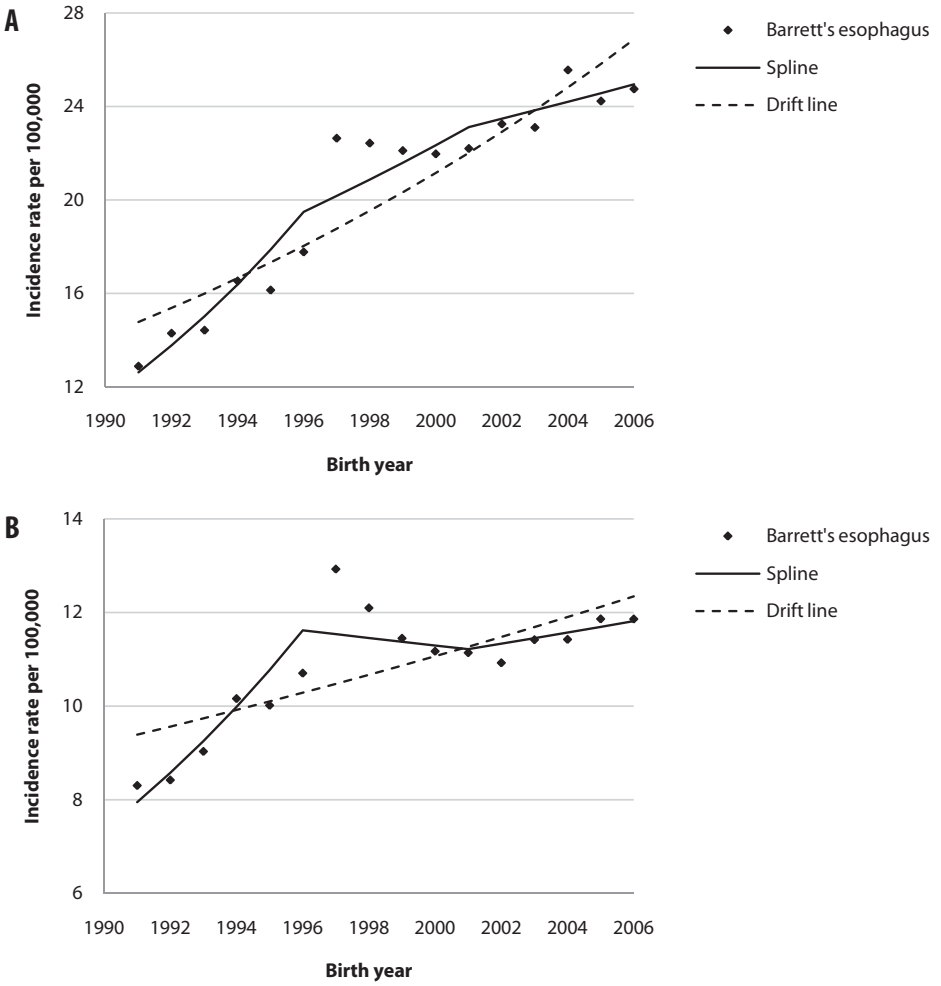

Figure 4. Period effects for Barrett's esophagus for (A) males and (B) females. Symbols refer to age standardized incidence rates. Drawn lines are the linear splines from the age-period model. The dashed lines show the results of the drift model.

for both genders, indicating that this rise in BE incidence could not be solely attributed to an increased use of upper GI endoscopy, but was for the larger part explained by altered circumstances for the general population.

Our results, based on data from the national registry of pathology reports, confirm the previously reported increase in incidence of BE in the Netherlands, based on data from a general practitioner database.[12] Other studies have drawn various conclusions as to whether the increasing incidence of BE in the general population reflected a true increase, or was merely due to the increased use of EGD. Our findings are in line with other observations showing true rises in BE incidence rates, independent of changes in endoscopy rates.[10, 11, 19, 20] On the

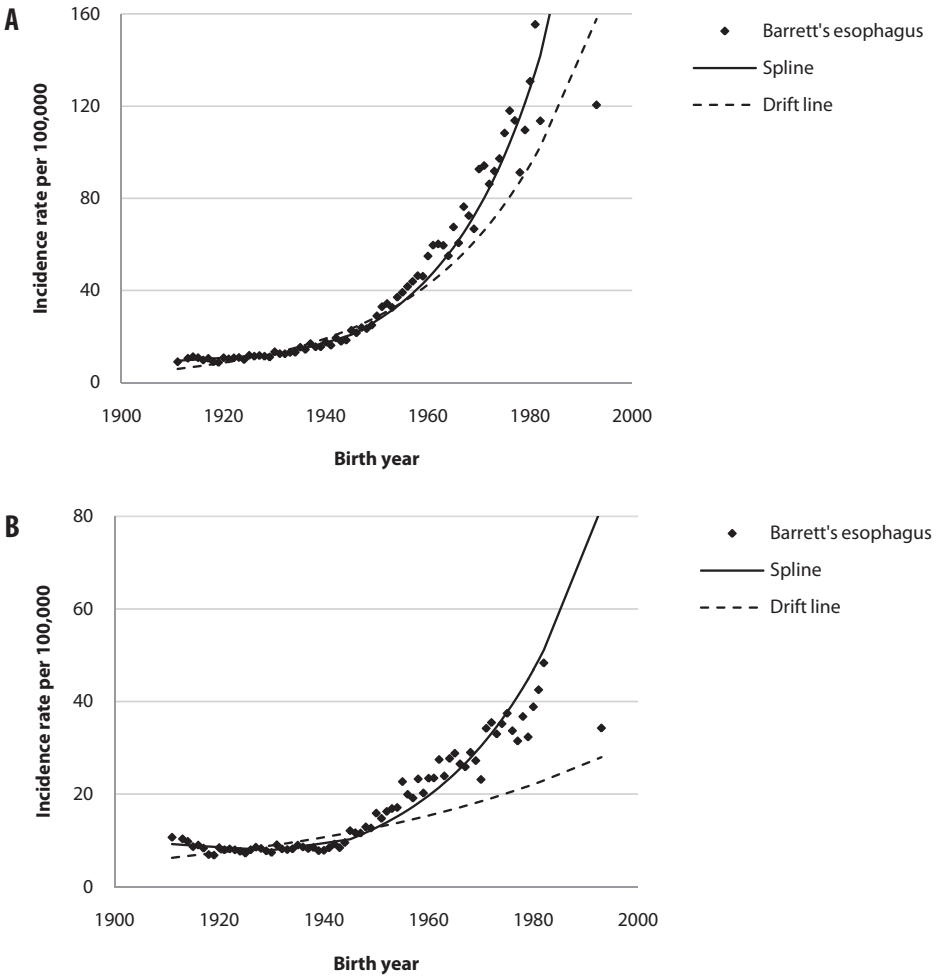

Figure 5. Cohort effects for Barrett's esophagus for (A) males and (B) females. Symbols refer to age standardized incidence rates. Drawn lines are the linear splines from the age-cohort model. The dashed lines show the results of the drift model.

other hand, a study from the USA did demonstrate a stable BE incidence despite increased EGD rates.[21] Differences in methodology or study population may have contributed to discrepancies between the aforementioned studies.

In order to gain more insight in possible causes of the increase in incidence of BE in the general Dutch population, we performed an age-period-cohort analysis. This demonstrated a significant period effect, in both males and females, consisting of a far steeper increase in newly diagnosed BE cases from 1991 to 1996, as compared to periods 1996 to 2001 and 2001 to 2006. To clarify this period effect several explanations can be considered. Firstly, an increased recognition of BE amongst gastroenterologists is plausible, in particular the rec-

ognition of SSBE during the 1990s,[22] a condition which has been first described in the late 1980s.[23] Unfortunately, as no data on the length of the Barrett's segment were available in our database, we were unable to ascertain such SSBE incidence trends in the Dutch population. Australian investigators, however, recently reported a marked increase in SSBE incidence between 1990 and 2002, resulting in a reduction in the mean length of BE [20]. This observation was in line with data from El-Serag *et al.*[24], who reported a similar secular decrease in the length of BE in the USA.

A second explanation for the marked period effect in the increase of BE incidence in the first half of the nineties could lie in changes in endoscopic technology, as fiberoptic endoscopes were largely replaced by video-endoscopes during this period, leading to considerably improved imaging. This has likely increased the sensitivity for detecting BE, especially with regard to patients with SSBE. Thirdly, changes in the endoscopy referral pattern by general practitioners, for instance under influence of manufacturers of proton pump inhibitors, may also have led to increased numbers of patients with symptoms of gastroesophageal reflux disease (GERD) being referred for EGD. The subsequent reduction of the number of EGDs is likely to have resulted at least in part from the introduction of Dutch primary care guidelines on dyspepsia in 1993, with a revision in 1996. These guidelines emphasized the restriction of referrals of dyspepsia patients without alarm symptoms for EGD.[25] A similar trend was previously observed in a Dutch study on the incidence of premalignant gastric lesions, showing a sharply increasing number of patients with a first gastric biopsy until 1998, followed by a decline until 2001, and a slower subsequent rise until 2005.[26] Our study showed a similar declining trend in the number of esophageal biopsies from 1996 to 1998, followed by a somewhat stronger incline after 2001 than that observed in the aforementioned study. This may have resulted from a mounting interest in histological confirmation of esophageal entities, such as eosinophilic esophagitis and non-erosive reflux disease. On the other hand, the ratio of the number of new BE cases to the total number of patients with a first esophageal biopsy rate actually increased during the study period. This supports the hypothesis that detection bias cannot be solely responsible for the increasing incidence of BE in the Netherlands, but that new or augmented risk factors also must have contributed to this rise.

This supposition was confirmed by the finding of a birth cohort effect for both genders revealing a clear increasing trend among cohorts born after World War II. The rise in incidence of BE was most pronounced in persons under 60 years of age, especially in males, which is in line with previous Dutch data.[12] In addition, similar cohort phenomena in the incidence of EAC have previously been reported in a study analyzing US SEER-data,[27] and more recently in the Dutch population.[3] For these trends several explanations seem plausible. Firstly, these patterns partly fit the secular changes of obesity,[28] which have also become evident in the Netherlands.[29] Yet, general obesity does not fully explain our birth cohort effects, as the prevalence of obesity is also increasing rapidly in demographic groups at relatively low risk of BE and EAC. Abdominal obesity alone explains some of the epidemiological features of BE.[30]

A recent study reported a consistent association between abdominal diameter and GERD symptoms in Caucasians, but not in African Americans or Asians.[31] Furthermore, the visceral component of abdominal obesity is thought to promote GERD-related disorders via humoral mechanisms, such as increased release of several pro-inflammatory cytokines,[32, 33] and lower serum levels of adiponectin, which has an anti-apoptotic and anti-proliferative effect.[34] In aggregate such factors tend to augment both inflammation and malignant transformation in patients with GERD. These findings, combined with the increased prevalence of abdominal obesity in men, suggest that increased obesity may disproportionately increase GERD and BE in Caucasians and particularly in males. This gender effect is reflected in the lower overall annual increase in BE incidence in females in our study, and the smaller cohort effect in females as compared to males. A second explanation for an increasing incidence of BE in subsequent birth cohorts could be found in the declining prevalence of *Helicobacter pylori* infection in the general population, as some data suggest that gastric *H. pylori* infection, in particular the more virulent CagA-positive strain, may protect the esophagus from the effects of acid reflux, perhaps by decreasing gastric acidity resulting from gastric atrophy or by enhancement of gastric emptying in especially younger persons.[35] A cohort phenomenon for the prevalence of *H. pylori* has previously been demonstrated by analyzing longitudinal data, which showed that the prevalence of chronic *H. pylori*-induced gastritis is much lower in younger birth cohorts and that *H. pylori* infection is rarely acquired after childhood. The observation that European and Asian countries, with far higher colonization rates of *H. pylori,* have considerably lower rates of BE supports this concept.[36, 37] However, a general problem with this concept is that the sex distribution of *H. pylori* does not match the different BE incidence trends between males and females.

In our cohort, BE was predominantly found in males, with a male to female ratio of 1.6:1.0, which is similar to reports by others.[38] The observed 10 year age shift between the parallel male and female BE age-incidence curves provides an explanation for the observed 1.6:1 sex ratio. The observed age-shift is, however, shorter than that observed in two previous studies from the U.K. and the Netherlands on the epidemiology of BE, reporting an age-shift of about 20 years.[39, 40] This discrepancy can be explained by differences in methodology, as in these studies BE incidence rates were calculated per 100 EGDs, with equal numbers of EGDs performed in males and females. Nevertheless, the observed age-shift strengthens the hypothesis that this shift is responsible for the observed increased risk of BE in males, and is in line with the male predominance among EAC patients.[41] The presence of an age-shift in the development of BE between males and females suggests a certain degree of protection against BE and EAC in females. This possibly relates to an endogenous effect associated with the female reproductive years, most likely being estrogen, which is known for its anti-inflammatory effects in certain tissues.[42] The 2:1 male to female ratio in the prevalence of reflux-esophagitis found by others again suggests a protective effect on inflammation of the GI tract in females.[43] In addition, it has been shown that the risk of gastric cancer is inversely

associated with both a delayed menopause and hormone replacement therapy.[44, 45] However, the effect of estrogen on esophageal disorders is unclear, as exogenous estrogen therapy was recently linked to symptomatic GERD,[46] although others could not confirm this association.[47, 48] Recently, a large-scale randomized clinical trial showed an increase in GERD symptoms following weight gain in patients who received menopausal hormone therapy.[49] Although estrogen therapy led to a smaller waist circumference, it did not affect the incidence or progression of GERD in these patients. This could suggest that the protective effect of hormones for females may disappear with the tendency to gain weight around their perimenopausal years. The interaction between estrogen and obesity needs further clarification.

Some limitations of this study need to be considered. Firstly, our observed BE incidence rates are only rough estimates. Most BE patients will not undergo EGD, due to absence of reflux symptoms. The actual BE incidence rate could therefore well be higher than that found in our study. However, trends in the detection rate of BE are presumably not influenced by this underestimation. Secondly, some patients with intestinal metaplasia of the cardia could have been misclassified as having BE in our cohort, as endoscopic data were not available. We believe, however, that such misclassifications would only have played a minor role, as only pathology excerpts specifically reporting Barrett's epithelium in esophageal biopsies were included. In addition, obtaining biopsy specimens from the gastric cardia is not routine practice during EGD or in the diagnosis of BE in the Netherlands.

In conclusion, this study shows an ongoing substantial rise in the incidence of histologically confirmed BE in the Dutch population over the past 16 years, affecting males even more than females. Period and cohort phenomena for BE were demonstrated for both genders, the former likely to have been explained by both an increasing awareness of BE among endoscopists and improved endoscopic techniques, the latter by changes in prevalence of environmental risk factors after World War II, such as the increasing prevalence of obesity and the declining prevalence of *H. pylori*. The increasing BE incidence is a harbinger of a further rise in the number of EACs of nearly 35% in males and 13% in females within the coming decade, and emphasizes the need of strategies that have a direct impact on cancer risk in BE.

APPENDIX

PALGA diagnosis codes used in the analysis:
Barrett's esophagus: T62310M73330, M73320
Dysplasia: M74000, M74006, M74007, M74008, M74009

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Chapter 3

Environmental risk factors in the development of adenocarcinoma of the esophagus or gastric cardia: a cross-sectional study in a Dutch cohort

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ABSTRACT

Background

Risk factors for adenocarcinoma of the esophagus (EAC) and gastric cardia (GCA) are not yet established.

Aim

To compare environmental risk factors between patients with EAC and GCA.

Methods

One-hundred and twenty-six patients with EAC, 43 with GCA and 57 with squamous cell carcinoma filled out a questionnaire with information on demographic and lifestyle characteristics, physical activity levels, family history, gastroesophageal reflux disease (GERD) symptoms, and medication use.

Results

EAC and GCA patients were similar with regard to male predominance and age, alcohol intake and smoking, use of fruits and vegetables, body posture and occupational activities ($p>0.05$). GCA patients less often had heartburn compared with EAC patients (odds ratio (OR) 0.5, 95% confidence interval (CI): 0.2-0.96) and had these symptoms less frequently and for a shorter period (OR 0.3, CI: 0.1-1.0 and OR 0.1, CI: 0.03-0.6, respectively). Former and current aspirin use was lower among GCA patients than EAC patients (OR 0.2, CI: 0.05-0.7 and OR 0.4, CI: 0.1-0.9, respectively), whereas no difference in non-steroidal anti-inflammatory drug use was detected.

Conclusion

Although EAC and GCA share several environmental risk factors, EAC is more frequently associated with a history of GERD, suggesting a more important role for gastroesophageal reflux in EAC compared with GCA.

INTRODUCTION

Over the past two decades, a dramatic increase in the incidence of esophageal adenocarcinoma (EAC) has been noticed in the Western world, which coincided with a less pronounced increase in the incidence of gastric cardia adenocarcinoma (GCA).[1] This suggests that common risk factors account for both disorders. More recent epidemiological data from the USA[2] and the Netherlands[3] investigating time trends with regard to these two malignancies, however, showed a declining incidence rate of GCA during the last decade, suggesting differences in risk factor profiles. Parallel to this trend, a moderate decrease in incidence of squamous cell carcinoma (SCC) and distal gastric cancer has been reported.

SCC on the one hand and EAC and GCA on the other hand show marked differences in pathogenesis, tumor biology and patient characteristics.[4] Differences in risk factor profiles and epidemiological features between EAC and GCA are, however, unclear. Various risk factors for these malignancies have been proposed, including gastroesophageal reflux disease (GERD), tobacco and alcohol use, dietary factors, medication, obesity and *Helicobacter pylori* infection.[5] In clinical practice, EAC and GCA often are considered as one entity with regard to staging, treatment and survival.[6-8] These carcinomas are also similar in histopathological features, such as pattern of lymph node metastases and overall prognosis. However, because of the different locations of these cancers, either the esophagus or stomach, the development of these two malignancies may involve alternative pathways and could therefore be triggered by different etiological factors.

Most studies on etiological factors with regard to the development of carcinomas of the proximal gastrointestinal (GI) tract have investigated the relationship between patients with either type of carcinoma and healthy controls. A direct comparison between patients with EAC and GCA by use of a questionnaire could add to a better insight in etiological differences between EAC and GCA. In addition, as EAC and SCC clearly have been shown to be different disease entities, a comparison between these two esophageal cancers could demonstrate the accuracy of the questionnaire.

We conducted a study in which we investigated the distributions of environmental risk factors among patients with either type of esophageal cancer or GCA.

MATERIALS AND METHODS

Patients

Between August 2002 and November 2005, all consecutive cases of esophageal carcinoma -EAC and SCC- and GCA who were evaluated for endosonographic staging in our unit were asked to participate in this study.

The type and location of the tumor were classified and determined by a team of an endoscopist, a GI pathologist and a surgeon. A cancer was classified as EAC, when the epicenter of the carcinoma was clearly situated in the distal esophagus and/or when the tumor originated from Barrett's esophagus.[9] The tumor was considered to be a GCA, when the epicenter was located at the junction in the absence of Barrett's epithelium, or if the center of the tumor was seen in the gastric cardia, defined as the area at or immediately below the gastroesophageal junction (GEJ). According to the Siewert classification, an EAC would refer to type I, whereas a GCA would be type II.[10] Patients with tumors originating from the fundus or corpus of the stomach but infiltrating into the cardia were excluded. All biopsy specimens and gross specimens, in case the patient underwent a resection, were evaluated by an expert GI pathologist (HvD). Barrett's epithelium was only diagnosed when specialized intestinal metaplasia was histologically present in the distal esophagus according to the guidelines of the American College of Gastroenterology.[11]

Data collection

All patients with EAC, SCC and GCA received a detailed questionnaire on the day of the endosonographic investigation. We have used this questionnaire previously in patients with Barrett's esophagus with or without EAC.[12] The questionnaire comprised questions on demographic factors (age, gender and race), body mass index (BMI), educational level, lifestyle (smoking habits, alcohol consumption and dietary patterns), physical activity levels (during work and spare time), working in a stooped posture, personal and family history of GI symptoms and diseases, and medication use (proton pump inhibitors (PPIs), histamin-2 receptor antagonists (H2RAs), non-steroidal anti-inflammatory drugs (NSAIDs), aspirin and antacids). In addition, data on BMI, dietary patterns, physical activity levels and working in a stooped posture were collected for the present situation as well as for that at age 20 and 10 years prior to filling out the questionnaire. Written informed consent was obtained from each subject. The study was approved by the Institutional Review Board of the Erasmus MC Rotterdam.

Statistical analysis

Baseline characteristics were compared using analysis of variance (ANOVA) for continuous variables and the chi-squared test for categorical variables. Logistic regression was performed to calculate odds ratios (OR) with 95% confidence (CI) intervals for comparison between EAC and GCA on the one hand and EAC and SCC on the other hand. OR<1 indicated a significant higher prevalence of a certain risk factor in EAC patients than in GCA or SCC patients. For all analyses, EAC was chosen as reference group. Univariable analysis was performed to obtain a first impression of the effect of risk factors. Variables with a *p*-value <0.2 were further selected for multivariable analysis with adjustments for age, sex, educational level, smoking and alcohol consumption. This set of confounders was applied to all models. When the variable of interest was also present in the multivariable model (*e.g.,* smoking status), then it was left

out from the analysis. Within domains of related variables (*e.g.,* smoking status and duration of smoking) we considered variables one by one. For the multivariable analysis, two-sided *p*-values <0.05 were considered statistically significant. Statistical analyses were conducted using SPSS software (SPSS 11.0, Chicago, Illinois, USA).

RESULTS

Of the 316 eligible patients, 236 (74.7%) patients gave informed consent and filled out the questionnaire, 73 (23.1%) patients refused participation and 7 (2.2%) patients were unable to fill out the questionnaire because of poor clinical condition.

Baseline characteristics of EAC, GCA and SCC patients are shown in Table 1. Age, race and educational level were not significantly different between the three groups. More female pa-

Table 1. Baseline characteristics of patients.

Variable	EAC (referent)	SCC	GCA	*p*-value
Participants	126	57	43	--
Age (years) (mean, sd)	61.8 (11)	62.9 (9)	65.3 (10)	0.148
Sex (male)	112 (89%)	37 (65%)	37 (86%)	<0.001
Race (causasian)	125 (100%)	56 (98%)	42 (98%)	--
Educational level				
Primary school	61 (48%)	28 (49%)	22 (51%)	0.986
High school	34 (27%)	17 (30%)	11 (26%)	
College/University	30 (24%)	12 (21%)	10 (23%)	
BMI (kg/m^2) (mean, sd)	26.3 (4)	22.7 (4)	27.4 (7)	<0.001
Ever smoked	113 (90%)	52 (91%)	38 (88%)	0.501
Ever alcohol use	116 (92%)	53 (93%)	39 (91%)	0.468
Fruit intake (p/week) (mean, sd)	8 (6)	7 (7)	6 (6)	0.239
Vegetable intake (p/week) (mean, sd)	6 (2)	6 (3)	6 (2)	0.864
Hot meal (p/week) (mean, sd)	6 (2)	6 (2)	6 (2)	0.664
Physical activity (h/week) (mean, sd)	8.6 (14)	8.1 (14)	2.3 (7)	0.031
Helicobacter pylori eradication	5 (4%)	1 (2%)	2 (5%)	0.733

EAC: esophageal adenocarcinoma, SCC: squamous cell carcinoma, GCA: gastric cardia adenocarcinoma, BMI: body mass index.
p-value from ANOVA / χ^2-test.

tients were diagnosed with SCC compared with EAC and GCA (35%, 11% and 14%, respectively, $p<0.001$). At the time of the endosonographic investigation, patients with SCC had a lower mean BMI as compared with EAC and GCA patients ($p<0.001$). GCA patients were less frequently physical active in their spare time than EAC and SCC patients ($p<0.031$). Of all the 126 EAC patients, only 4 patients were known with a previous history of BE and participated in a surveillance program (3%). With regard to GCA patients, 1 patient had a clinical diagnosis of BE prior to the development of GCA (2%). None of the SCC patients was previously diagnosed with BE.

Alcohol consumption and smoking behavior

Patients with SCC more often used alcohol compared with EAC patients (OR 6.4 (95%CI: 1.1-38)) and had used this for a period of 1-20 years (OR 8.2 (95%CI: 1.0-72)) (Table 2). With regard to tobacco use, both current and former uses were comparable between the three groups. In addition, no differences in duration of tobacco use were detected.

Body mass index

SCC patients were less often overweight (BMI>25) compared with EAC patients, both at age 20 as well as 10 years prior to the endosonographic investigation (OR 0.2 (95%CI: 0.1-0.8) and OR 0.3 (95%CI: 0.1-0.6), respectively). Although GCA patients less often had a BMI>25 compared with EAC patients (16% vs. 21%), this difference was statistically not significant (Table 2).

Gastroesophageal reflux and family history

A history of heartburn was less often indicated by SCC and GCA patients than by patients with EAC (OR 0.3 (95%CI: 0.1-0.6) and OR 0.5 (95%CI: 0.2-0.96), respectively) (Table 3). SCC and GCA patients less frequently had longstanding (>12 years) symptoms of heartburn (OR 0.1 (95%CI: 0.05-0.6) and OR 0.1 (95%CI: 0.03-0.6), respectively) and also had more frequent episodes of heartburn per week (OR 0.1 (95%CI: 0.03-0.5) and OR 0.3 (95%CI: 0.1-1.0), respectively) than EAC patients. However, regurgitation was equally indicated by patients with GCA and EAC (OR 0.4 (95%CI: 0.2-1.1)), whereas SCC patients less often had symptoms of regurgitation than EAC patients (OR 0.4 (95%CI: 0.2-1.0)). A family history of heartburn and regurgitation was less common in SCC than in EAC (OR 0.1 (95%CI: 0.03-0.4) and OR 0.3 (95%CI: 0.1-0.9), respectively). However, no differences in family history were detected between EAC and GCA patients.

Medication use

SCC patients less often had been using PPIs prior to diagnosis than EAC patients (OR 0.2 (95%CI: 0.1-0.6)). In addition, current use of H2RAs was also less common in SCC patients (OR 0.07 (95%CI: 0.01-0.6)) than in EAC patients (Table 4). Although use of PPIs and H2RAs was

Table 2. Body mass index, use of alcohol and use of tobacco in relation to the risk of esophageal and gastric cardia cancer development.

Variable	EAC cases (percent)*	SCC cases (percent)	GCA cases (percent)	Univariable OR SCC (95%CI)	Multivariable OR SCC (95%CI)**	Univariable OR GCA (95%CI)	Multivariable OR GCA (95%CI)
Smoking							
Never	13 (10%)	3 (5%)	5 (12%)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
Former	44 (35%)	26 (47%)	12 (28%)	2.6 (0.7-9.8)	2.5 (0.5-11)	0.7 (0.2-2.4)	0.8 (0.2-2.7)
Current	67 (53%)	27 (46%)	26 (61%)	1.7 (0.5-6.6)	3.2 (0.7-14)	1.0 (0.3-3.1)	0.9 (0.3-3.3)
Duration of smoking							
Never	12 (10%)	3 (5%)	4 (9%)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
1-20 yr	25 (20%)	5 (9%)	7 (16%)	0.8 (0.2-3.9)	1.3 (0.2-7.7)	0.8 (0.2-3.4)	0.9 (0.2-4.1)
20-40 yr	48 (38%)	29 (51%)	13 (30%)	2.4 (0.6-9.3)	3.1 (0.7-14)	0.8 (0.2-2.9)	0.8 (0.2-3.3)
>40 yr	35 (28%)	15 (26%)	16 (37%)	1.7 (0.4-7.0)	2.1 (0.4-10)	1.4 (0.4-4.9)	1.3 (0.3-5.4)
Alcohol							
Never	10 (8%)	2 (4%)	6 (14%)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
Former	92 (73%)	46 (81%)	28 (65%)	2.5 (0.5-12)	6.4 (1.1-38)	0.5 (0.2-1.5)	0.5 (0.1-1.7)
Current	21 (17%)	8 (14%)	6 (14%)	1.9 (0.3-11)	4.8 (0.7-27)	0.5 (0.1-1.9)	0.4 (0.1-1.8)
Duration of alcohol							
Never	10 (8%)	2 (4%)	5 (12%)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
1-20 yr	6 (5%)	5 (9%)	4 (9%)	4.2 (0.6-29)	8.2 (1.0-72)	1.3 (0.3-7.0)	1.5 (0.2-8.7)
20-40 yr	49 (39%)	22 (39%)	6 (14%)	2.2 (0.5-11)	4.1 (0.6-28)	0.3 (0.1-1.0)	0.3 (0.1-1.2)
>40 yr	53 (42%)	22(39%)	24 (56%)	2.1 (0.4-10)	4.0 (0.6-27)	0.9 (0.3-2.9)	0.8 (0.2-3.0)
BMI at age 20							
<25	87 (69%)	49 (86%)	34 (79%)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
>25	26 (21%)	4 (7%)	7 (16%)	0.3 (0.1-0.8)	0.2 (0.1-0.8)	0.7 (0.3-1.7)	0.7 (0.3-1.9)
BMI 10 yrs before questionnaire							
<25	36 (29%)	33 (58%)	14 (33%)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
>25	84 (67%)	20 (35%)	28 (65%)	0.3 (0.1-0.5)	0.3 (0.1-0.6)	0.9 (0.4-1.8)	0.7 (0.3-1.6)

EAC: esophageal adenocarcinoma, SCC: squamous cell carcinoma, GCA: gastric cardia adenocarcinoma, BMI: body mass index, OR: odds ratio, CI: confidence interval.

* Total percentages may not be 100 because of missing values.

** In the multivariable analysis, odds ratios for smoking status and duration of smoking were both adjusted for age, sex, educational level and alcohol consumption. Odds ratios for alcohol use and duration of alcohol use were both adjusted for age, sex, educational level and smoking. Odds ratios for BMI were adjusted for age, sex, educational level, smoking and alcohol consumption.

not different between EAC and GCA patients, former antacid use was less frequent in GCA patients than in EAC patients (OR 0.3 (95%CI: 0.1-0.9)).

SCC patients less often were former NSAID users (OR 0.3 (95%CI: 0.1-0.9)) for a short period (<6 months) (OR 0.2 (95%CI: 0.05-0.8)) than EAC patients (Table 4), whereas usage patterns of NSAIDs were comparable between EAC and GCA patients. On the other hand, GCA patients

Table 3. Symptoms of reflux and family history in relation to the risk of esophageal and gastric cardia cancer development.

Variable	EAC cases (percent)*	SCC cases (percent)	GCA cases (percent)	Univariable OR SCC (95%CI)	Multivariable OR SCC (95%CI)**	Univariable OR GCA (95%CI)	Multivariable OR GCA (95%CI)
Heartburn							
No	61 (48%)	43 (75%)	30 (70%)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
Yes	64 (51%)	12 (21%)	13 (30%)	0.3 (0.1-0.6)	0.3 (0.1-0.6)	0.4 (0.2-0.9)	0.5 (0.2-0.96)
Duration of heartburn							
Never	64 (51%)	44 (77%)	32 (74%)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
<12 yr	26 (21%)	8 (14%)	8 (19%)	0.5 (0.2-1.1)	0.3 (0.1-0.9)	0.6 (0.3-1.5)	0.7 (0.3-1.7)
>12 yr	32 (25%)	3 (6%)	2 (5%)	0.1 (0.04-0.5)	0.1 (0.05-0.6)	0.1 (0.03–0.6)	0.1 (0.03-0.6)
Frequency of heartburn							
0x	65 (52%)	45 (79%)	31 (72%)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
1x	14 (11%)	1 (2%)	2 (5%)	0.1 (0.01-0.8)	0.1 (0.02-1.1)	0.3 (0.1-1.4)	0.3 (0.06-1.5)
2-3x	16 (13%)	6 (11%)	6 (14%)	0.5 (0.2-1.5)	0.6 (0.2-1.7)	0.8 (0.3-2.2)	1.1 (0.4-3.1)
>3x	27 (21%)	3 (5%)	4 (9%)	0.2 (0.05-0.6)	0.1 (0.03-0.5)	0.3 (0.1-0.1.0)	0.3 (0.1-1.0)
Regurgitation							
No	88 (70%)	46 (81%)	36 (84%)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
Yes	37 (29%)	9 (16%)	7 (16%)	0.4 (0.1-0.98)	0.4 (0.2-1.0)	0.5 (0.2-1.1)	0.4 (0.2-1.1)
Duration of regurgitation							
Never	90 (71%)	46 (81%)	36 (84%)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
<12 yr	13 (10%)	4 (7%)	5 (12%)	0.6 (0.2-1.8)	0.4 (0.1-1.6)	1.0 (0.3-2.9)	1.0 (0.3-3.1)
>12 yr	20 (16%)	5 (9%)	2 (5%)	0.5 (0.2-1.3)	0.6 (0.2-1.7)	0.3 (0.1–1.1)	0.2 (0.1-1.1)
Frequency of regurgitation							
0x	90 (71%)	47 (83%)	36 (84%)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
1x	9 (7%)	2 (4%)	0 (0%)	0.4 (0.1-1.9)	0.8 (0.2-4.2)	2.0 (0.5-7.9)	2.5 (0.6-10)
2-3x	5 (4%)	4 (7%)	4 (9%)	1.4 (0.4-5.6)	1.9 (0.5-7.7)		
>3x	20 (16%)	2 (4%)	3 (7%)	0.2 (0.04-0.8)	0.1 (0.03-0.7)	0.4 (0.1-0.1.3)	0.3 (0.1-1.2)
Family history of heartburn							
No	47 (37%)	33 (58%)	21 (49%)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
Yes	49 (39%)	7 (12%)	10 (23%)	0.2 (0.1-0.5)	0.1 (0.03-0.4)	0.5 (0.2-1.1)	0.5 (0.2-1.2)
Family history of regurgitation							
No	56 (44%)	35 (61%)	22 (51%)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
Yes	32 (25%)	8 (14%)	6 (14%)	0.4 (0.2-1.0)	0.3 (0.1-0.9)	0.5 (0.2-1.3)	0.5 (0.2-1.5)

EAC: esophageal adenocarcinoma, SCC: squamous cell carcinoma, GCA: gastric cardia adenocarcinoma, OR: odds ratio, CI: confidence interval.

* Total percentages may not be 100 because of missing values.

** In the multivariable analysis, odds ratios were adjusted for age, sex, educational level, smoking and alcohol consumption.

Table 4. Medication use in relation to the risk of esophageal and gastric cardia cancer development.

Variable	EAC cases (percent)*	SCC cases (percent)	GCA cases (percent)	Univariable OR SCC (95%CI)	Multivariable OR SCC (95%CI)**	Univariable OR GCA (95%CI)	Multivariable OR GCA (95%CI)
PPI use							
Non	79 (63%)	45 (79%)	30 (70%)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
Former	27 (21%)	5 (9%)	7 (16%)	0.6 (0.2-2.1)	0.2 (0.1-1.1)	0.5 (0.1-2.3)	0.4 (0.1-2.0)
Current	11 (9%)	4 (7%)	2 (5%)	0.3 (0.1-0.9)	0.2 (0.1-0.6)	0.7 (0.3-1.7)	0.6 (0.2-1.7)
Duration							
Never	86 (68%)	45 (79%)	31 (72%)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
<6 months	13 (10%)	4 (7%)w	3 (7%)	0.2 (0.2-1.9)	0.3 (0.1-1.4)	0.7 (0.3-2.1)	0.8 (0.2-3.1)
>6 months	19 (15%)	5 (9%)	5 (12%)	0.5 (0.2-1.4)	0.3 (0.1-1.1)	0.6 (0.2-2.3)	0.5 (0.2-1.6)
H2RA use							
Non	98 (78%)	53 (93%)	34 (79%)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
Former	2 (2%)	0 (0%)	3 (7%)	0.1 (0.01-0.7)	0.07 (0.01-0.6)	4.3 (0.7-27)	6.5 (0.96-44)
Current	21 (17%)	1 (2%)	4 (9%)			0.5 (0.2-1.7)	0.5 (0.2-1.6)
Duration							
Never	102 (81%)	53 (93%)	34 (79%)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
<6 months	8 (6%)	1 (2%)	1 (2%)	0.1 (0.03-2.0)	0.2 (0.02-1.7)	0.1 (0.05-3.1)	0.3 (0.04-2.8)
>6 months	11 (9%)	0 (0%)	6 (14%)			1.6 (0.6-4.8)	1.6 (0.5-4.7)
NSAID use							
Non	72 (57%)	39 (68%)	29 (67%)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
Former	18 (14%)	6 (11%)	3 (7%)	0.5 (0.2-1.2)	0.3 (0.1-0.9)	0.7 (0.3-1.8)	0.8 (0.3-1.9)
Current	30 (24%)	8 (14%)	9 (21%)	0.6 (0.2-1.7)	0.5 (0.2-1.7)	0.4 (0.1-1.5)	0.4 (0.1-1.7)
Duration							
Never	96 (76%)	46 (81%)	37 (86%)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
<6 months	18 (14%)	4 (7%)	2 (5%)	0.3 (0.1-1.4)	0.2 (0.05-0.8)	0.4 (0.1-1.3)	0.2 (0.1-1.2)
>6 months	6 (5%)	3 (5%)	2 (5%)	1.0 (0.3-4.4)	1.0 (0.2-5.0)	0.9 (0.2-4.5)	0.7 (0.1-4.0)
Aspirin use							
Non	61 (48%)	32 (56%)	31 (72%)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
Former	32 (25%)	15 (26%)	7 (16%)	0.3 (0.1-0.8)	0.6 (0.2-1.7)	0.2 (0.06-0.8)	0.2 (0.05-0.7)
Current	27 (21%)	6 (11%)	3 (7%)	0.4 (0.2-1.1)	0.8 (0.3-1.8)	0.5 (0.2-1.1)	0.4 (0.1-0.9)
Duration							
Never	92 (73%)	46 (81%)	36 (84%)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
<6 months	7 (6%)	0 (0%)	2 (5%)	0.6 (0.2-1.6)	0.5 (0.2-1.6)	0.7 (0.1-3.7)	0.8 (0.2-4.3)
>6 months	21 (17%)	6 (11%)	3 (7%)			0.4 (0.1-1.3)	0.3 (0.08-1.1)
Antacids							
Non	57 (45%)	36 (63%)	27 (63%)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
Former	23 (18%)	8 (14%)	6 (14%)	0.1 (0.1-0.8)	0.3 (0.1-0.9)	0.2 (0.1-0.8)	0.3 (0.1-0.9)
Current	38 (30%)	8 (14%)	5 (12%)	0.6 (0.2-1.5)	0.6 (0.2-1.7)	0.6 (0.2-1.4)	0.5 (0.2-1.3)
Duration							
Never	90 (71%)	46 (81%)	34 (79%)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
<6 months	6 (5%)	3 (5%)	2 (5%)	1.0 (0.2-4.1)	0.9 (0.2-4.2)	0.9 (0.2-4.5)	1.1 (0.2-6.2)
>6 months	22 (18%)	3 (5%)	2 (5%)	0.3 (0.08-0.9)	0.3 (0.08-1.2)	0.3 (0.05-1.1)	0.3 (0.06-1.4)

EAC: esophageal adenocarcinoma, SCC: squamous cell carcinoma, GCA: gastric cardia adenocarcinoma, OR: odds ratio, CI: confidence interval, PPI: proton pump inhibitor, H2RA: histamine-2 receptor antagonist, NSAID: non-steroidal anti-inflammatory drug.

* Total percentages may not be 100 because of missing values.

** In the multivariable analysis, odds ratios were adjusted for age, sex, educational level, smoking and alcohol consumption.

less often were former as well as current users of aspirin as compared to EAC patients (OR 0.2 (95%CI: 0.05-0.7) and OR 0.4 (95%CI: 0.1-0.9) respectively).

DISCUSSION

Previous studies on the incidence of EAC and GCA investigated risk factors that could have additional value in preventive strategies for these two different types of cancer. So far, the close proximity of EAC to GCA around the gastroesophageal junction, the identical staging and treatment protocols for both malignancies and the fact that tumor location is not an independent risk factor with regard to prognosis,[5, 6, 13] have made that these cancers are regarded as one-disease entity. In contrast, because of its distinct histopathological differences, SCC on the one hand and EAC and GCA on the other hand are regarded as separate disorders, but again with largely common staging and treatment strategies. Previous epidemiological studies, however, suggested that EAC and GCA were indeed different entities and therefore worth to be evaluated separately.[2, 3, 14]

In the present study, the majority of the evaluated risk factors did not show differences between EAC and GCA patients, as has been reported previously.[15] Patients were similar with regard to male predominance and age at the time of diagnosis (middle-aged). In addition, no differences were found in alcohol intake and smoking, use of fruits and vegetables, body posture and occupational activities (Tables 1 and 2). Nonetheless, patients with EAC more often experienced symptoms of heartburn and had more often longstanding symptoms of heartburn compared with GCA patients (Table 3). Reflux symptoms have previously been shown to be weakly associated with the risk of GCA, but more strongly with the risk of developing EAC.[16] Chak *et al.*[17] reported that nearly two-thirds of patients with GCA did not recall chronic GERD symptoms. This is in agreement with our findings. The differential association with the symptoms of reflux may imply that EAC and GCA are different disease entities.

In accordance with findings from others,[18, 19] EAC patients were significantly more often obese throughout their life than SCC patients, whereas EAC and GCA patients were almost similar, however, with a slightly higher percentage of EAC patients having a BMI>25 than GCA patients (Table 2). Obesity is thought to induce a decrease in lower esophageal sphincter functioning combined with increased abdominal pressure which has been shown to induce gastroesophageal reflux.[20] It has convincingly been shown that GERD is closely associated with the development of EAC,[16] most likely through the induction of Barrett's esophagus.[21]

As expected, patients with EAC more frequently used both PPIs and H2RAs than SCC patients, most likely because of differences in GERD symptoms (Table 4). Although the use of acid-suppressive drugs was not different between EAC and GCA patients, GCA patients less often were antacid users.

Remarkably, in contrast to NSAID use, EAC patients more often used aspirin than GCA patients, both at the time of diagnosis and during the 10 years prior to diagnosis (Table 4). Aspirin could either have been used as painkiller (high-dose) by some patients or for the prevention of cardiovascular events (low-dose) by others. Unfortunately, we have no information with regard to dosage patterns among aspirin users. If aspirin was used as painkiller by EAC patients, then it seems likely that they also should have used more NSAIDs than GCA patients, which was not the case. It can be speculated that patients with a tumor located in the esophagus (EAC or SCC) experience more pain than GCA patients, because of a higher risk of tumor tissue growing into surrounding tissues as compared to GCA patients. If this would have been the case, then it is difficult to understand why EAC patients more frequently were former NSAID users than SCC patients (Table 4).

A study on the effect of aspirin on the development of gastric cancer has shown a reduced risk on both distal gastric cancer and cardia cancer.[22] In addition, selective NSAIDs have been suggested to protect against the development of Barrett's esophagus and subsequent EAC.[23, 24] This protective effect is thought to exert its action through inhibition of cyclo-oxygenase-2 (COX-2). The COX-2 selective agents have been shown to decrease proliferation and increase apoptosis *in vitro* in Barrett's epithelial cells and in human Barrett-associated EAC cell lines.[25, 26] Moreover, selective inhibition of COX-2 has been shown to decrease proliferation in metaplastic Barrett's mucosa *in vivo*.[27] For that reason, we would have expected that, at least in EAC and GCA patients, use of aspirin and NSAID would have been similar.

EAC and SCC have been shown to be different disease entities with distinct risk factors. Until now, the majority of reported studies have compared risk factors for these two carcinomas with a control group.[28, 29] However, both patient groups have an intrinsic carcinogenic potential and genetic factors might well contribute to the development of these carcinomas. Our study showed that both EAC and SCC patients were in some ways comparable with regard to baseline factors with the exception of a male gender difference in EAC compared to SCC patients. In addition, SCC patients more often used alcohol and, if so, for a longer period than EAC patients, whereas no differences were detected in smoking habits. These findings are in agreement with previous studies in which smoking was found to be a risk factor for both types of cancer, whereas alcohol intake was primarily related to the development of SCC.[5]

Some limitations of our study warrant consideration. First, the reported results may have been subjected to recall bias. However, this faulty recall is unlikely to be related to the type of carcinoma and therefore should be random. Second, although our current findings are in agreement with previous reports in the literature, we might not have been able to detect important differences because of the relatively small sample size of GCA and SCC patients. Third, misclassification of EAC and GCA tumors could have influenced our results. However, we tried to minimize the risk of misclassification by centrally reviewing all endoscopy, pathology and surgery reports. Fourth, our questionnaire was not validated. However, during the development of the questionnaire, we did a great effort to minimize the possibility of

ambiguities and misinterpretations of the different questionnaire items in order to ensure valid responses. Moreover, the same questionnaire has recently been used in another study investigating risk factors for EAC development by comparing patients with GERD, BE and EAC.[12] Finally, we might have increased the risk of false-positive results (type 1 error) by multiple statistical testing.

Strengths of our study include the size of the study population, the single center design, and the collection of data on several potential environmental risk factors that could increase or decrease the risk of cancer development at the GEJ and distal esophagus. In addition, associated risk factors were adjusted for potential confounding factors. Moreover, patients were uniformly classified and strict and consistent criteria were used to make a final diagnosis of carcinoma. Finally, the comparison between patients with EAC and SCC clearly demonstrated that the questionnaire is able to accurately detect epidemiological differences. This justifies its application for the comparison between patients with EAC and GCA.

In conclusion, although several risk factors, such as alcohol, smoking, obesity, occupational activities, and fruit and vegetable intake are similar in EAC and GCA patients, EAC patients more often have a present or past history of GERD. This suggests a more important role for gastroesophageal reflux in EAC compared with GCA.

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Chapter 4

Capsule endoscopy for the detection of esophageal mucosal disorders: a comparison of two different ingestion protocols

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ABSTRACT

Background

To assess the accuracy of a new ingestion protocol for capsule endoscopy (CE) in evaluating patients with gastroesophageal reflux disease (GERD).

Methods

Esophagogastroduodenoscopy (EGD) was performed 1 week prior to CE. The first 28 subjects swallowed the capsule following the original ingestion protocol (OIP) and the subsequent 30 subjects following a simplified ingestion protocol (SIP). CE videos were reviewed by two independent investigators who were blinded to the EGD findings.

Results

Of 48 patients included, 24 were diagnosed with reflux esophagitis (67% male, mean age 49.5 ± 13 yrs), and 24 with Barrett's esophagus (BE) (88% male, 55.6 ± 10 yrs) by EGD. In addition, 10 asymptomatic healthy controls (50% male, 45.8 ± 7.1 yrs) were included. Esophageal transit time was faster in patients using the SIP compared to the OIP (126 ± 26 seconds vs. 214 ± 33, p=0.04). Complete evaluation of the Z-line was possible in 19/28 (68%) of the OIPs compared to 28/30 (93%) of the SIPs (p=0.04). Sensitivity for detecting any esophageal abnormality was higher in the SIP group than in the OIP group (97% vs. 89%, p=0.11). Overall, CE detected esophagitis in 22/24 patients (sensitivity, 92%; specificity, 88%) and BE in 23/24 patients (sensitivity, 96%; specificity, 91%). Furthermore, 41/44 (93%) preferred CE over EGD and experienced less discomfort and pain during CE.

Conclusion

CE is an accurate method for detecting mucosal esophageal abnormalities. The new ingestion protocol improves the visualization of the Z-line, which is likely to increase the diagnostic yield of CE.

INTRODUCTION

Gastroesophageal reflux disease (GERD) is a common entity that has been estimated to affect around 10 to 20% of the Western population.[1] The disease is associated with significant impairment of quality of life and frequently requires endoscopy for the evaluation of symptoms or to screen for complications such as erosive esophagitis, strictures, and Barrett's esophagus (BE).[2]

Screening for BE in patients with symptomatic GERD is performed in an attempt to identify patients with an increased risk of developing esophageal adenocarcinoma. Esophagogastroduodenoscopy (EGD) with biopsies is the preferred technique for evaluating the esophageal mucosa and is the standard for BE evaluation. However, screening for BE and other esophageal pathologies in the general population is considered not to be realistic because of the costs associated with EGD.[3] In addition, the procedure may be burdensome to patients[4] and carries a small potential risk of complications.[5, 6] Consequently, some reflux patients are known to be reluctant to undergo BE screening even when it is indicated. The use of an accurate, safe and alternative visualization method, especially in the setting of screening, is therefore needed.

As an alternative to sedated EGD, ultra-thin video endoscopes have been developed, which can easily be passed transorally or transnasally without sedation. These instruments can provide an efficient, cost-effective alternative to standard endoscopy, and can be offered as an option to conventional sedated examination, particularly in the setting of screening.[7-9] However, use of unsedated small-caliber endoscopy is not yet accepted on a wide scale.

Another newly introduced diagnostic modality is capsule endoscopy (CE), which offers a method of visualizing the esophagus without the need for sedation, and without the discomfort and risks of EGD. Initial pilot studies demonstrated a high diagnostic yield of CE in the detection of BE.[10, 11] However, other investigators reported less optimal test characteristics, probably due to limitations of the video capsule itself and deviations from the recommended ingestion.[12, 13] Recently, a new simplified ingestion protocol (SIP) was developed and shown to be superior to the original ingestion protocol (OIP) with regard to visualization of the Z-line in healthy controls.[14] So far, clinical studies in appropriate disease-targeted populations using the SIP have not yet been performed.

In the present study, we aimed to compare the accuracy of CE with EGD in evaluating patients with symptomatic GERD using a new simplified ingestion protocol. Secondary aims were to investigate the burden of endoscopy and patient satisfaction with CE, and to estimate costs of both EGD and CE.

METHODS

Patients

From March 2006 to March 2007, patients at the Erasmus MC - University Medical Center Rotterdam and IJsselland Hospital, Capelle aan den IJssel in the Netherlands, referred for EGD for evaluation of symptomatic GERD or for surveillance of BE were eligible to participate in this study. Consecutive patients with reflux esophagitis (RE) or BE at the time of EGD were invited to undergo CE within one week after EGD. In addition, healthy controls without a history of GERD symptoms were enrolled. Absence of GERD symptoms in these patients was confirmed by scoring negatively on the GERD-HRQL questionnaire.[15, 16] Exclusion criteria were dysphagia, known Zenker diverticulum, or known/suspected intestinal obstruction. In addition, pregnant women, patients with a cardiac pacemaker or patients who were scheduled for MRI examination within 7 days after capsule ingestion were excluded from the study. All participants signed an informed consent form and the study was approved by the Institutional Review Board of the Erasmus MC Rotterdam.

Protocol

Patients were not allowed to use anti-secretory medication in the period between the EGD and CE; however, if needed, the use of antacids was permitted. EGD was performed with a small-diameter forward-viewing endoscope (Olympus Evis Exera GIF-160). Conscious sedation with midazolam was not routinely given. During EGD, the endoscopist recorded images of the Z-line, gastroesophageal junction, and diaphragmatic impression. Patients with RE were classified using the Los Angeles (LA) classification.[17, 18] Long segment BE (LSBE) was defined as BE length > 3 cm and short segment BE (SSBE) was defined as BE length ≤ 3 cm.[19] All procedures were performed by a total of three senior endoscopists experienced in conventional EGD.

CE was performed using the PillCam ESO capsule (Given Imaging, Yoqneam, Israel), which is an ingestible, disposable capsule equipped with two miniature cameras. It has a size of 11 x 26 mm and collects video images at both ends of the capsule at a rate of 14 frames (fr.)/sec. (7 fr. /sec. x 2 cameras) during its passage through the esophagus. The images are transmitted via radiofrequency signalling to a portable data recorder unit, which is carried by the study participant. The recorded data are available for processing and interpretation by the physician.

Two different ingestion protocols were compared in this study. Before the examination a fast of at least 6 hours was required. The first 28 patients swallowed the capsule following the original ingestion protocol (OIP), in which the patient ingests the capsule in a supine position (with the head flexed forward), followed by gradually elevating the trunk in a series of inclinations over a total of 6 minutes.[11] The remaining 30 patients swallowed the capsule following a simplified ingestion protocol (SIP). In this new protocol, the capsule is ingested with the pa-

tient in a right lateral supine position while taking sips of water (15 mill each) approximately every 30 seconds.[14] Patients were asked not to talk or make sudden movements during both protocols. One week following CE, patients were contacted by telephone for confirmation of capsule excretion and documentation of adverse events.

Capsule endoscopy analysis

One experienced gastroenterologist and one gastroenterology fellow, independently from each other and blinded to the patient's history and diagnostic findings at EGD, reviewed the CE videos. In the event of disagreement, a third independent investigator was asked for a review. Since none of the three investigators had reviewed any esophageal CE video prior to start of the study, all three investigators participated in a training session in which 25 representative clips of BE and RE were discussed. The investigators documented the esophageal transit time, visualization of the circumferential Z-line, presence or absence of BE, estimated length of suspected BE (*i.e.*, SSBE or LSBE), presence or absence of RE, LA grade of RE or any other abnormal findings. Esophageal transit time was calculated by subtracting the time of the first esophageal image from the time of the first gastric image.

Questionnaire

Patients' experience of the burden of endoscopy and CE was assessed longitudinally by use of a validated questionnaire at four time-points: 1 week before the EGD (baseline), on the day of, but before the EGD, on the day of, but before the CE and 1 week after the CE. The 4 different components of the questionnaire, included: 1) Demographic data at baseline, including patients' judgement of their own health, using the EuroQol–5D[20, 21], an instrument for measuring health-related quality of life; 2) Pain and discomfort experienced during the procedure, measured both 1 week after EGD and 1 week after CE. Question items related to the different stages of the procedures (*e.g.*, introduction of the endoscope, ingestion of the capsule, the endoscopic exploration itself, removal of the endoscope), with three response options (not, quite, and very painful/unpleasant). Discomfort was additionally assessed with regard to fasting and, if applicable, for waking up after sedation. Moreover, we asked patients to rate the overall burden of the procedures (very, somewhat, not burdensome), and to identify the most and the least burdensome parts of the procedures; 3) Physical symptoms caused by the EGD and CE were compared with regard to 10 symptoms at baseline and 1 week after both procedures, *i.e.,* throat ache, heartburn, regurgitation, flatulence or feeling bloated, vomiting, haematemesis, dysphagia for solid foods, dysphagia for fluids, diarrhoea, and constipation. Questions were composed with four answer categories (not at all, and lasting for 1 day, 2-3 days, or 4 or more days); 4) The Hospital Anxiety and Depression (HAD) scale, which was used to assess anxiety (7 items) and depression (7 items) at each time point.[22, 23] Scores per subscale range from 0 to 21, scores of 11 or higher indicating clinical, and 8-10 borderline anxiety or depression.

Cost calculations

Calculation of the full cost price of EGD and CE consisted of a detailed measurement of investments in manpower, equipment, materials, housing and overhead. For CE, both the duration of the examination itself (20 min) and the duration of the reviewing process of the CE video (9 min) were considered in the calculation of personnel costs. The salary schedules of hospitals and other health-care suppliers were used to estimate costs per hour for each caregiver. Taxes, social securities and vacations were included, as well as costs related to the time that could not be assigned to other patients. The costs of equipment included those of depreciation, interest and maintenance. For the most important cost items, unit prices were determined by following the micro-costing method ,[24] which is based on a detailed inventory and measurement of all resources used.

Statistical analysis

CE test characteristics were estimated by calculating sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV). These were assessed for the detection of the esophageal findings RE and BE. The gold standard in this study was defined as the diagnostic findings observed at the time of the EGD. Chi-square tests and *t*-tests were used to compare the categorical and continuous characteristics, respectively. Symptoms before and after both EGD and CE were compared using the McNemar test. Statistical significance was considered to be present if a two-sided p was <0.05. Statistical analyses were conducted using SPSS software (10.1, Chicago, Ill, USA).

Results

Of forty-eight consecutive patients who underwent both EGD and CE, 24 patients (50%) were diagnosed with RE (67% male, mean age 49.5 ± 13 years) and 24 (50%) with BE (88% male, 55.6 ± 10 years) by EGD. In addition, both EGD and CE were performed in 10 asymptomatic healthy controls (50% male, mean age 45.8 ± 7.1 years). The patients had no difficulty swallowing the capsule while in the supine position, and no adverse advents were noted during or after capsule ingestion.

Technical characteristics

The mean total recording time for CE videos was 1218 sec (range 380 - 1305) (Table 1). The mean esophageal transit time was longer in patients ingesting the capsule using the OIP than in those following the SIP (214 ± 33 vs. 126 ± 26 sec, p=0.04). A complete study of the Z-line (all 4 quadrants) was possible in 19/28 (68%) of the OIP videos and in 28/30 (93%) of the SIP videos (p=0.04). Incomplete visualization of the Z-line was mainly caused by the presence of bubbles or a rapid passage through the distal part of the esophagus. The SIP did not result in less interference due to bubbles at the Z-line (p=0.41).

Table 1. Technical characteristics of capsule endoscopy (CE) comparing the original ingestion protocol (OIP) with the simplified ingestion protocol (SIP).

Measure	OIP (*n*=28)	SIP (*n*=30)	*p*-value*
Mean esophageal transit time (seconds ± SD)	214 (33)	126 (26)	0.04
Z-line visualization (%)			
All quadrants	19 (68%)	28 (93%)	0.04
3 quadrants	7 (25%)	2 (7%)	
≤ 2 quadrants	2 (7%)	0 (0%)	
Interference of bubbles Z-line (%)			
Major interference	1 (4%)	0 (0%)	0.41
Moderate interference	3 (11%)	1 (3%)	
Minor interference	4 (14%)	3 (10%)	
No interference	20 (71%)	26 (87%)	

*p-values from t-test /chi-square test

Diagnostic characteristics

Based on EGD findings, 24 patients were diagnosed with RE (LA grade A: 20, and grade B: 4) and 24 patients with BE (SSBE: 6, LSBE: 18). When comparing the OIP with the SIP with regard to diagnostic accuracy of CE, sensitivity for detecting any esophageal abnormality (RE and BE) in the 30 subjects who followed the SIP (EGD: RE 17, BE 13) was non-significantly higher compared to the OIP group (EGD: RE 7, BE 11, healthy controls 10) (97% vs. 89%) (p=0.11). In 7 RE patients who followed the OIP, RE was found in 6 (sensitivity 86%). In the remaining 17 RE patients who followed the SIP, RE was detected in 16 (sensitivity 94%). In 11 BE patients who followed the OIP, BE was found in 10 (sensitivity 91%). In the remaining 13 BE patients who followed the SIP, BE was detected in all (sensitivity 100%). Of all 10 healthy controls included in the OIP group, one subject was diagnosed as having RE grade A.

In the overall analysis, CE had a sensitivity of 92%, a specificity of 88%, a NPV of 94% and a PPV of 85% for the detection of RE (Table 2). RE could not be identified by CE in 2 patients. The grade of RE was correctly assessed in 14 of 22 patients (64%) at CE, with none of the patients being scored with RE grade C or D.

With regard to overall accuracy of CE for detecting BE, CE had a sensitivity of 96%, a specificity of 91%, a NPV of 97% and a PPV of 88% (Table 2). BE could not be identified by CE in 1 patient. The length of the BE segment was correctly scored by CE in 19 of 23 patients (83%).

The sensitivity for the detection of RE was higher when CE videos were reviewed by an experienced gastroenterologist compared to a trainee (92% vs. 83%, p=0.02). However, the sensitivity for detecting BE was not significantly different (96% vs. 88%, p=0.13). Overall, disagreement between the two investigators occurred in 5 CE videos, requiring review by a third independent investigator.

Table 2. Diagnostic test characteristics of capsule endoscopy (CE) for the diagnosis of reflux esophagitis (RE) and Barrett's esophagus (BE).

RE	EGD - positive	EGD – negative	CE (%)
CE – positive	22	4	
CE - negative	2	30	
Sensitivity RE			92
Specificity RE			88
Positive predictive value RE			85
Negative predictive value RE			94

BE	EGD - positive	EGD – negative	CE (%)
CE – positive	23	3	
CE - negative	1	31	
Sensitivity BE			96
Specificity BE			91
Positive predictive value BE			88
Negative predictive value BE			97

EGD: esophagogastroduodenoscopy.

Patients' experiences

The questionnaires were completed by 44/48 (92%) patients. Eleven patients (25%) underwent conscious sedation with midazolam during EGD. Pain during EGD was reported by 19 patients (43%), whereas only 1 patient experienced pain during capsule ingestion (2%) (Figure 1). The majority of the patients reported discomfort during EGD (73% at the introduction of the endoscope and 75% during the endoscopy itself), whereas 4 patients (9%) experienced discomfort during CE. Overall, EGD and CE were reported to be burdensome procedures by 39 (89%) and 2 (4%) patients, respectively (Figure 2). Only a few patients reported symptoms before and after the EGD (Table 3). Symptoms that were reported by more than 25% of patients at baseline were: heartburn (45%), regurgitation (59%), and flatulence (50%). EGD resulted in a significant increase in throat ache (64%), regurgitation (82%) and flatulence (75%) (all $p<0.05$). On the other hand, CE did not cause an increase in symptoms. There was even a significant decline in reported symptoms after CE, which is likely to be explained by patients starting anti-secretory drugs after CE. In general, depression scores did not differ significantly before and after both procedures, whereas anxiety scores were significantly lower after CE as compared to those ascertained at baseline (5.4 vs. 4.0, $p=0.01$). On the day of endoscopy, 5 patients (12%) had scores indicative of clinical anxiety and 6 (14%) of borderline anxiety. Scores indicative of clinical depression were seen in 2 patients (5%) and scores indicative of borderline depression in 6 (14%). Of the 44 patients questioned, 41 preferred CE above EGD, none preferred EGD and three had no preference.

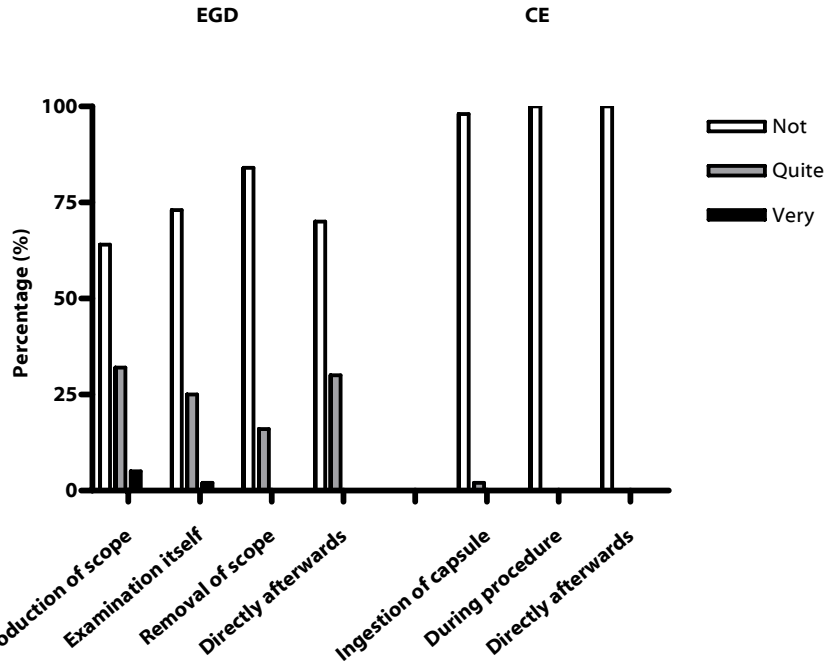

Figure 1. Pain reported by patients at different stages of esophagogastroduodenoscopy (EGD) and capsule endoscopy (CE).

Costs

The initial cost price of treatment, based on real resource use, was much higher for CE (€647) than for EGD (€126). The main cause for this difference was the high purchase costs of the PillCam Eso capsule (€590) (Table 4).

DISCUSSION

Several gastroenterological societies have suggested to screen patients with chronic GERD for the presence of BE by using EGD and performing surveillance endoscopy in those with BE in an attempt to detect early stage esophageal cancer. Newly introduced screening modalities such as CE, which do not require sedation and can be performed in an office setting in less than 30 minutes, could potentially enhance the acceptability of BE screening and improve compliance. Studies reporting on the application of CE for esophageal disorders, however, have shown conflicting results, with sensitivities and specificities for detecting esophageal pathology varying from 38% to 100% and from 78% to 95%, respectively.[10-13] Poor visualiza-

Table 3. Symptoms reported by patients, both prior as well as after esophago-gastroduodenoscopy (EGD) and capsule endoscopy (CE).

Symptom	Baseline *n* (%)	After EGD *n* (%)	*p*-value	After CE *n* (%)	*p*-value
Throat ache	8/44 (18%)	28/44 (64%)	0.001	5/44 (11%)	0.250
Heartburn	20/44 (45%)	15/44 (34%)	0.302	5/44 (11%)	0.001
Regurgitation	26/44 (59%)	36/44 (82%)	0.013	10/44 (23%)	0.001
Flatulence	22/44 (50%)	33/44 (75%)	0.003	11/44 (25%)	0.021
Vomiting	6/44 (14%)	4/44 (9%)	0.727	2/44 (5%)	0.219
Dysphagia with solid foods	11/44 (25%)	8/44 (18%)	0.375	5/44 (11%)	0.109
Dysphagia with liquid foods	2/44 (5%)	4/44 (9%)	0.500	3/44 (7%)	1.000
Diarrhoea	10/44 (23%)	4/44 (9%)	0.109	8/44 (18%)	0.774
Constipation	5/44 (11%)	7/44 (16%)	0.500	6/44 (14%)	1.000

**p*-values from McNemar test.

Table 4. Full cost price (€, 2007) of esophagogastroduodenoscopy (EGD) and esophageal capsule endoscopy (CE).

Cost Category	EGD	CE
Personnel	59	26
Equipment	33	16
Materials	2	590
Housing/ Overhead	32	15
Total costs	126	647

tion of the Z-line and inconvenience of capsule ingestion in the supine position were suggested as explanations for these variations. Advances in CE technology, such as increasing frame rates, a higher image resolution, but also new ingestion protocols have been proposed to improve its diagnostic accuracy.

In the current study, CE identified the presence of RE and BE with a high degree of accuracy (Table 2). In addition, CE had a high NPV for excluding the presence of these disorders. The new ingestion protocol significantly improved visualization of the Z-line and resulted in a better detection of esophageal abnormalities as compared to the OIP, although the latter was not statistically significant. Furthermore, nearly all patients preferred CE over EGD, and experienced less discomfort and pain during CE as compared to EGD (Figures 1 and 2).

The high accuracy rates for the detection of both RE and BE in our study are in agreement with previous reports.[10, 11] A pilot study in 17 patients with suspected esophageal disorders reported a sensitivity and specificity of 100% and 80%, respectively, and a positive and negative predictive value of 92% and 100%, respectively, for CE detecting RE and BE.[10] In a larger, multicenter trial, Eliakim *et al.*[11] compared the accuracy of CE with EGD in 106 patients with

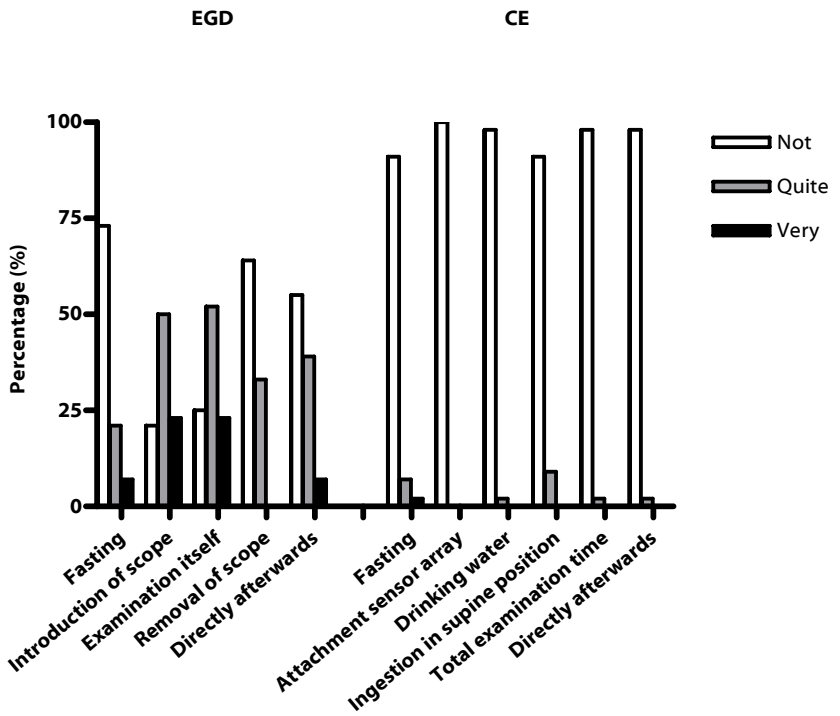

Figure 2. Discomfort reported by patients at different stages of esophagogastroduodenoscopy (EGD) and capsule endoscopy (CE).

symptomatic GERD. CE identified RE or BE in 61 of the 66 patients (sensitivity 92%, specificity 95%).

In contrast to these findings, lower accuracy rates for the detection of RE and BE have been reported in two recent studies.[12, 13] In one of them, comparing CE with EGD for the detection of BE and RE in 90 patients with chronic GERD, a sensitivity of 67% and a specificity of 84% in identifying BE were reported.[12] Moreover, the sensitivity of identifying RE was only 38%, with a specificity of 86%. Similarly, in another prospective study evaluating CE in 94 symptomatic GERD patients, Sharma *et al.*[13] reported that CE had a sensitivity and specificity of only 50% and 90% for RE and 79% and 78% for BE, respectively.[13]

A possible explanation for these discrepancies in accuracy rates could be poor visualization of the Z-line resulting from the OIP that was used. In the current study, a new SIP was tested in patients with either RE or BE. Although the new SIP resulted in a significant shorter esophageal transit time as compared to the OIP, the SIP improved visualization of the Z-line, with an increase in the Z-line circumferential view (Table 1). Re-examination of those OIP videos that were characterized by a long esophageal transit time (>1 minute) revealed that

in the majority of these an excess presence of saliva/ bubbles disturbed a clear view of the esophageal mucosa.

Our findings are in agreement with a study from Gralnek *et al.*[14], who also showed that the SIP was superior to the OIP in visualization of the Z-line in healthy volunteers. In addition, the SIP resulted in a higher sensitivity for the detection of RE and BE, although these differences were not statistically significant.

Not surprisingly, CE was shown to be more convenient to patients than EGD, and was associated with less discomfort and less pain. In addition, CE did not cause symptoms during the procedure, whereas EGD did (Table 3). A relatively high number of patients experienced symptoms during EGD and directly afterwards. This can most likely be explained by the fact that no conscious sedation was administered in the majority of patients (46/58 (79%)), which is common practice in patients undergoing upper endoscopy on an outpatient basis in the Netherlands. All patients ingested the capsule without difficulty and the vast majority preferred CE to EGD. This has also been demonstrated previously.[11] Furthermore, a recent study reported that EGD is burdensome and causes moderate distress in patients undergoing repeat endoscopies for surveillance of BE.[4] Therefore, if screening for BE is considered, the short-term burden and distress of EGD for patients should be taken into account.

CE was more expensive than EGD, which was mainly due to the high purchase costs of the capsule (Table 4). One solution to lower CE costs is the application of string CE, which allows multiple uses after disinfection. String CE has been shown to be an accurate and acceptable procedure.[25] Based on our full cost prices, the capsule would need to be reused at least 5 times in order to equal EGD costs. However, we acknowledge that cost-effectiveness of CE versus EGD requires a formal economic analysis. Recently, two studies analyzed the cost-effectiveness of CE for BE screening. Both studies compared CE with EGD with biopsies, and concluded that the latter was superior and was also less costly to CE when the detection of BE was the end-point.[26, 27]

Some limitations of the current study need to be considered. First, the test characteristics of CE might have been affected by the relatively high prevalence of esophageal findings at EGD as compared to other studies.[11-13] If CE had been performed prior to EGD, less patients with esophageal findings at EGD would have been included. However, this study was not primarily designed to asses CE performance in BE screening. It was designed to compare diagnostic accuracy of two different swallowing protocols, requiring a high prevalence of esophageal findings. Second, since all BE patients participated in a surveillance program and were histologically confirmed to have this diagnosis, we were unable to investigate diagnostic accuracy rates for suspected BE and histologically confirmed BE separately. Third, performing CE 1 week after EGD could have obscured and altered findings during CE, as EGD with biopsies was the initial investigation in all patients. However, the 1-week interval should have been sufficient for successful healing of esophageal biopsy lesions. In addition, symptomatic patients were only allowed to take antacids in the period between EGD and CE, whereas

the more potent acid-inhibitory drugs were prescribed after CE. Finally, our sample size was rather small, which might have limited the ability to detect significant differences between the SIP and OIP.

Strengths of our study include the prospective blinded design, the evaluation of a new ingestion protocol, and the formal assessment of patient satisfaction and burden of endoscopy using validated questionnaires. In addition, differences in accuracy rates between an experienced gastroenterologist and a trainee in the interpretation of CE findings were investigated. Moreover, calculation of EGD and CE costs consisted of detailed measurements of real medical costs instead of obtaining cost estimates from a third-party payer perspective.

In conclusion, CE seems an accurate and patient friendly method for screening patients with reflux symptoms for esophageal abnormalities including BE. The new ingestion protocol improves visualization of the Z-line and is therefore likely to increase the diagnostic yield of CE in evaluating patients with chronic GERD. Future large, prospective, blinded trials utilizing CE with this new ingestion protocol are required to study whether the diagnostic accuracy of CE can be further improved.

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Chapter 5

Risk of esophageal adenocarcinoma and mortality in patients with Barrett's esophagus: a systematic review and meta-analysis

Submitted for publication

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ABSTRACT

Background and aim

Patients with Barrett's esophagus (BE) are at increased risk for esophageal adenocarcinoma (EAC). The magnitude of the annual cancer risk in BE remains uncertain, as published estimates are highly heterogeneous. Moreover, mortality due to EAC in BE patients under surveillance is supposed to be low. As EAC risk and mortality in BE are important determinants of the cost-effectiveness of BE surveillance, clarification of these factors is essential. We performed a systematic review and meta-analysis to determine the incidence of EAC and mortality due to EAC in BE patients.

Methods

Pubmed, EMBASE and Web of Science databases were searched for relevant cohort studies in English language published between 1966 and September 2008 that reported on EAC risk and mortality due to EAC in BE. Studies had to include patients with histologically proven BE, documented follow-up, and histologically proven EAC on surveillance. We used a random effects model for the meta-analysis, with assessment of heterogeneity by the I^2 statistic and of publication bias by Begg's and Egger's tests.

Results

Fifty-one studies were included in the main analysis. The overall mean age of BE patients was 61 years (40 studies); the mean overall proportion of males was 64% (37 studies). In total, these studies included 13,777 patients followed up for 60,688 person-years, during which 303 patients developed EAC. The pooled estimate for EAC incidence was 6.2/1,000 pyrs (95%CI: 4.9-7.8) with considerable heterogeneity ($p<0.001$; $I^2=63\%$). Nineteen studies reported data on mortality due to EAC. These studies included 7,930 patients followed up for 33,022 pyrs, with 88 deaths due to EAC and 1,271 deaths due to other causes. The pooled incidence of fatal EAC was 3.0/1,000 pyrs (95%CI: 2.2-3.9) with no evidence for heterogeneity ($p=0.4$; $I^2=7\%$). Evidence of publication bias was found for studies from the USA.

Conclusion

Patients with BE have a low risk of malignant progression and predominantly die from other causes than EAC. This undermines the cost-effectiveness of BE surveillance, and supports the search for valid risk stratification tools to identify the minority of patients that is likely to benefit from surveillance.

INTRODUCTION

Barrett's esophagus (BE) is a well recognized premalignant condition,[1] which carries a 30-125 fold higher risk of esophageal adenocarcinoma (EAC) than the general population.[2, 3] The incidence of BE as well as the incidence of EAC are increasing in the Western world.[4-6] EAC usually portrays a poor prognosis, with a 5-year survival rate of less than 15%.[7] Hence, surveillance endoscopy is recommended for patients with BE, in order to detect early stage neoplasia and subsequently to improve survival.[8]

It has been reported that BE patients in whom EAC was detected within a surveillance program had both earlier stage disease and a better survival than patients with EACs detected outside surveillance programs.[9] Nevertheless, there is little evidence that surveillance programs have prevented deaths from EAC,[10, 11] as most patients with BE die from other causes than EAC.[12] This questions the cost-effectiveness of a strict surveillance strategy, which is in particular dependent on cancer risk and risk of cancer-specific mortality.[13] The true annual incidence of EAC in BE patients remains unclear, as it shows considerable variation among cohort studies, ranging from 0.2% to almost 3.5% per year.[14, 15] These rates could have been overestimated as a result of publication bias in published BE surveillance studies, with evidence of selective publication of small studies with high cancer incidence rates.[16] In addition, some studies have reported an overall increased mortality in BE patients compared to the general population,[17, 18] whereas others could not confirm this.[19] Moreover, EAC-specific mortality rates in BE patients show contrasting results in various studies.[18, 20]

Clarification of these factors is essential in re-appraising the value of surveillance endoscopy in BE. As randomized controlled trials comparing surveillance with non-surveillance in BE patients in terms of cancer-related deaths are not likely to be performed, a meta-analysis on both the risk of cancer and cancer-related deaths in BE provides an alternative to answer this question. So far, four reviews have been published on the risk of cancer in BE.[16, 21-23] One of these reviews included also patients who had undergone surgery and evaluated the difference in cancer incidence between medically and surgically treated BE patients.[23] The most recent review reported an EAC incidence rate of 6.1/1,000 pyrs of follow-up.[21] However, all four risk analyses were limited to incidence rates of cancer in BE, while none investigated overall mortality rates in BE, nor the risk of mortality from EAC specifically. Therefore, we performed an updated systematic review and meta-analysis of various surveillance studies to determine not only the risk of EAC and of EAC and high-grade dysplasia (HGD) combined, but also to determine the risk of cancer-related deaths in patients with BE.

MATERIALS AND METHODS

Search strategy

PubMed, EMBASE and Web of Science databases were systematically searched for cohort studies reporting on EAC risk and mortality due to EAC in patients with BE, published between 1966 and September 2008. The following keywords were used for: (1) BE: Barrett's esophagus, Barrett's metaplasia, Barrett's mucosa, Barrett's epithelium, columnar-lined esophagus (CLE), specialized intestinal metaplasia (SIM); (2) EAC: esophageal adenocarcinoma, esophageal cancer, esophageal neoplasm, esophageal malignancy, esophageal neoplasia; and (3) Mortality: mortality, death. Both American and British spellings were applied, and results of keyword searches were combined using the Boolean terms "and/or". Each abstract was independently reviewed by two investigators (MS, PdJ), and from those reporting EAC risk and/or mortality in patients with BE, the full text was reviewed. References from these selected articles were scrutinized for additional articles for inclusion. In addition, previous meta-analyses on cancer risk in BE were checked for articles that were not identified with our search strategy. [16, 21-23]

Study selection

Studies were included if they met the following criteria: (1) written in English; (2) histologically proven BE (CLE or SIM); (3) documented follow-up data either in person-years (pyrs) or mean follow-up period; and (4) histologically proven EAC on surveillance. Studies were excluded if they were available as abstracts only, if they were written in languages other than English, if they lacked data on follow-up, or if they reported solely on patients who underwent endoscopic ablation or surgery. If serial studies from a single center reported cancer risk or mortality in the same cohort, only the most recent publication was included.

Data extraction

Two investigators (MS and PdJ) independently collected the following data from each study: country, year and type of study; definition of BE used; number of patients in the study with documented follow-up; mean follow-up period; person-years of follow-up; mean age at entering surveillance; sex ratio; number of prevalent and incident cancers; number of prevalent and incident HGDs; number of patients who died during the study; and number of patients who died due to EAC. In addition, where available, data on the proportion of patients with SSBE and low-grade dysplasia at baseline BE diagnosis were also extracted. Where possible, we excluded patients with baseline HGD for this analysis. In case of disagreement, a third independent investigator was asked for a review (EJK).

Data analysis

Incidence rates of both EAC and EAC/HGD combined in BE were calculated by dividing the number of EACs/HGDs by the total number of person-years of follow-up. In case the latter

was not provided in a study, it was estimated by multiplying the number of patients who underwent surveillance by their mean duration of follow-up. For this analysis we only used incident cancers and HGDs. Mortality rates due to EAC (or the incidence of fatal EAC) in BE were calculated similarly. The corresponding 95% confidence intervals (CI) were calculated using exact methods and assuming a Poisson distribution. When the number zero was present in the data, a continuity correction of 0.5 was used for the purpose of calculations, as has previously been described.[24]

The heterogeneity between studies was calculated using the chi-square test and measured by the I^2 statistic.[24, 25] The pooled estimates with 95%CIs were obtained from a random-effects model, and log incidence rates of EAC/HGD and fatal EAC with corresponding standard errors.[26]

Assessment of publication bias was performed using Begg's and Egger's tests, and by exploring funnel diagrams.[27, 28] All statistical analyses were performed by using STATA software (version 10.0; Stata corporation, College station, Texas, USA), using the "metan" and "metabias" commands.

Results

The search strategy yielded 7,200 abstracts, of which 190 were relevant to the review topic and subsequently reviewed. Following evaluation of the full text papers, fifty-one articles met the inclusion criteria and were included in the final analysis.[2, 11, 12, 14, 15, 17, 18, 29-69]

Study characteristics

Of the 51 studies included, 20 were from the United Kingdom (UK), 16 from the United States (US), 13 from other European countries, and two from Australia. Baseline characteristics of the study cohorts are given in Tables 1 and 2. Forty studies provided data on mean age, the overall mean age was 61.3 years (range 40.0-70.0).[2, 11, 12, 14, 15, 17, 18, 20, 29-37, 39, 41, 46-53, 56-59, 61-67, 69-71] Sex ratio was reported in 37 studies and the overall male proportion was 64%.[2, 11, 12, 14, 15, 17, 18, 20, 29, 33-39, 41, 44, 46-49, 51, 54, 55, 57-59, 61-65, 67, 69, 70] Initial Barrett's length was reported in 23 studies, rendering a mean length of 5.3 cm (range 1.5-8.1 cm).[11, 12, 31, 34-36, 39, 41, 43, 46, 48-52, 54, 59, 62, 63, 67, 69, 70, 72] In 49 studies, a length of 3 cm was used as a cut-off to classify patients as having LSBE or SSBE. The overall prevalence of patients with LSBE was 95% and with SSBE 5%. The definition of BE showed variation between studies. In 21 studies it was defined as SIM-positive,[11, 12, 18, 29, 37, 39, 40, 43, 44, 48, 50, 52, 57, 59, 61-64, 66, 69, 70] in 6 studies as SIM-positive and CLE,[15, 17, 20, 53-55] in 18 studies as CLE or SIM only,[2, 14, 30-36, 41, 45-47, 49, 51, 67, 68, 72] and in 6 studies it was unclear [38, 42, 56, 58, 60, 65]. In total 9,897 (78%) patients were SIM-positive. Presence of baseline LGD was reported in 30 studies, with an overall prevalence of 11%.[2, 11, 12, 29, 30, 32-34, 36, 38-41, 43, 45-48, 52, 54-57, 59, 61, 62, 65, 66, 69, 71] Baseline HGD was reported in ten studies and could not be excluded from the baseline analysis. [2, 12, 29, 32, 33, 39, 47, 48, 55, 65, 69] The overall baseline prevalence of HGD was 3%.

Table 1. Characteristics of the included studies on the incidence of EAC and mortality in BE.

First author	Year	Geography	Total *n* under FU	Male (%)	mean age
Spechler[29]	1984	USA	105	NA	58
Cameron[14]	1985	USA	104	67	59.6
Robertson[30]	1988	UK	56	55	62
Ovaska[31]	1989	Finland	26	NA	59.2
Hameeteman[2]	1989	NL	50	60	59.3
Miros[32]	1991	Australia	81	NA	63.3
Williamson[33]	1991	USA	176	65	56
Iftikhar[34]	1992	UK	102	61	63
Attwood[35]	1992	UK	26	46	70
Ortiz[36]	1996	Spain	27	74	40
Wright[37]	1996	UK	166	65	58.5
Komorowski[38]	1996	USA	14	79	NA
Sharma[39]	1997	USA	32	98	63.1
Younes[40]	1997	USA	61	NA	NA
Katz[41]	1998	USA	102	83	63
Streitz[42]	1998	USA	136	NA	NA
Schoenfeld[43]	1998	USA	123	NA	NA
Teodori[44]	1998	Italy	30	60	NA
Wilkinson[45]	1999	UK	12	NA	NA
MacDonald[46]	2000	UK	143	60	57
Reid[47]	2000	USA	327	81	62
Srinivasan[48]	2000	USA	9	89	60
Eckardt[49]	2000	UK	357	58	63
Bani Hani [71]	2001	Germany	60	NA	61
Conio[50]	2001	USA	154	70	62.3
Rana[51]	2001	UK	44	73	58
Fitzgerald[52]	2001	UK	96	NA	65
Spechler[53]	2001	USA	108	NA	NA
Conio[11]	2003	Italy	166	81	59.9
Parilla[54]	2003	Spain	43	77	NA
Murray[55]	2003	UK	2969	57	NA
Anderson [20]	2003	UK	2373	58	58.2
Hurschler[56]	2003	Switzerland	207	NA	64.4
Hillman[57]	2003	Australia	353	71	59.2
Hage[12]	2004	NL	105	55	63.4
Solaymani[58]	2004	UK	1656	61.6	63.6
Basu[59]	2004	UK	138	74	62.1
Meining[60]	2004	Germany	148	NA	NA
Aldulaimi[15]	2005	UK	126	76	63
Dulai[61]	2005	USA	575	99	60
Murphy[62]	2005	UK	178	71	57
Oberg[63]	2005	Sweden	140	74	57.3
Gladman[64]	2006	UK	195	55	62.9
Vieth[65]	2006	Germany	748	68	62.6
Sharma[66]	2006	USA	618	NA	60.9
Cook[17]	2007	UK	502	55	58.8
Olithselvan[67]	2007	UK	121	70	60.2
Gatenby[68]	2008	UK	807	NA	NA
Musana[69]	2008	USA	216	76	62
Moayyedi[18]	2008	UK	1272	63	66.6
Martinek[70]	2008	Czech RR.Republic	135	76	59.4

*BE: Barrett's esophagus, LSBE: long segment Barrett's esophagus, SSBE: short segment Barrett's esophagus, SIM: specialized intestinal metaplasia, LGD: low-grade dysplasia, HGD: high-grade dysplasia, EAC: esophageal adenocarcinoma, †: mortality, NA: non-applicable (data not available).

SIM+ (%)	LGD (*n*)	HGD (*n*)	LSBE (%)	SSBE (%)	Pyrs of FU	Incident EAC (*n*)	Total † (*n*)	EAC †(*n*)	other †(*n*)
100	35	10	NA	NA	350	2	16	1	15
NA	NA	NA	100	0	884	2	25	1	24
77	8	NA	100	0	162	3	4	0	4
NA	NA	NA	100	0	166	3	NA	NA	NA
68	6	1	100	0	260	5	NA	NA	NA
NA	10	3	100	0	289	3	21	2	19
NA	20	0	100	0	497	5	NA	NA	NA
NA	2	NA	100	0	462	4	6	1	5
NA	NA	NA	100	0	90	1	NA	NA	NA
85	0	NA	100	0	127	1	NA	NA	NA
NA	NA	NA	100	0	461	6	NA	NA	NA
79	7	NA	93	7	70	2	NA	NA	NA
91	5	0	0	100	99	1	NA	NA	NA
100	25	NA	100	0	201	5	NA	NA	NA
100	5	NA	100	0	563	3	19	2	17
NA	NA	NA	100	0	510	7	NA	NA	NA
NA	0	NA	54	46	323	2	NA	NA	NA
100	NA	NA	100	0	350	4	NA	NA	NA
NA	1	NA	100	0	57	0	NA	NA	NA
NA	0	NA	100	0	629	5	33	3	30
100	122	76	100	0	979	9	NA	NA	NA
100	3	1	89	0	36	0	NA	NA	NA
86	NA	NA	NA	NA	594	2	NA	NA	NA
0	0	NA	100	0	1293	12	11	0	11
100	NA	NA	76	24	585	4	35	1	34
68	NA	NA	100	0	418	2	20	2	18
71	6	NA	100	0	375	0	NA	NA	NA
100	NA	NA	100	0	1037	4	NA	NA	NA
100	16	NA	64	36	1100	5	18	3	15
100	3	NA	100	0	258	0	NA	NA	NA
56	171	19	100	0	11068	29	NA	NA	NA
54	NA	NA	100	0	7413	NA	253	12	241
45	19	NA	100	0	966	10	NA	NA	NA
100	50	NA	100	0	1588	9	NA	NA	NA
100	11	0	100	0	1329	6	72	4	68
NA	NA	NA	100	0	2615	13	111	13	98
0	3	NA	88	12	405	1	NA	NA	NA
67	NA	NA	100	0	376	0	NA	NA	NA
100	NA	NA	100	0	338	12	NA	NA	NA
100	134	NA	100	0	2775	2	164	3	161
100	33	NA	81	19	613	3	NA	NA	NA
100	NA	NA	100	0	946	3	NA	NA	NA
100	NA	NA	90	10	1068	4	21	1	20
100	19	10	42	33	4875	15	NA	NA	NA
100	101	NA	100	0	2546	12	NA	NA	NA
86	NA	NA	100	0	5247	14	246	13	233
NA	NA	NA	100	0	424	2	NA	NA	NA
NA	NA	NA	100	0	3912	23	NA	NA	NA
100	45	7	52	25	691	4	NA	NA	NA
100	NA	NA	100	0	5705	47	245	25	220
100	NA	NA	36	64	700	2	NA	NA	NA

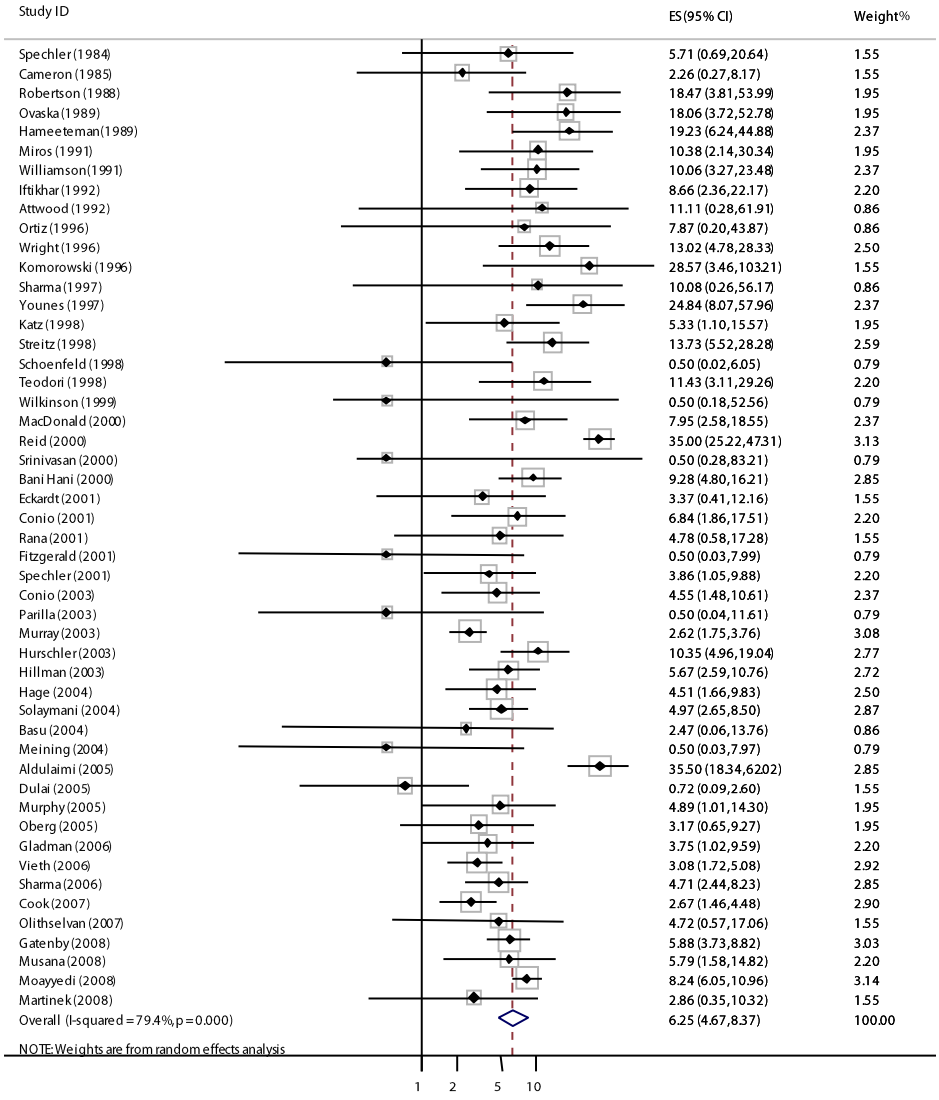

Figure 1. Forrest plot showing the overall incidence of EAC in 50 studies. The cancer incidence rate is a log scale on the x-axis (1, 2, 5 denote 1/1,000, 2/1,000 and 5/1,000 person-years of follow-up, respectively).

Incidence of EAC

Fifty studies reported on the incidence of EAC and were used in the analysis (Table 1). In total, these studies included 14,109 patients followed up for 61,804 person-years. During this follow-up 344 incident EACs were diagnosed. A random effects models produced a pooled estimate

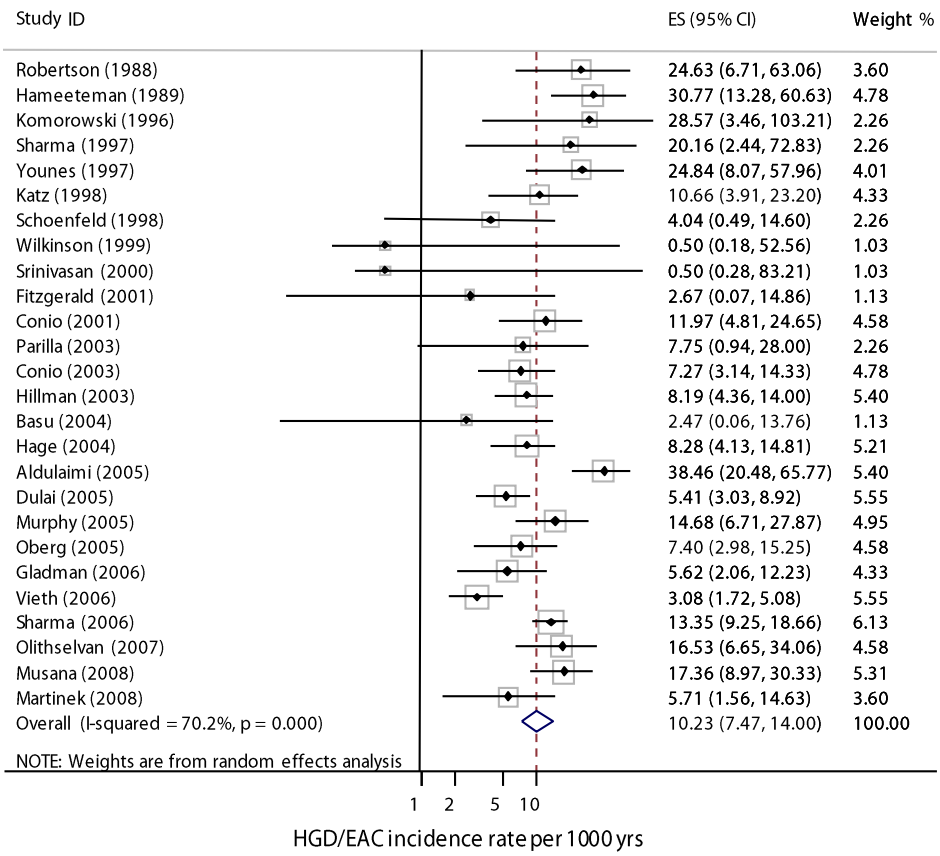

Figure 2. Forrest plot showing the overall incidence of HGD and EAC in 26 studies. The HGD/EAC incidence rate is a log scale on the x-axis (1, 2, 5 denote 1/1,000, 2/1,000 and 5/1,000 person-years of follow-up, respectively).

for EAC incidence in BE of 6.3/1,000 pyrs (95%CI: 4.7-8.4) (Figure 1). There was, however, considerable heterogeneity in incidence rates between studies (χ^2=238.2; df=49; p<0.001; I^2=79%).

The mean incidence of EAC in studies from the UK was 6.3/1,000 pyrs (95%CI: 4.2-9.3), in those from the US 6.5/1,000 pyrs (95%CI: 3.4-12.4), in other European studies 5.6/1,000 pyrs (95%CI: 3.5-9.2), and in Australian studies 6.5/1,000 pyrs (95%CI: 3.5-12.2). On exclusion of studies with less than 500 pyrs of follow-up, the overall incidence of EAC was 5.3/1,000 pyrs (95%CI: 3.7-7.6). If only studies with CLE or SIM-positive BE patients or well defined BE were included, the overall EAC incidence was 5.0/1,000 pyrs (95%CI: 3.4-7.3).

Incidence of HGD and EAC

Twenty-six studies reported both on the incidence of HGD and EAC in their BE patients (Table 1).[2, 11, 12, 15, 30, 38-41, 45, 48, 50, 52, 54, 57, 59, 61-67, 69, 70, 72] In total, these studies included 4,528 patients fol-

lowed-up for 22,559 pyrs, with 103 incident cases of EAC and 91 incident cases of HGD during follow-up. The pooled estimate of incidence of both EAC and HGD combined was 10.2/1,000 pyrs (95%CI: 7.5-14.0). Again, there was marked evidence of heterogeneity (χ^2=83; df= 25, p<0.001; I^2=70%) (Figure 2).

The overall incidence of HGD/EAC was lowest in other European countries than the UK (7.3/1,000 pyrs (95%CI: 3.6-15.0)), and higher in the US (11.0/1,000 pyrs (95%CI: 6.9-17.5)) and the UK (13.0/1,000 pyrs (95%CI: 7.4-22.8)).

Mortality due to EAC

Nineteen studies reported on EAC-related mortality in BE patients (Table 1). [11, 12, 14, 17, 18, 20, 29, 30, 32, 34, 41, 46, 50, 51, 61, 64, 71, 73, 74] These studies included 7,930 patients followed up for 33,022 pyrs, with 88 deaths due to EAC and 1,271 deaths due to other causes. The pooled incidence of fatal EAC was 3.0/1,000 pyrs (95%CI: 2.2-3.9), with no evidence of heterogeneity (χ^2=19.3; df= 18; p=0.4; I^2=7%) (Figure 3).

Cause-specific mortality

In 17 studies the total number of patients who died during surveillance was reported.[11, 12, 14, 17, 18, 20, 29, 30, 32, 34, 41, 46, 49-51, 61, 73] Only in 12 studies the cause-specific mortality was provided.[11, 12, 14, 17, 18, 20, 29, 32, 46, 49, 50, 73] These studies included 4,207 patients followed up for 24,959 pyrs, with 921 deaths. Sixty-four of 921 deaths (7%) were due to EAC and 857 (92%) due to other causes. The pooled estimate of the mortality rate due to other causes than EAC was 37.1/1,000 pyrs (95%CI: 31.6-43.6), with evidence of large heterogeneity (χ^2= 91.7; df=17; p<0.001; I^2= 82%). Figure 4 shows the cause-specific mortality in BE patients. Cardiovascular disease was the most common cause of death, with 320 deaths (34%) in patients with BE.

Table 2. Summary of characteristics of BE patients included in the analysis.

Variable	Number of studies	Cumulative number of patients	Number of patients with selected variable	Overall percentage
Males	37	13,930	8,904	64
SIM positive	35	12,641	9,897	78.3
Baseline LGD	26	7,539	860	11.4
Baseline HGD	8	4,505	127	2.8
LSBE	49	16,056	15,177	95
SSBE			640	5

***** BE: Barrett's esophagus, LSBE: long segment Barrett's esophagus, SSBE: short segment Barrett's esophagus, SIM: specialized intestinal metaplasia, LGD: low-grade dysplasia, HGD: high-grade dysplasia, NA: non-applicable (data not available).

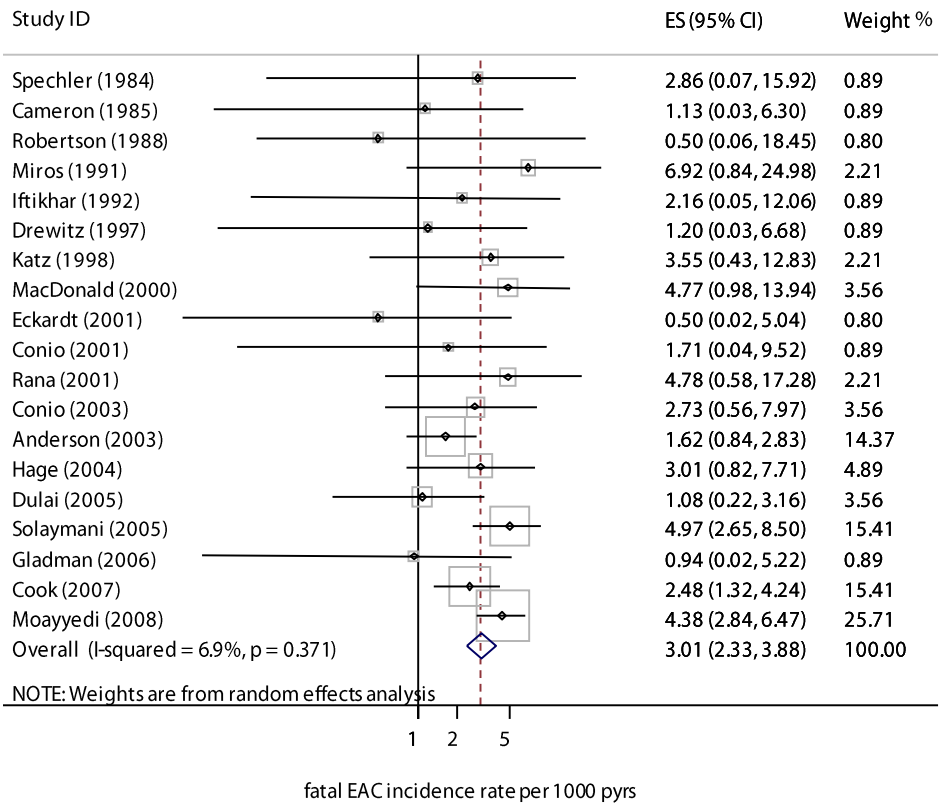

Figure 3. Forrest plot showing the overall incidence of fatal EAC in 18 studies. The fatal EAC incidence rate is a log scale on the x-axis (1, 2, 5 denote 1/1,000, 2/1,000 and 5/1,000 person-years of follow-up, respectively).

Publication bias

In Figure 5, EAC incidence rates were plotted against person-years of follow-up. The funnel plot demonstrated smaller incidence rates in the larger studies, which was largely confirmed by tests of funnel plot asymmetry (Begg's test, p=0.075; Egger's test, p=0.051). Publication bias was present among studies from the US (p=0.001), but was not found among studies from the UK and other European countries. Publication bias was also assessed among studies reporting both HGD and EAC incidence and mortality. There was no evidence of such a bias among those studies.

DISCUSSION

The cost-effectiveness of surveillance of BE remains a matter of discussion. In the absence of data from randomized controlled trials on surveillance in BE patients, cost-efficacy estimates have to

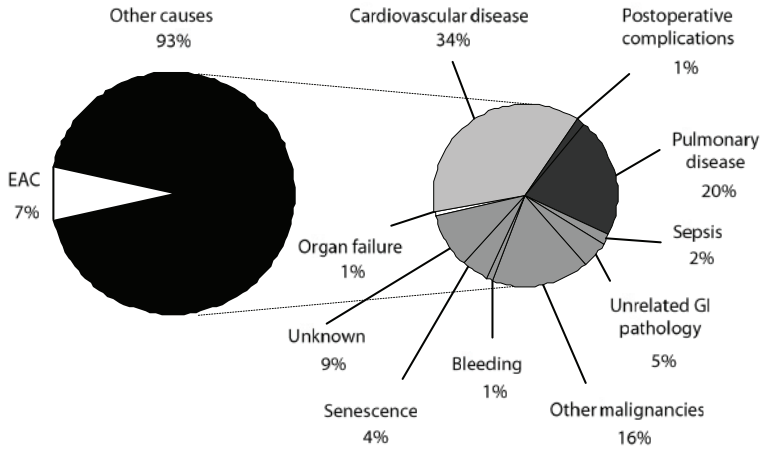

Figure 4. Causes of mortality in BE patients.

be based on mathematical models. These show that both the incidence rate of progression to EAC and mortality due to EAC are critical factors for the cost-effectiveness of surveillance.[13, 19]

Our meta-analysis showed that the overall estimate of the incidence of EAC in patients with BE was 6.3 cases per 1,000 pyrs of follow-up and that the overall incidence of EAC and HGD combined was 10.2/1,000 pyrs, which corresponded to an annual risk of 0.6% and 1.0%, respectively. The overall estimate of the EAC incidence declined to 5.3/1,000 pyrs upon exclusion of studies with less than 500 pyrs of follow-up, and to 5.0/1,000 pyrs when only studies with a robust definition for BE diagnosis were included. Furthermore, the overall estimate of the mortality rate due to EAC in patients with BE was 3.0/1,000 pyrs of follow-up. This is low, as expected, and correlates to one fatal case of EAC per 333 pyrs. The mortality rate due to other causes than EAC was 10-fold higher with an estimate of 37 deaths per 1,000 pyrs, as compared to the mortality rate due to EAC.

So far, four systematic reviews have been published on cancer risk in patients with BE. The most recent review included publications up to 2006.[21] Our review on EAC incidence was an update of that review with inclusion of studies up to October 2008. Our findings are in agreement with those from two recent reviews, reporting annual EAC risks of 0.6% and 0.7%, respectively.[21, 22] In addition, both studies showed a decline in EAC incidence to 5/1,000 pyrs[22] and 4.4/1,000 pyrs[21] when small studies were excluded from the analysis, as was also the case in our study. The presence of geographic variation in BE cancer risk has previously been suggested by others.[5, 75], which is in line with other studies.[21, 22] There were very small differences in EAC incidence between different geographic regions, with only a slightly higher EAC incidence in the US and UK compared to other European countries.

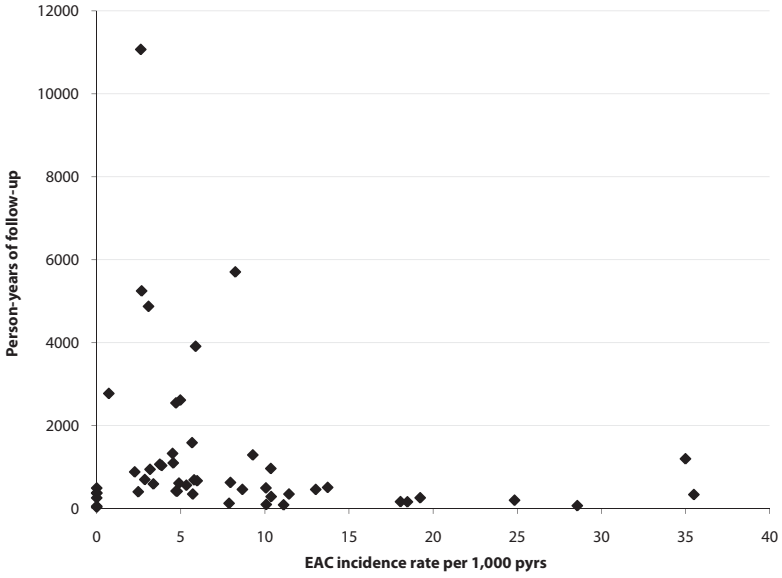

Figure 5. Funnel plot of the EAC incidence rate against the number of person-years of follow-up.

Reports on the combined incidence of HGD and EAC can be even more valuable than those on EAC risk alone, as the detection of HGD is an important outcome of surveillance programs. At present, HGD can be eradicated by advanced endoscopic techniques, which are less invasive than esophagectomy, and could prevent further progression to cancer.[76, 77] The overall pooled estimate of combined HGD/EAC incidence in our study was 10.2 per 1,000 pyrs of follow-up which corresponded to one case per 98 pyrs. This is slightly higher than those reported by others, who found rates of 9/1,000 pyrs[22] and 10.0/1,000 pyrs[21]. Compared to these studies, we included a larger number of studies in which progression to HGD was used as an outcome, which could explain the small difference in HGD/EAC incidence rate.

To our knowledge, this is the first systematic review analyzing studies that report on mortality rates in patients with BE. Although the risk of EAC is clearly elevated in BE patients as compared to the general population, the majority of patients will not develop EAC. Moreover, it has been suggested that even few will die from it.[20, 74] The magnitude of the mortality risk due to EAC in patients with BE remains, however, uncertain. Also the benefit of surveillance in preventing EAC mortality and the impact of this condition on overall life expectancy remains unclear.[18] A truly low risk of death due to EAC would undermine the cost-effectiveness of generalized BE surveillance. The overall pooled estimate of fatal EAC incidence in our review was 3.0 per 1,000 pyrs which corresponded to an annual risk of 0.3%. As there was no evidence of heterogeneity in this analysis, this is a reliable estimate of the mortality rate due to EAC for

patients with BE under surveillance. Mortality due to other causes than EAC was more than ten times increased in patients with BE than mortality due to EAC. When examining cause-specific mortality in BE patients, only 7% of the total number of patients died from EAC and 93% died due to other causes. Cardiovascular disease (including stroke) accounted for 34% of the total number of patients who died, followed by 20% due to pulmonary disease and 16% due to other malignancies. This emphasizes that EAC mortality in patients with BE under surveillance is relatively low. From our analysis, we can only speculate whether the natural course of EAC in patients with BE is slow, or that other explanations are more important. One explanation could be length time bias. Another explanation could be that further progression to invasive EAC was prevented because early endoscopic or surgical treatment was performed. The incidence of EAC estimated in this meta-analysis approximates the incidences used in published cost-effectiveness analyses on BE surveillance[13, 19] and confirms that the benefit of generalized BE surveillance is questionable. Our findings support the search for valid risk stratification tools to identify the minority of patients who are likely to benefit from surveillance.

Marked heterogeneity was present in the analyses on EAC incidence and the combined HGD/EAC incidence. Publication bias was not a clear explanation for this. Other explanations could be differences in cohort compositions regarding age, gender and period of inclusion selected for this review or differences in surveillance endoscopies and biopsy protocols. Another explanation could be that in small studies selected patient groups with a high cancer risk were included. Also, in large studies EACs could have been missed by sampling error during surveillance.

Several limitations of this study need to be considered. Firstly, as the majority of included studies did not report accurately on demographic and clinical patient characteristics, we were unable to adjust for confounding variables. Secondly, with regard to our analysis on mortality rates in BE patients, we were unable to compare these rates with overall mortality rates in the general population. Thirdly, causes of death due to other causes than EAC could have been misclassified, as ICD-classes or death certificates were not used in all studies reporting mortality.

In conclusion, the rate of progression in BE to EAC or HGD and EAC combined is low (0.6% and 1.0% annually, respectively) and the rate of mortality due to EAC is even much lower (0.3% annually). Our findings question the effectiveness of generalized BE surveillance programs, and emphasize the need of large studies from other unselected populations to develop valid risk stratification.

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Chapter 6

Risk of malignant progression in patients with Barrett's esophagus: a Dutch nationwide cohort study

Submitted for publication

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ABSTRACT

Background

Reported incidence rates of esophageal adenocarcinoma (EAC) in Barrett's esophagus (BE) vary widely. As the effectiveness of BE surveillance is crucially dependent on this rate, its clarification is essential.

Aim & Methods

To estimate the rate of malignant progression in BE patients, all patients with a first diagnosis of BE with no dysplasia (ND) or low-grade dysplasia (LGD) between 1991 and 2006 were identified in the Dutch nationwide registry of histopathology (PALGA). Follow-up data were evaluated until November 2007.

Results

In total, 42,207 BE patients were included, 4,132 (8%) of them had LGD. Re-evaluation endoscopies at least 6 months after initial diagnosis were performed in 16,365 patients (39%), who were significantly younger than those not re-examined (58±13 vs.63±16 yrs, $p<0.001$). These patients were followed-up for a total of 78,131 person years (pyrs), during which 666 (4%) HGD/EACs occurred, affecting 4% of the surveillanced patient population (mean age: 69±12 yrs, 76% male). After excluding HGD/EAC cases detected within 1 year after BE diagnosis (*n*=212, 32%), incidence rates per 1,000 pyrs were 4.3 (95%CI: 3.4-5.5) for EAC and 5.8 (95%CI: 4.6-7.0) for HGD/EAC combined. Risk factors for HGD/EAC were increased age (*e.g.*, >75 years HR: 12: 95%CI: 8.0-18), male gender (2.01: 1.68-2.60), and presence of LGD at baseline (1.91: 1.53-2.40).

Conclusion

In this largest reported cohort of unselected BE patients, the annual risk of EAC was 0.4%. Male gender, older age, and LGD at diagnosis are independent predictors of malignant progression, and should enable an improved risk assessment in BE.

INTRODUCTION

Barrett's esophagus (BE) is an acquired condition, in which the squamous epithelium lining the distal esophagus is replaced by columnar intestinal-type mucosa.[1] It is considered to be a complication of longstanding gastroesophageal reflux and constitutes the prime risk factor for esophageal adenocarcinoma (EAC).[2, 3] EAC usually portrays a poor prognosis, with a 5-year survival rate of less than 15%.[4] Hence, in order to detect early-stage cancers suitable for curative treatment, surveillance endoscopy of patients with BE is advised, at intervals dictated by the absence or presence and grade of dysplasia.[5]

The effectiveness of surveillance of BE is, however, equivocal. Increased survival has been observed in patients with EAC enrolled in BE surveillance programs. This may have resulted from early detection of cancers. On the other hand, this effect may also have resulted from lead-time bias, as in particular young patients without concomitant diseases were included in surveillance programs.[6-11] In addition, most patients with BE die from unrelated causes, as according to a cohort follow-up study from our department only 5.6% of total mortality in BE patients was related to EAC.[10] Moreover, some patients may not be fit for surgery even if EAC is detected at an early stage.[12, 13]

A primary determinant of the cost-effectiveness of BE surveillance strategies is the risk of progression to EAC.[14-16] Unfortunately, published estimates of the annual risk of cancer in BE patients are highly heterogeneous, ranging from 0% to 2.9% per annum.[17] These estimates were based primarily on patients referred to tertiary centers, whose cancer risk may exceed that for patients managed by non-referral centers. Moreover, published data predominantly come from small retrospective cohort studies with relatively short follow-up, showing higher cancer incidence than may be observed in larger surveillance studies. Consequently, U.S. investigators reported evidence of publication bias in surveillance studies favoring publication of small studies with high cancer incidence rates.[18]

As a result of the low degree of ascertainment of BE in the general population,[19, 20] there is a lack of both large scale and long-term follow-up studies of BE patients, providing more reliable risk estimates for malignant progression. Such studies are essential, both in reappraising the potential value of surveillance endoscopy for BE patients and in optimizing the recommended follow-up intervals. We therefore estimated the progression rate of BE to high-grade dysplasia (HGD) and EAC in a nationwide cohort of BE patients in the Netherlands, and assessed the value of the factors age, sex and initial histology as predictors of malignant progression in BE.

METHODS

Histopathology database

In the Netherlands all histopathology and cytopathology reports are collected in a national archive (PALGA database), which encompasses all sixty-four pathology laboratories in the Netherlands. Since 1991, PALGA has had nationwide coverage and currently contains about 42 million excerpts from nearly 10 million patients.[21] Every excerpt in the database contains encrypted patient identification, a part of the summary of the original pathology report and diagnostic codes similar to the Systematized Nomenclature of Medicine (SNOMED) issued by the College of American Pathologists.[22] This diagnostic code contains a topological term, the type of sample, and a morphological term describing the finding, *e.g.*, 'esophagus*biopsy*intestinal metaplasia'. The SNOMED morphology codes are identical to the codes in the International Classification of Diseases for Oncology (ICD-O-2; World Health Organization).[23] Details with regard to the number and intra-esophageal location of biopsies, or information on the indication for performing an endoscopic procedure, are not uniformly registered. Each pathology report can, however, be traced to an individual patient with a unique identifier, allowing follow-up of subsequent histology, irrespective of where subsequent biopsies were taken or resections were performed.[24] For each report, gender, date of birth, date of pathology review, summary text and diagnostic codes were made available. It was not, however, possible to access additional clinical data. The present study was based on data recorded in the PALGA database between 1991 and 2007.

Data collection

All patients registered in the database between 1991 and 2006 with an initial, histological diagnosis of BE with no baseline dysplasia (ND), or maximally low-grade dysplasia (LGD), were identified.[25] Codes that were used to classify biopsies as BE are described in the Appendix. Patients with a baseline diagnosis of high-grade dysplasia in BE were excluded, as were patients with either gastric or esophageal surgery or malignancy, registered prior to, or simultaneously with the first diagnosis of BE.

For each patient admitted to the cohort, all pathology excerpts concerning esophageal biopsies from the first diagnosis of BE to the end of the study period (November 2007) were retrieved. Follow-up excerpts were scrutinized for codes indicating HGD and/or EAC. Where present, the pathology reports of the surgical resection specimens were reviewed; only carcinomas of which the bulk was macroscopically located above the gastroesophageal junction and those clearly originating from BE, were diagnosed as EAC.[26, 27]

Data analysis

Person-years of follow-up were calculated for each member of the cohort. Here we distinguished between patients who underwent one or more re-evaluation endoscopies with bi-

opsy sampling, and patients without further histology after BE diagnosis. The former group was censored either at the date of HGD or EAC diagnosis or of last patient contact with biopsy sampling, the latter at date of death. The date of death of patients registered in the PALGA database is, however, not uniformly recorded, unless an autopsy has been performed, reports of which are also registered in the system, but are a fairly rare in this country. Therefore, for the latter cohort, censoring was imputed to evaluate the number of person-years at risk, as they were unlikely to have developed symptomatic EAC throughout the study period. As the ascertainment of symptomatic EAC in the Netherlands is presumed to be very high,[28] the disease practically always results in histological analysis, which is registered in the PALGA archive. Survival data from the general Dutch population were collected, starting from age and gender of the patients, and calendar year (Dutch Cancer Registry 2007). A dataset with an approximately unbiased number of person years at-risk was subsequently created, by drawing from a binomial distribution for every year, as was done previously.[29, 30] Multiple imputation did not significantly change results. Finally, imputed survival estimates were corrected for an assumed 16% risk of excess mortality in BE patients, based on previous reports.[31]

Within the first analysis, EAC risk and combined HGD/EAC risk were calculated for those patients with actual documented follow-up in the PALGA registry. Within the second analysis, EAC risk was calculated for the whole cohort of patients, including those who did not receive one or more follow-up endoscopies with biopsies after initial BE diagnosis. To avoid misclassification of prevalent cases of HGD/EAC, only those cases that were detected at least one year after initial BE diagnosis were included in cancer risk analysis. Crude EAC and combined HGD/EAC incidence rates are presented as number of cases per 1,000 patient-years of follow-up. Kaplan-Meier survival analysis was performed to evaluate the interval between initial BE diagnosis and occurrence of HGD/EAC. Survival curves for BE patients without baseline dysplasia were compared to those with baseline LGD, using the log-rank test, at the $p<0.05$ level of significance. Cox-regression analyses were performed to identify independent risk factors for progression of BE to HGD/EAC. Estimates of relative risks are expressed as hazard ratios (HRs) with 95% confidence intervals (CIs). Statistical analyses were conducted using S-PLUS (S-PLUS 6.0 Insightful Corp, Seattle, WA, USA) and SPSS software (SPSS 16.0, Chicago, IL, USA).

RESULTS

Study cohort

In total, 42,207 patients with a first diagnosis of BE were identified in the PALGA database, with a 1.0/0.6 male to female ratio (Table 1). Of those, 4,132 (9.8%) were classified with LGD at baseline. Patients with LGD were significantly older than patients without dysplasia (mean age ± SD: 64±14 vs. 61±15 yrs, $p<0.001$) and more often male (63% vs. 61%, $p=0.003$). In ad-

Table 1. Baseline characteristics of the study population.

Variable	Total	No dysplasia	Low-grade dysplasia
Number of BE patients (%)	42,207	38,075 (90%)	4,132 (9.8%)
Male/ Female	1.0 / 0.6	1.0 / 0.6	1.0 / 0.6
Age (years)			
Median	62.1	61.8	65.0
10th-90th percentile	41 – 80	41 - 80	46 - 81

BE: Barrett's esophagus.

dition, men were also significantly younger than women at initial diagnosis of BE without dysplasia (58±15 vs. 65±15 yrs, $p<0.001$), and BE with LGD (61±14 vs. 67±13 yrs, $p<0.001$).

Follow- up endoscopies with biopsy sampling

Follow-up upper GI endoscopy with biopsy sampling, at least 6 months after the initial diagnosis, was performed in 16,365 BE patients (39%). Mean length of total follow-up in these patients was 4.8 years (SD3), for a total of 78,131 patients-years. In total, 43,568 re-evaluation endoscopies were performed, with an average of 3 (range: 1-22) endoscopies per patient. Patients with histological follow-up were significantly younger than those not undergoing follow-up (58±13 vs.63±16 yrs, $p<0.001$), and more often male (65% vs. 59%, $p<0.001$). Only 38% of patients without dysplasia at baseline underwent at least one histological follow-up, as against 52% after a diagnosis of baseline LGD ($p<0.001$). The mean interval between initial and follow-up endoscopy was 2.0±2 yrs for BE patients without baseline dysplasia, and 1.4±2 yrs for those with LGD at baseline ($p<0.001$).

Diagnosis of high-grade dysplasia and/or esophageal adenocarcinoma

Malignant progression was observed in 666 (1.6%) patients (HGD: 161, EAC: 505). Five additional patients were diagnosed with an adenocarcinoma of which the bulk was located below the gastroesophageal junction. These cases were excluded from the analysis. Mean age of patients at HGD/EAC development was 69.0 years (SD 12), and the majority of HGD/EACs occurred in males (76%). Male patients were significantly younger at diagnosis of HGD/EAC than females (67±11 vs.74±11, $p<0.001$). This overall male predominance gradually disappeared with increasing age, as the rise in incidence rate of HGD/EAC in males leveled off over age 85, whereas in women, especially in those aged above 85, it continued to rise with age, especially in those aged above 85 (Figure 1 and 2).

Risk of high-grade dysplasia and/or esophageal adenocarcinoma

The distribution of detected HGD/EAC cases in relation to the duration of follow-up is shown in Figure 3. During the study period, 180 (0.4%) BE patients developed HGD/EAC within 6 months, and 32 patients (0.08%) within the subsequent 6 months after BE diagnosis. Another

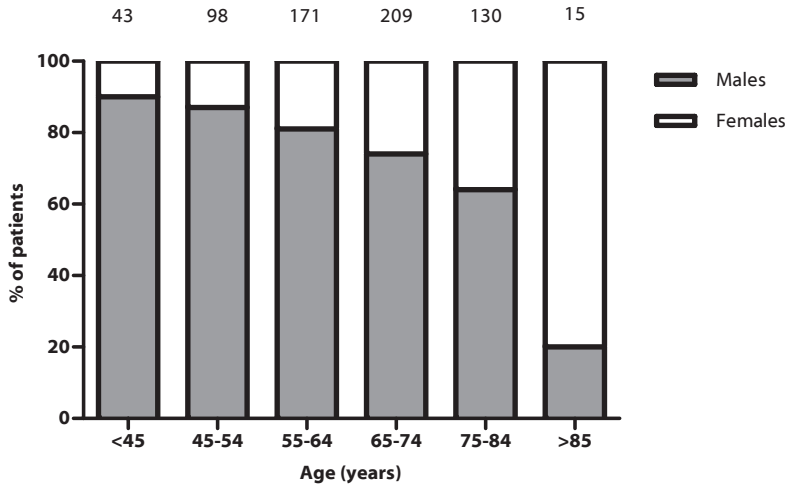

Figure 1. Age-distribution of 666 detected cases of HGD/EAC in 42.207 patients with Barrett's esophagus during the study period January 1991 till November 2007.
HGD: high-grade dysplasia, EAC: esophageal adenocarcinoma.

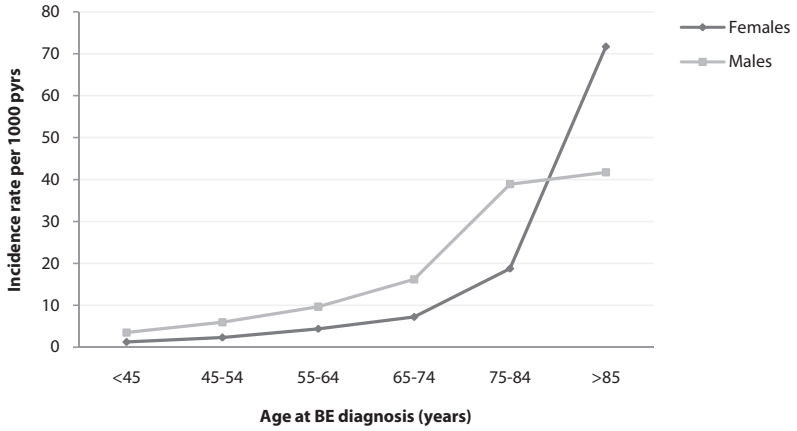

Figure 2. Age-specific incidence rates of HGD/EAC for males and females with a first diagnosis of BE.
HGD: high-grade dysplasia, EAC: esophageal adenocarcinoma.

215 patients (0.5%) progressed to HGD/EAC between the second and fifth year after BE diagnosis and the remaining 239 (0.6%) patients after 5 years. In the majority (*n*=357, 54%) of patients with malignant progression, HGD/EAC was already detected at the second endoscopy. In 53% of these patients, this second investigation with biopsy sampling was performed after more than one year after initial BE diagnosis, and in 21% after more than five years.

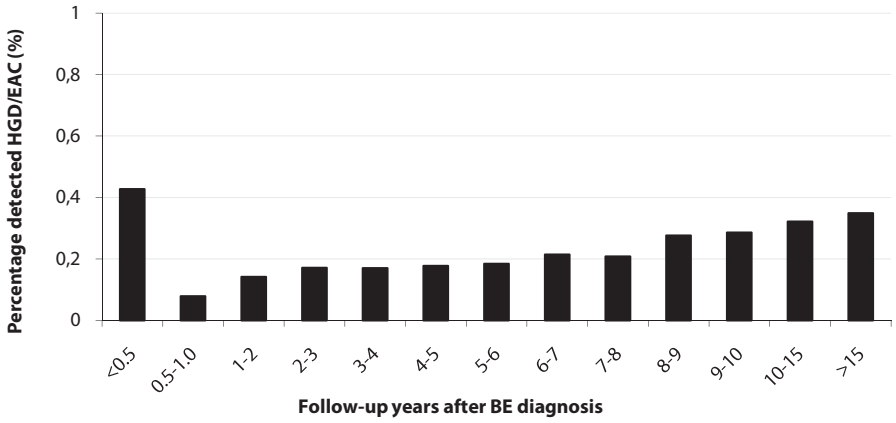

Figure 3. Time distribution of 666 detected cases of HGD/EAC in 42,207 patients with Barrett's esophagus during the study period January 1991 till November 2007 (number of cases relative to number of BE patients (%) with corresponding years of follow-up). HGD: high-grade dysplasia, EAC: esophageal adenocarcinoma.

The remaining 309 (46%) patients with malignant progression underwent one or more surveillance endoscopies with biopsies yielding negative for HGD/EAC, before being diagnosed with HGD/EAC. Patients with prior negative endoscopies were significantly younger at BE diagnosis than those in whom HGD/EAC was diagnosed at the second endoscopy (60±12 vs. 67±11, $p<0.001$). Furthermore, the mean latent period from BE to HGD/EAC was significantly longer in those with prior intermittent endoscopies as compared to those without (7.0±4 vs. 2.5±3, $p<0.001$).

After excluding prevalent cases of HGD/EAC (detection <1 year after BE diagnosis), crude EAC and combined HGD/EAC incidence rates among 16,333 BE patients with at least one re-evaluation endoscopy were calculated, on the basis of a total of 78,105 patient-years of follow-up (Table 2). Overall incidence rates per 1,000 pyrs at risk were 4.3 (95%CI: 3.4-5.5) for EAC and 5.8 (95%CI: 4.6-7.2) for HGD/EAC combined. Survival curves for BE patients (n=16,333) with or without baseline LGD are shown in Figure 4. Within 2, 5, and 10 years of follow-up after initial diagnosis, HGD/EAC was diagnosed in respectively 1%, 2%, and 6% of BE patients without baseline dysplasia, and in respectively 2%, 4% and 13% of BE patients with baseline LGD ($p<0.001$). Men with baseline LGD experienced a faster progression to HGD/EAC as compared to women (both $p<0.001$). The same difference was observed between men and women without baseline dysplasia ($p<0.001$).

For patients who did not undergo re-evaluation and were unlikely to have developed symptomatic EAC throughout the study period, life expectancy based on general survival

Table 2. Crude incidence rates of EAC and combined HGD/EAC in BE patients (*n*=16,333).

Variable	Follow-up (pyrs)	EAC cases (*n*)	HGD/ EAC cases (*n*)	EAC IR (per 1000 pyrs) (95%CI)	HGD/EAC IR (per 1000 pyrs) (95%CI)	EAC Annual risk (95%CI)	HGD/EAC Annual risk (95%CI)
BE all	78,105	337	454	4.31 (3.37-5.52)	5.81 (4.70-7.18)	0.43% (0.34-0.55)	0.58% (0.47-0.72)
Males	51,576	259	345	5.02 (3.80-6.65)	6.69 (5.25-8.53)	0.50% (0.38-0.67)	0.67% (0.53-0.85)
Females	26,529	78	109	2.94 (1.76-4.90)	4.11 (2.67-6.33)	0.29% (0.18-0.49)	0.41% (0.27-0.63)
BE ND	68,700	265	354	3.86 (2.93-5.09)	5.15 (4.06-6.55)	0.39% (0.29-0.51)	0.52% (0.41-0.66)
Males	45,217	210	274	4.64 (3.41-6.35)	6.06 (4.62-7.96)	0.46% (0.34-0.64)	0.61% (0.46-0.80)
Females	23,483	55	80	2.34 (1.27-4.30)	3.41 (2.06-5.66)	0.23% (0.13-0.43)	0.34% (0.21-0.57)
BE LGD	9,405	72	100	7.66 (4.50-13.1)	10.6 (6.77-16.7)	0.77% (0.45-1.31)	1.06% (0.68-1.67)
Males	6,359	49	71	7.70 (4.04-14.7)	11.2 (6.54-19.0)	0.77% (0.40-1.47)	1.12% (0.66-1.90)
Females	3,046	23	29	7.55 (2.95-19.3)	9.52 (4.12-22.0)	0.76% (0.30-1.93)	0.95% (0.41-2.20)

BE: Barrett's esophagus, EAC: esophageal adenocarcinoma, HGD: high-grade dysplasia, LGD: low-grade dysplasia, ND: no dysplasia, CI: confidence interval, pyrs: person-years, IR: Incidence rate.

data of the Dutch population was imputed. This rendered a total follow-up of 234,821 pyrs for the whole cohort of 42,207 BE patients. Overall incidence rates of EAC per 1,000 patient-years were 1.4 (95%CI: 1.2-1.6) for both sexes combined, 1.9 (95%CI: 1.7-2.1) for males and 0.8 (95%CI: 0.7-1.0) for females. For BE patients with baseline LGD, the EAC incidence rate rose to 3.6 per 1,000 patient-years (95%CI: 2.5-4.2), versus 1.2 (95%CI: 1.1-1.5) for those without baseline dysplasia ($p<0.05$).

Risk factors for malignant progression

Multivariate Cox regression analysis showed that male gender was independently associated with an increased risk of progression to HGD/EAC (HR 2.12, 95%CI: 1.70 – 2.65) (Table 3). In addition, older age as well as the presence of LGD at initial BE diagnosis were independently associated with development of HGD/EAC.

DISCUSSION

In this, to our knowledge, largest reported cohort of BE patients, the overall annual risk of cancer in BE was 0.4% (95%CI: 0.3-0.6), and the annual risk of cancer and high grade dysplasia combined only 0.6% (95%CI: 0.5-0.7). These figures are lower than previously published estimates.[17] In fact, the annual cancer risk decreased to 0.14%, in case cancer risk for all BE patients was analyzed, regardless of whether any follow-up was performed. Against this background, male gender, older age and LGD at initial BE diagnosis were identified as independent predictors for malignant progression.

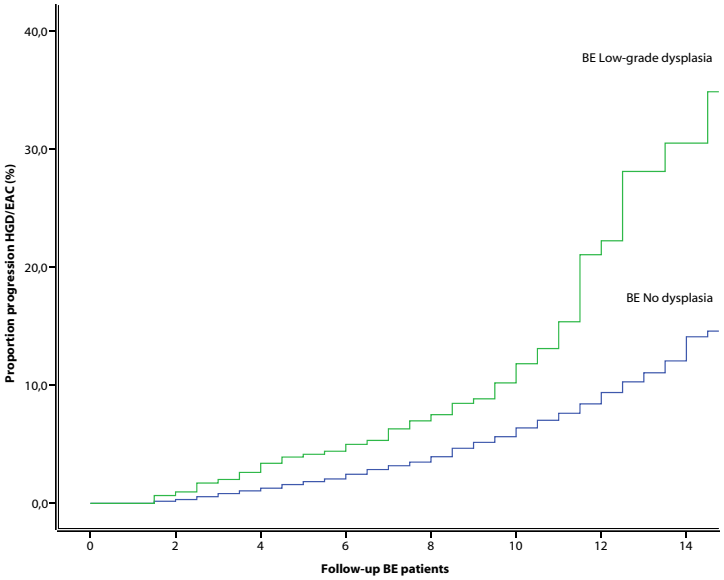

Figure 4. Progression rate to HGD/EAC in 16,333 patients with Barrett's esophagus.
HGD: high-grade dysplasia, EAC: esophageal adenocarcinoma.

Table 3. Risk factors for malignant progression in Barrett's esophagus (*n*=16,333).

Variable	HR univariate	95%CI	HR multivariate	95%CI
Sex				
Female	1.00	--	1.00	--
Male	1.58	1.27 – 1.96	2.12	1.70 – 2.65
Age				
<40 years	1.00	--	1.00	--
40-49 years	1.77	1.03 – 3.05	1.81	1.05 – 3.11
50-59 years	2.56	1.54 – 4.30	2.64	2.90 – 3.96
60 -69 years	3.32	2.00 – 5.51	3.57	1.58 – 4.41
70-79 years	8.77	5.27 – 14.6	10.0	5.99 – 16.7
>80 years	16.38	8.70 – 30.9	21.0	11.1 – 39.7
Histopathology				
BE with ND	1.00	--	1.00	--
BE with LGD	2.24	1.80 – 2.80	1.92	1.54 – 2.40

BE: Barrett's esophagus, ND: no dysplasia, LGD: low-grade dysplasia, HR: hazard ratio, CI: confidence interval.

To date, there have been four published systematic reviews on the incidence of cancer in BE.[17, 18, 32, 33] Our finding of an annual cancer risk of 0.4% in BE is lower than those reported in these studies. Several explanations may account for this discrepancy. Firstly, the population of our BE cohort consisted of a large number of unselected BE patients with long-term follow-up, from all hospitals in the Netherlands. Our cohort therefore reflects daily clinical ascertainment and management of BE. This nationwide registry minimizes selection bias, which is a particular drawback of small retrospective studies. This obviously differs from previous studies describing referral-based cohorts and from small studies with short follow-up, which may have included a biased selection of BE patients with high cancer risk. For instance, only three studies with over 1000 patient-years of follow-up were included in a review from the U.S.[18] A large study size results in lower reported cancer risks, as was also previously demonstrated in two systematic reviews.[17, 33] Another explanation for variations in outcome can be found in our strict exclusion of HGD and EAC cases occurring within the first year after initial BE diagnosis, and the exclusion of BE patients with HGD at baseline. Only two reviews excluded incident cancers occurring within the first year after initial BE diagnosis.[17, 33] The inclusion of prevalent cancers will obviously inflate cancer risk for patients with uncomplicated BE. In the most recently published review, annual cancer incidence decreased to 0.41%, after limiting the analysis to the occurrence of only incident cancers, which is in line with our estimate of annual cancer risk.[33]

Several authors have used mathematical models to explore the cost-effectiveness of BE surveillance.[14-16] U.S. researchers concluded that, for a cancer risk of 0.5% per annum, surveillance every 4 years was indicated and, if the annual risk was 0.4%, surveillance every 5 years was the only strategy that increased quality of life.[16] Others reported that screening 50-year-old men with GERD, followed by surveillance of those with dysplasia only, is probably cost-effective, but that surveillance of BE, even at 5-yearly intervals, is very expensive even though more QALYs may be gained.[15] According to a British study using an economic model, an annual cancer risk of 0.5% would mean that surveillance conferred less benefit and more costs than no surveillance at all, irrespective of the surveillance interval used.[14] The overall annual cancer risk obtained from our study is even lower than those incorporated in these models, especially with regard to the annual cancer risk of the whole cohort of BE patients (0.14%), including those who did not receive any histological follow-up, and who did in all probability not develop symptomatic EAC. Our findings indicate that both quality of life benefit and cost-effectiveness of Barrett's surveillance is highly questionable unless it can be targeted at those BE patients who are at the highest risk of cancer.

Despite the development of new cancer biomarkers, the presence and grade of dysplasia in random esophageal biopsies obtained at BE surveillance still remain the best indicators of cancer risk.[34] This was confirmed in our study by the fact that, despite its generally criticized lack of reproducibility, the initial diagnosis of LGD by a large variety of pathologists proved to have been a predictor of a twofold increased risk of malignant progression as compared to BE

patients without dysplasia. This was consistent with other studies.[35-41] It is likely that an even better accuracy of risk prediction may be achieved by a consensus LGD diagnosis of more than one pathologist.[37, 40] In addition, the extent of the spread of LGD has been suggested to be another significant risk factor for the development of EAC.[42]

Male gender and older age at initial diagnosis were identified as other important independent risk factors for progression to HGD/EAC. Moreover, men showed a significantly faster progression of BE to HGD/EAC as compared to women ($p<0.001$). This observation remained unchanged after stratifying patients by age. In addition, BE was diagnosed at a significantly older age in women. These findings are in agreement with others,[35, 43-50] and are in accordance with the male predominance in EAC incidence, as we have demonstrated previously.[44, 51, 52] This is compatible with the concept of women entering the carcinogenic cascade at an older age,[53, 54] and suggests that women progress less rapidly through subsequent stages of LGD and HGD. This observation has also been made in a recent study from Scotland.[55] However, currently age and gender have not been routinely included in planning BE surveillance programs.

Some limitations of our study warrant consideration. Firstly, as both sampling error and inter-observer variability in the interpretation of dysplasia between non-expert and expert GI pathologists exist,[56] misclassification of baseline dysplasia status in our patients could have influenced our results. However, differential misclassification to absence of baseline dysplasia would bias the difference between non-dysplastic and dysplastic BE patients towards the null value, thereby rather underestimating than overestimating cancer risk in BE patients with baseline LGD, without affecting the overall cancer risk. In addition, the very large number of BE patients in this study is likely to have compensated for biopsy sampling error and inter-observer variation. Secondly, as data on the length of the Barrett's segment, presence of hiatal hernia, presence of esophagitis, body mass index and use of selected medications were not available, cancer risk could not be stratified for these factors. Unfortunately, it was also impossible to collect information with regard to the indication of performing re-evaluation endoscopies in these patients. As a result, no clear distinction could be made between patients participating in a surveillance program and those undergoing re-evaluation endoscopies for investigation of symptoms. This could have led to an underestimation of our reported annual risk of cancer and HGD combined, as asymptomatic HGD cases might have been overlooked in this cohort. Nevertheless, we think that this has had only little influence on our findings, as a recent systematic review reported an incidence rate of cancer and HGD combined of 7.7 per 1,000 person-years, which is only slightly higher as compared to our findings.[33] Thirdly, in order to calculate progression rates to EAC for the whole cohort of patients, a virtual life expectancy was calculated for all patients without follow-up until November 2007, based on the life expectancy of the general population. This assumption may have led to a slight underestimation of cancer risk, as there is conflicting evidence of increased co-morbidity and mortality in BE patients as compared to the general population.[8, 9, 31, 57-59] However, as we

assumed a high, 16%, increased risk of all-cause mortality in patients with BE, as reported by Cook *et al.*[31], it is highly unlikely that this caused underestimation of our incidence rates.

In conclusion, in this large nationwide cohort of unselected patients with histologically confirmed Barrett's esophagus, the annual risk of malignant progression was lower than previously reported. This further undermines the cost-effectiveness of generalized BE surveillance, of which an even smaller minority of patients than previously estimated is likely to benefit. However, we have demonstrated three independent predictors of neoplastic progression enabling tailoring of surveillance programs towards a better selection of high-risk patients. Future large studies from other unselected populations should be performed to develop further risk stratification. A multicenter trial with randomization of patients to varying surveillance intervals based on age, gender and the presence and grade of dysplasia is urgently needed.

APPENDIX

PALGA diagnosis codes used in the analysis:
Barrett's esophagus: T62310M73330, M73320
Low-grade dysplasia: M74000, M74006, M74007
High-grade dysplasia and esophageal adenocarcinomas: M74008, M80003, M80011, M80101, M80102, M80103, M80104, M80105, M80123, M80193, M80203, M80213, M80413, M81403, M81404, M81453, M82003, M82113, M82603, M84303, M84803, M85603

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Chapter 7

Risk factors for the development of esophageal adenocarcinoma in patients with Barrett's esophagus

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ABSTRACT

Objective

To identify risk factors for esophageal adenocarcinoma (EAC) in patients with Barrett's esophagus (BE).

Methods

A hospital-based case-control study was performed in which 91 cases with EAC and 244 controls with histologically confirmed BE (>2 cm) with no dysplasia or low-grade dysplasia were included. Information on demographic, anthropometric, and lifestyle characteristics, physical activity levels, working posture, family history, gastroesophageal reflux disease (GERD) symptoms and medication use was collected by questionnaire.

Results

Cases more often were current smokers (odds ratio, 3.7, 95% confidence interval 1.4-9.9), more often had a body mass index >25 assessed at age 20 (2.6, 1.2-5.5), and more frequently had been working in a stooped posture at age 20 (2.0, 1.1-3.9), compared to controls. In addition, cases less often experienced symptoms of heartburn (0.3, 0.2-0.5) and less frequently used proton pump inhibitors (0.1, 0.05-0.2), compared to controls, whereas use of non-steroidal anti-inflammatory drugs/aspirin was more common among cases (1.8, 1.1-3.2). Cases more often were men, compared to controls (91% vs. 67%, $p<0.001$).

Conclusion

In patients with BE, the risk of EAC is related to risk factors for GERD, which is, however asymptomatic. As these risk factors are common in Western countries, they are probably not helpful in individualization of surveillance intervals.

INTRODUCTION

The incidence of adenocarcinoma of the esophagus and gastroesophageal junction has increased rapidly during the last two decades. This increase was mainly observed in the United States and Western Europe and was most pronounced in Caucasian men over 50 years of age.[1, 2] Up to about 1970, more than 95% of esophageal cancers were squamous cell carcinomas; however, currently, at least 60% of all esophageal cancers are adenocarcinomas.[3] Established risk factors for the development of esophageal adenocarcinoma (EAC) include chronic gastroesophageal reflux disease (GERD) and Barrett's esophagus (BE).[4, 5]

BE is characterized by the replacement of the squamous epithelium by metaplastic columnar epithelium as a consequence of chronic exposure to gastroesophageal reflux. This may lead to an incomplete form of intestinal metaplasia, named specialized intestinal metaplasia (SIM), which predisposes to the development of EAC.[5, 6] The excess risk of developing EAC in BE relative to the general population ranges between 30- and 60-fold.[7-10] Hence, surveillance endoscopy of patients with BE is advised at intervals based on the presence or absence and grade of dysplasia.[11]

Many clinicians consider this strategy to be justified in patients with BE, since cancers detected during surveillance endoscopy are more likely to be at an early stage compared to those cancers detected by upper gastrointestinal (GI) endoscopy in symptomatic patients.[12] However, the cost-effectiveness of surveillance is controversial since EAC is an uncommon cause of death in this patient population. In a cohort follow-up study from our department, only 5.6% of total mortality in BE patients was related to EAC.[9] Furthermore the incidence of BE is rapidly increasing, which augments the burden of surveillance.[13]

While risk factors for the development of EAC in the general population have been well investigated,[14] it is largely unknown which patients with BE have an increased risk for malignant progression. Age, hiatal hernia and length of the Barrett's segment are all established risk factors for development of EAC in BE.[15-17] Identification of additional risk factors for the development of adenocarcinoma, that could be obtained without endoscopy, could be of use to guide surveillance recommendations and thus improve cost-effectiveness of surveillance.

We conducted an epidemiologic investigation on associations between BE and EAC. The aim was to identify risk factors, that could be used to discriminate between low-risk and high-risk BE patients for the development of EAC.

METHODS

Patients

We performed a hospital-based case-control study in two university hospitals and five regional hospitals within the southwest of the Netherlands.

Between January 2003 and February 2005, all new diagnosed cases of adenocarcinoma of the distal esophagus in the Erasmus MC Rotterdam were enrolled. At least 90% of the patients with an incident EAC within the southwest of the Netherlands are referred to our clinic for an endoscopic ultrasound (EUS) examination. In order to reduce misclassification of cases with regard to the site of the tumor, all available pathology, surgery and endoscopy reports of the patients were centrally reviewed. Adenocarcinomas that were primarily located in the distal esophagus and had histological evidence of adjacent Barrett's epithelium were classified as EAC. If Barrett's epithelium was not detected, the tumor was classified by a team of an endoscopist, a gastrointestinal (GI) pathologist, and a surgeon, based on the location of the bulk of the tumor. Adenocarcinomas were classified as EAC if the lesion was located for at least 50% in the esophagus.[18] Patients with a squamous cell carcinoma of the esophagus, an adenocarcinoma of the gastric cardia or an adenocarcinoma of the corpus or antrum of the stomach were excluded.

BE patients were retrieved from the Erasmus MC Rotterdam, Ikazia Hospital Rotterdam, Sint Franciscus Hospital Rotterdam, Albert Schweitzer Hospital Dordrecht, IJsselland Hospital Capelle aan den IJssel and Free University Medical Center Amsterdam. These patients participate in a multicenter endoscopic follow-up study, currently being conducted, in which the diagnostic and prognostic value of baseline flow cytometry in relation to individualization of endoscopic surveillance intervals is being investigated. BE was defined as the presence of columnar cell metaplasia of the specialized type with characteristic goblet cells.[6] In order to be included as a control, the length of the Barrett's segment needed to be at least 2 cm with no or at most low-grade dysplasia on histological investigation. Exclusion criteria for the control group were BE with high-grade dysplasia, or the presence of cancer of the esophagus or stomach.

Biopsy specimens from BE patients were examined by a pathologist from the regional hospital where the BE patient was first identified. Then, the biopsy specimens were mailed to a GI pathologist from a panel of five experienced GI pathologists for review. If there was disagreement between the local and the expert GI pathologist on the histological diagnosis, another member of the panel, who was blinded to the previous findings, was asked for a review. Only if at least two pathologists agreed on the diagnosis (intestinal metaplasia with goblet cells, with or without dysplasia) a final diagnosis was made. Biopsy specimens from EAC patients were also examined by an expert gastrointestinal pathologist from the above-mentioned panel.

Data collection

All EAC patients and BE patients received a nonvalidated questionnaire that was developed specifically for this study (for details, see http://www.gastrolab.nl/pjdejonge.html). This self-administered questionnaire could be completed at home or in the hospital in less than 15 minutes. EAC patients received the questionnaire on the day that an EUS examination was performed. BE patients received the questionnaire within 1 year after having been included in the above-mentioned prospective follow-up study. Information was collected on demographic factors (age, gender, race), anthropometric characteristics (body mass index (BMI)), socioeconomic status (educational level), lifestyle (smoking habits, alcohol consumption, dietary patterns), physical activity levels (at work and free time), working in a stooped posture (*e.g.*, pavers and gardeners), personal and family history of GI symptoms and diseases, and medication use (proton pump inhibitors (PPIs), histamin-2 receptor antagonists (H2RAs), nonsteroidal anti-inflammatory drugs (NSAIDs) and aspirin). In addition, data on BMI, dietary patterns, physical activity levels, and working in a stooped posture were collected for different life time periods, *i.e.*, for the actual time period as well as for age 20 and for a period of 10 yr prior to filling out the questionnaire. Written informed consent was obtained from each subject. The study was approved by the Institutional Review Board of the Erasmus MC Rotterdam.

Statistical analysis

Chi-square tests and *t*-tests were used to compare the categorical and continuous characteristics. Logistic regression was performed to calculate odds ratios (ORs) with 95% confidence intervals (CIs), which were used as estimates of relative risks. In the baseline model, adjustments were made for age and gender. For each risk factor, other confounders considered were race, educational level (three levels), BMI (in quartiles), reflux symptoms, fruit intake (numbers of fruits/week: 0, <6, 6-10, >10), vegetable intake (numbers of vegetables/week: 0, <4, 4-6, >6), smoking status (never, former, current) and alcohol use (number of drinks/week: none, <5, 5-15, >15). These confounders were further selected by backward stepwise selection for a reduced multivariable model using the Akaike's Information Criterion (equivalent to $p<0.157$ for predictors with one degree of freedom).[19] This set of confounders was applied to all models. When the variable of interest was also present in the multivariable model, *e.g.*, smoking status, then it was left out from the analysis. Within domains of related variables, *e.g.*, smoking status and duration of smoking, we considered variables one by one.

Missing values of confounding variables occurred in 49 patients with 68 missings among 8 confounders (2%). These were imputed using the 'aregImpute' routine from the Hmisc library in S+ software (version 2000, Insightful Inc., Seattle WA). Analyses with exclusion of patients with missing values gave very similar results (not shown). Statistical analyses were conducted using SPSS software (SPSS 10.1, Chicago, Illinois, USA).

RESULTS

Ninety-one patients of 120 (76%) eligible patients with EAC (cases) and 244 patients of 350 (70%) eligible patients with BE (controls) filled out the questionnaire. Reasons for nonparticipation among cases were a poor clinical condition (*n*=6) or unwillingness to participate (*n*=23). Nonparticipation among controls most often was unwillingness to participate (*n*=102) rather than physical impediments (*n*=4). Baseline characteristics of the participants are shown in Table 1. Men presented 91% of the cases and 67% of the controls ($p<0.001$). All cases and almost all controls (99%) were Caucasian.

Table 1. Characteristics of participants.

Variable	Esophageal adenocarcinoma (cases)	Barrett's esophagus (controls)	*p*-value
Participants			
number enrolled	91	244	--
Age (years)			
mean (SD)	63 (10.9)	62 (11.7)	0.76
Male sex			
number (%)	83 (91%)	164 (67%)	<0.001
Years of schooling after primary school			
mean (SD)	4.8 (3.1)	4.5 (3.4)	0.43
Race			
Caucasian	91 (100%)	242 (99%)	0.54
Asian	0 (0%)	1 (0.4%)	
missing	0 (0%)	1 (0.4%)	
Current BMI (kg/m^2)			
mean (SD)	26 (5.7)	27 (2.9)	0.65
Fruit intake (numbers/ week)			
mean (SD)	8.3 (7.5)	9.0 (7.1)	0.44
Vegetable intake (numbers/ week)			
mean (SD)	5.7 (1.6)	5.9 (1.4)	0.31
Hot meals (numbers/week)			
mean (SD)	5.8 (1.8)	6.0 (1.4)	0.46
Physical activity (hours/week)			
mean (SD)	5.9 (8.2)	5.8 (8.5)	0.92

*Total percentages may not be 100 because of rounding.

***p*-value from chi-square tests/ *t*-tests

Tobacco smoking and alcohol consumption

After adjustment for age, gender, educational level, alcohol use and reflux symptoms, cases more often were current smokers (OR 3.7 (95%CI: 1.4-9.9)) than controls (Table 2). In addition, former smoking was also more common in cases than in controls (OR 2.6 (95%CI: 1.1-6.4)). Cases had more frequently smoked for a prolonged period of time compared to controls.

Table 2. Association between tobacco smoking and alcohol consumption, and esophageal adenocarcinoma in Barrett's esophagus.

Variable	Esophageal adenocarcinoma (cases) (percent)	Barrett's esophagus (controls) (percent)*	Age- and gender-adjusted OR (95%CI)	Multivariable adjusted OR (95%CI)**
Smoking status of cigarettes				
Non	8 (9%)	62 (25%)	1.0 (referent)	1.0 (referent)
Former	56 (62%)	139 (57%)	2.8 (1.2 – 6.7)	2.6 (1.1 – 6.4)
Current	26 (29%)	42 (17%)	4.5 (1.7 – 11)	3.7 (1.4 – 9.9)
			p=0.009	*p=0.032*
Duration of smoking				
None	8 (9%)	61 (25%)	1.0 (referent)	1.0 (referent)
1 – 20 years	17 (19%)	59 (24%)	2.2 (0.9 – 5.5)	2.2 (0.9 – 5.5)
21- 40 years	36 (40%)	85 (35%)	2.3 (1.0 – 5.4)	1.9 (0.8 – 4.8)
>40 years	27 (30%)	32 (13%)	5.3 (2.0 – 14)	4.7 (1.7 – 13)
			p=0.005	*p=0.017*
Alcohol use				
Never	7 (8%)	43 (18%)	1.0 (referent)	1.0 (referent)
Former	24 (26%)	34 (14%)	2.9 (1.1 – 8.0)	3.4 (1.2 – 10)
Current	58 (64%)	155 (64%)	1.3 (0.5 – 3.3)	1.6 (0.6 – 4.3)
			p=0.028	*p=0.036*
Duration of alcohol consumption				
None	7 (8%)	41 (17%)	1.0 (referent)	1.0 (referent)
1 – 20 years	5 (6%)	13 (5%)	2.1 (0.5 – 8.8)	2.4 (0.5 – 11)
21- 40 years	36 (40%)	83 (34%)	1.8 (0.7 – 4.7)	2.2 (0.8 – 6.3)
>40 years	38 (42%)	83 (34%)	1.4 (0.5 – 3.6)	1.6 (0.6 – 4.6)
			p=0.64	*p=0.50*

OR: odds ratio, CI: confidence interval.

*Total percentages may not be 100 because of missing values.

**Odds ratios for smoking status and duration of smoking were both adjusted for age, gender, educational level, alcohol use and reflux symptoms. Odds ratios for alcohol use and duration of alcohol consumption were both adjusted for age, gender, educational level, smoking status and reflux symptoms.

For example, the OR was 4.7 (95%CI: 1.7-13) for smoking more than 40 yr compared to non-smoking. There were no clear differences in alcohol use between cases and controls and no association with duration was found.

GERD symptoms and family history

After adjustment for age, gender, educational level, smoking status and alcohol use, cases experienced fewer symptoms of heartburn and regurgitation than controls (OR 0.3 (95%CI: 0.2-0.5) and OR 0.4 (95%CI: 0.3-0.8)), respectively (Table 3). When we combined heartburn and regurgitation, we still found a significant difference between cases and controls (OR 0.3 (95%CI: 0.2-0.5)).

In addition, cases less frequently had longstanding symptoms of reflux. For example, the ORs between cases and controls were 0.2 (95%CI: 0.1-0.5) and 0.4 (95%CI: 0.2-0.8), respectively, for symptoms of heartburn and regurgitation lasting more than 20 yr. A positive family history of regurgitation was also less common in cases than in controls (OR 0.4 (95%CI: 0.2-0.8)).

Use of PPIs and NSAIDs/aspirin

After adjustment for age, gender, educational level, smoking status, alcohol use, and reflux symptoms, cases had less frequently used PPIs (OR 0.09 (95%CI: 0.05-0.2)) than controls (Table 4). Moreover, cases less frequently used PPIs for a prolonged period compared to controls. For example, the OR between cases and controls was 0.05 (95%CI: 0.02-0.1) for using PPIs for at least 6 months.

After adjustment for age, gender, educational level, smoking status, alcohol use and reflux symptoms, cases more frequently used NSAIDs/aspirin (OR 1.8 (95%CI: 1.1–3.2)), but for a shorter period compared to controls. For example, the OR was 2.4 (95%CI: 1.3-4.4) for using NSAIDs/aspirin less than 6 months.

Gender, BMI, work in a stooped posture and educational level

The risk associated with BMI and work in a stooped posture was examined at different lifetime periods (Table 5). After adjustment for age, gender, educational level, smoking status, alcohol use, and reflux symptoms, cases more often had a BMI of at least 25 at age 20 (OR 2.6 (95%CI: 1.2-5.5)) than controls. BMI at age 20 could, however, be a less reliable measurement since it was collected from the distant past. We therefore also examined the association between a high BMI 10 yr prior to filling out the questionnaire and the risk of development of EAC. A significant association was also found for a BMI of at least 10 yr before filling out the questionnaire (OR 1.8 (95%CI: 1.1-3.3)).

After adjustment for age, gender, educational level, smoking status, alcohol use and reflux symptoms, cases more frequently had been working in a stooped posture at age 20 (OR 2.0 (95%CI: 1.1-3.9)) than controls. This association was also significant for working in a stooped

Table 3. Association between GI symptoms and family history of GI symptoms, and esophageal adenocarcinoma in Barrett's esophagus.

Variable	Esophageal adenocarcinoma (cases) (percent)	Barrett's esophagus (controls) (percent)*	Age- and gender-adjusted OR (95%CI)	Multivariable** adjusted OR (95%CI)
Heartburn				
No	43 (47%)	47 (19%)	1.0 (referent)	1.0 (referent)
Yes	47 (52%)	196 (80%)	0.3 (0.2 – 0.5)	0.3 (0.2 – 0.5)
Frequency of heartburn (# per week)				
0x	45 (50%)	48 (19%)	1.0 (referent)	1.0 (referent)
1x	7 (8%)	19 (8%)	0.4 (0.2 – 1.2)	0.5 (0.2 – 1.4)
2-3x	11 (12%)	60 (25%)	0.2 (0.1 – 0.4)	0.2 (0.1 – 0.5)
>3x	24 (26%)	111 (46%)	0.3 (0.1 – 0.5)	0.3 (0.1 – 0.5)
			p<0.001	*p<0.001*
Duration of heartburn				
None	45 (50%)	49 (20%)	1.0 (referent)	1.0 (referent)
<12 years	17 (19%)	83 (34%)	0.3 (0.1 – 0.5)	0.3 (0.1 – 0.6)
12- 20 years	12 (13%)	38 (16%)	0.4 (0.2 – 0.8)	0.4 (0.2 – 1.0)
>20 years	13 (14%)	67 (28%)	0.2 (0.1 – 0.5)	0.2 (0.1 – 0.5)
			p<0.001	*p<0.001*
Regurgitation				
No	61 (67%)	114 (47%)	1.0 (referent)	1.0 (referent)
Yes	29 (32%)	129 (53%)	0.5 (0.3 – 0.8)	0.4 (0.3 – 0.8)
Frequency of regurgitation (# per week)				
0x	62 (68%)	114 (47%)	1.0 (referent)	1.0 (referent)
1x	6 (7%)	15 (6%)	0.8 (0.3 – 2.2)	0.8 (0.3 – 2.5)
2-3x	4 (4%)	36 (15%)	0.2 (0.1 – 0.6)	0.2 (0.1 – 0.7)
>3x	17 (19%)	72 (30%)	0.5 (0.3 – 0.9)	0.5 (0.2 – 0.9)
			p=0.012	*p=0.011*
Duration of regurgitation				
None	63 (69%)	115 (47%)	1.0 (referent)	1.0 (referent)
<12 years	10 (11%)	53 (22%)	0.4 (0.2 – 0.9)	0.4 (0.2 – 0.9)
12-20 years	6 (7%)	24 (10%)	0.5 (0.2 – 1.2)	0.5 (0.2 – 1.4)
>20 years	10 (11%)	48 (20%)	0.4 (0.2 – 0.8)	0.4 (0.2 – 0.8)
			p=0.011	*p=0.019*

table is continued on the next page

Family history of heartburn				
No	30 (33%)	55 (23%)	1.0 (referent)	1.0 (referent)
Yes	36 (40%)	131 (54%)	0.6 (0.3 – 1.1)	0.7 (0.4 – 1.3)
Family history of regurgitation				
No	39 (43%)	63 (26%)	1.0 (referent)	1.0 (referent)
Yes	24 (26%)	108 (44%)	0.4 (0.2 – 0.8)	0.4 (0.2 – 0.8)

OR: odds ratio, CI: confidence interval.

*Total percentages may not be 100 because of missing values.

**Odds ratios for heartburn and regurgitation, frequency of heartburn and regurgitation, duration of heartburn and regurgitation, and family history of heartburn and regurgitation were all adjusted for age, gender, educational level, smoking status and alcohol consumption.

Table 4. Association between use of PPIs and use of NSAIDs/aspirin, and esophageal adenocarcinoma in Barrett's esophagus.

Variable	Esophageal adenocarcinoma (cases) (percent)	Barrett's esophagus (controls) (percent)*	Age- and gender-adjusted OR (95%CI)	Multivariable** adjusted OR (95%CI)
Use of PPIs				
No	44 (48%)	17 (7%)	1.0 (referent)	1.0 (referent)
Yes	43 (47%)	227 (93%)	0.08 (0.04 – 0.16)	0.09 (0.05 – 0.2)
Duration of PPI use				
None	44 (48%)	17 (7%)	1.0 (referent)	1.0 (referent)
< 6 months	17 (19%)	3 (1%)	2.7 (0.7 – 11)	2.6 (0.6 – 11)
> 6 months	18 (20%)	190 (78%)	0.04 (0.02 – 0.09)	0.05 (0.02 – 0.1)
			p<0.001	*p<0.001*
Use of NSAIDs/aspirin				
No	40 (44%)	136 (56%)	1.0 (referent)	1.0 (referent)
Yes	50 (55%)	108 (44%)	1.7 (1.0 – 2.9)	1.8 (1.1 – 3.2)
Duration of NSAID/ aspirin use				
None	40 (44%)	136 (56%)	1.0 (referent)	1.0 (referent)
< 6 months	36 (40%)	58 (24%)	2.3 (1.3 – 4.1)	2.4 (1.3 – 4.4)
> 6 months	13 (14%)	50 (20%)	0.9 (0.5 – 2.0)	1.1 (0.5 – 2.2)
			p=0.01	*p=0.17*

OR: odds ratio, CI: confidence interval, PPI: proton pump inhibitor, NSAID: non-steroidal anti-inflammatory drug.

*Total percentages may not be 100 because of missing values.

**Odds ratios for use of PPIs, duration of PPI use, use of NSAIDs/ aspirin and duration of NSAID use were all adjusted for age, gender, educational level, smoking status, alcohol use and reflux symptoms.

Table 5. Association between BMI, work in a stooped posture and educational level, and esophageal adenocarcinoma in Barrett's esophagus.

Variable	Esophageal adenocarcinoma (cases) (percent)	Barrett's esophagus (controls) (percent)*	Age- and gender-adjusted OR (95%CI)	Multivariable** adjusted OR (95%CI)
BMI at age 20				
<25	63 (69%)	184 (75%)	1.0 (referent)	1.0 (referent)
>25	20 (22%)	24 (10%)	2.7 (1.3 – 5.4)	2.6 (1.2 – 5.5)
BMI 10 years before questionnaire				
<25	29 (32%)	123 (50%)	1.0 (referent)	1.0 (referent)
>25	58 (64%)	107 (44%)	2.0 (1.2 – 3.5)	1.8 (1.1 – 3.3)
Work in a stooped posture at age 20				
No	24 (26%)	98 (40%)	1.0 (referent)	1.0 (referent)
Yes	51 (56%)	107 (44%)	2.1 (1.2 – 3.7)	2.0 (1.1 – 3.9)
Work in a stooped posture 10 years before questionnaire				
No	30 (33%)	109 (45%)	1.0 (referent)	1.0 (referent)
Yes	46 (51%)	97 (40%)	1.8 (1.1 – 3.2)	1.8 (0.9 – 3.3)
Educational level				
Primary school	19 (21%)	31 (13%)	1.0 (referent)	1.0 (referent)
High school	50 (55%)	158 (65%)	0.4 (0.2 – 0.8)	0.4 (0.2 – 0.9)
College/University	22 (24%)	53 (22%)	0.4 (0.2 – 1.0)	0.5 (0.2 – 1.2)
			p=0.034	*p=0.088*

OR: odds ratio, CI: confidence interval, BMI: body mass index.

*Total percentages may not be 100 because of missing values.

**Odds ratios for BMI, working in a stooped posture were adjusted for age, gender, educational level, smoking status, alcohol use and reflux symptoms. Odds ratios for educational level have been adjusted for age, gender, smoking status, alcohol use and reflux symptoms.

posture 10 yr before filling out to the questionnaire (age- and gender-adjusted OR 1.8 (95 % CI: 1.1-3.2)), but was not significant after multivariable adjustment.

After adjustment for age, gender, smoking status, alcohol use and reflux symptoms, high school education was less common among cases than controls (OR 0.4 (95%CI: 0.2-0.9)) (Table 5). However, college/university education was not significantly less common among cases (OR 0.4 (95%CI: 0.2-1.2)).

DISCUSSION

In order to make surveillance of BE more efficient, identification of high-risk groups for the development of EAC in BE is important. Although flow cytometry and molecular typing techniques might be able to distinguish between high-risk and low-risk groups,[20-22] identification of easily applicable and simple epidemiological factors could be of help in daily clinical practice.

This study identified risk factors that are associated with an increased risk of developing EAC. We found that tobacco smoking, a BMI above 25, work in a stooped posture and male gender were such risk factors. In addition, we also found significant differences in GI symptoms and family history of GI symptoms, and in PPI-use and NSAID/aspirin-use between EAC patients and BE patients. To our knowledge, only a few case-control studies have been reported in literature so far in which patients with BE instead of healthy control subjects were selected as controls.[23-25] In comparison to these studies, we investigated a considerable larger number of cases and controls. We collected data on several potential factors that could increase or decrease the risk of EAC development in BE. Moreover, our cases and controls were uniformly classified and strict and consistent criteria were used for the diagnosis EAC and BE. Finally, ORs were adjusted for the most relevant confounding variables.

Several case-control studies have investigated the role of smoking and alcohol consumption in the development of EAC.[23-28] Most of these studies concluded that the association with smoking was of moderate strength and that alcohol consumption was not associated with an increased risk of development of EAC. However, in only three studies patients with BE were selected as controls whereas in the other studies healthy subjects served as controls. Two of these three studies[23, 25] also reported that EAC patients more often smoked than BE patients without EAC. In contrast, another study[24] did not find such an association. The results of this third study were based on a limited number of cases and consequently probably a lack of power to detect a significant association.

The relationship between a high BMI and the risk of development of EAC has been reported previously in various case-control studies.[28-32] Lagergren *et al.*[29] found an OR of 16.2 for subjects with a BMI above 30 compared to subjects with a BMI less than 22 for the risk of development of EAC. In another study[31] the risk for development of EAC increased significantly with an increasing BMI. However, in none of these case-control studies was the association between a high BMI in BE patients and EAC patients compared. Risk comparison in those studies was performed on healthy control subjects. Hence, to our knowledge, our study is the first case-control study that shows that a BMI above 25 at different time periods in life increases the risk of development of EAC in BE.

Working in a stooped posture in the past increased the risk of development of EAC in BE patients as well. This is a new finding and has not been reported previously. It is highly plau-

sible that working in a stooped posture increases gastroesophageal reflux, which has been reported to play a role in the pathogenesis of both BE[5] and EAC[4].

It has well been recognized that there is a male and Caucasian race predominance among patients with EAC.[33] This is also confirmed in our study, in which cases were more often men compared to controls. Although it is difficult to make a valid conclusion on the effect of race based on our findings as the Dutch population consists of approximately 10% non-Caucasians, our data at least seem to support the fact that EAC is largely restricted to the Caucasian population.

The use of PPIs and the presence of GERD symptoms were more common in controls compared to cases (Table 3 and 4). It is important to emphasize that we should be careful to consider these findings as risk factors for EAC development. The results may be a consequence of selection bias, since participation was related to the presence of GI symptoms, both in cases (dysphagia) and in controls (heartburn, regurgitation). It has been reported that 40% of patients with EAC have no history of heartburn, while most known BE patients are seen initially for GERD symptoms.[4] This is also in agreement with our findings. Nonetheless, the ORs for use of PPIs between patients with EAC and BE also were highly significant, which may suggest a protective effect of PPIs in the development of EAC in BE. This is in agreement with one study[34] reporting a lower risk of developing dysplasia in BE patients receiving PPI therapy, compared to BE patients not being treated with PPIs. Further data, preferably from randomized clinical trials, are needed to show whether PPIs decrease the risk of EAC development in BE.[35]

As a last observation, we found that EAC patients more often had been using NSAIDs/aspirin compared to BE patients. This finding is in contrast to the literature, in which a protective effect of these drugs on the progression to EAC in BE patients has been reported.[36] Possibly, EAC patients experienced more pain compared to BE patients before a formal diagnosis of EAC was made. This is supported by the observation that EAC patients were more likely to have used NSAIDs/aspirin for only a short time period compared to BE patients. When examining the relationship between NSAID/aspirin use for at least 6 months and the risk of EAC development, a nonsignificant age- and gender-adjusted OR of 0.9 was found.

Several possible limitations of this study warrant consideration. First, our questionnaire was not validated. The results should therefore be interpreted with some caution. However, as we constructed the questionnaire, we made a great effort to minimize the possibility of ambiguities and misinterpretations of the questionnaire items in order to ensure valid responses. Second, we were not able to identify whether all EAC cases arose from preexisting BE, as BE was not always detectable at endoscopy when that EAC was diagnosed. It has been suggested that this is most likely due to tumor overgrowth.[37] This could have introduced a bias, as, according to our protocol, we hypothesized that EAC had evolved from Barrett's epithelium in those cases where at least 50% of the tumor was located in the distal esophagus. We believe, however, that this bias is only of limited magnitude because the predominant opinion pres-

ently suggests that most, if not all, EACs arise in Barrett's metaplasia.[38-40] The final proof for the presence of EAC without BE would be the finding of an EAC originating from nonmetaplastic columnar epithelium, *i.e.*, submucosal esophageal mucous glands. A number of such cases have been published in reports from the 1960s, each of which included only one case of EAC, suggesting that, at that time, this was the most common type of EAC.[41, 42] However, only two such cases have been published in the more recent literature.[43] This indicates that the impact of these tumors on current EAC epidemiology is probably negligible. Third, information bias, a well-known disadvantage of case-control studies, may have led to the collection of invalid data. However, this faulty recall is unlikely to be related to the presence of disease (*i.e.*, EAC and BE) and hence should be random. Therefore, our results probably show, at the most, somewhat diluted effects.[44, 45] Fourth, we were not able to define an index date, since this information for the diagnosis of BE in EAC patients was unknown. As in another study,[46] approximately 95% of patients with EAC were not known with a diagnosis of BE prior to a diagnosis of malignancy. Adjustment for age was performed to control for the natural course of EAC development in BE. However, no significant differences in age were present between EAC and BE patients. This is somewhat remarkable since the mean age of EAC patients is usually higher compared to that of BE patients. Fifth, nonparticipation among cases, especially among those who were too ill to complete the questionnaire, may have introduced bias. However, it is unlikely that their physical impediments were related to the risk factors that we studied. Their impediments were rather related to an advanced tumor disease. Finally, the risk of false-positive results (a type 1 error) may be increased by multiple statistical testing, and the power of our study may have been too limited to identify important risk factors.

In summary, our study showed that tobacco smoking, a BMI above 25 and working in a stooped posture in the past, as well as male gender and less frequent symptoms of GERD and PPI use increased the risk of the development of EAC in patients with BE. The clinical implication seems, however, not high as these factors are common in Western countries and therefore probably not helpful in individualizing surveillance intervals in BE patients.

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Chapter 8

Risk of colorectal cancer in patients with Barrett's esophagus: a Dutch population-based study

Submitted for publication

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ABSTRACT

Background

The association between Barrett's esophagus (BE) and colorectal cancer (CRC) is disputed. Population-based studies on the risk of CRC in BE are scarce.

Aim

To determine the risk of CRC in a Dutch nationwide cohort of BE patients in the Netherlands with long-term follow-up.

Methods

Patients diagnosed with BE between 1991 and 2006 were identified in the Dutch nationwide histopathology registry (PALGA). The incidence of CRC observed in these patients was compared with that in the general Dutch population aged over 40 years. Relative risks (RR) and 95% confidence intervals (95%CI) were calculated by a Poisson model.

Results

A total of 42,207 patients with a first diagnosis of BE were included. During a mean follow-up of 5.6 years (SD 4), 713 patients (1.7%) were diagnosed with CRC (overall rate 3.4/1000 person years at risk), at a mean age of 73.7 years (SD 10). All CRCs occurred in BE patients aged above 40 years, and the majority (96%) in those over 50 years of age. Of those CRCs, 317 (44%) were detected within the first year after initial BE diagnosis, and 396 (54%) thereafter. For all patients with BE, CRC risk was 1.70 (95%CI: 1.58-1.83), as compared to the general Dutch population aged over 40 years. CRC risk within the first year of follow-up after BE diagnosis (RR: 4.76 (95%CI: 4.26-5.31) was significantly higher as compared to one to five years of follow-up (RR: 0.99 (95%CI: 0.86-1.14) or more than five years of follow-up (RR: 1.28 (95%CI: 1.11-1.47) (p<0.001).

Conclusion

This population-based study shows an overall increased risk of CRC in patients with BE as compared to the Dutch general population, which cannot be solely attributed to the presence of diagnostic bias, as CRC incidence in BE patients at long-term follow-up was still significantly increased. Based on these findings, we suggest that screening colonoscopy could be recommended for male BE patients aged above 50 years, in case they do not already participate in other CRC screening programs.

INTRODUCTION

Barrett's esophagus (BE), presumably acquired through long-standing gastroesophageal reflux, is the cardinal precursor of esophageal adenocarcinoma (EAC).[1, 2] The excess risk of developing EAC in BE relative to the general population ranges between 30- and 60-fold.[3-5] In most Western countries, the incidence of EAC has increased rapidly over the past two decades and now comprises at least 60% of all esophageal cancer cases.[6-9]

Besides this acknowledged risk of EAC, BE has also been associated with an increased risk of colorectal cancer (CRC). In 1985, a 5.5% prevalence rate of malignant colonic neoplasms in patients with BE was reported, which appeared to be in excess of what would be expected in the general population.[10] However, since then, several studies addressing this issue have reported conflicting results, some of which confirmed the association while others did not.[11-21] These discrepancies may be explained by low BE patient numbers in most studies, lack of histologic confirmation in the endoscopic diagnosis of BE, diagnostic bias, use of an inappropriate control group or even its complete absence.

Elucidation of the proposed association between BE and CRC is important. If a true association exists, it would substantiate the demand for colonoscopy in BE patients, and it would provide a basis for searching common causal factors, possibly pointing to a genetic or environmental factor. High dietary fat and alcohol intake, as against reduced fruit and vegetable use, are environmental factors implicated in CRC and possibly in BE.[22, 23] In addition, several studies have implicated bile acids as a stimulus for the development of both BE and CRC.[24-26] Furthermore, some genetic abnormalities are common to both conditions, such as p53 mutations and allelic loss of chromosomes 5q, 17p, and 18q, which are associated with progression of the adenoma-carcinoma sequence, both in the human colon and in BE.[27, 28]

A theoretically desirable design for studying an association between CRC and BE would be a long-term follow-up study of CRC incidence among a large cohort of patients with BE. However, valid epidemiological data on CRC incidence in BE patients are scarce, and published studies have included relatively small numbers of patients with limited follow-up. In the present study, we addressed this question by using a large, nationwide cohort of Dutch BE patients with long-term follow-up.

METHODS

Histopathology database

In the Netherlands all histopathology and cytopathology reports are collected in a national archive (PALGA database), which encompasses all sixty-four pathology laboratories in the Netherlands. Since 1991, PALGA has had nationwide coverage and currently contains about 42 million excerpts from nearly 10 million patients.[29] Every excerpt in the database contains

encrypted patient identification, a summary of the original pathology report and diagnostic codes similar to the Systematized Nomenclature of Medicine (SNOMED) issued by the College of American Pathologists.[30] This diagnostic code contains a topological term, the type of sample, and a morphological term describing the finding, *e.g.,* 'esophagus*biopsy*intestinal metaplasia'. The PALGA morphology codes are identical to the codes in the International Classification of Diseases for Oncology (ICD-O-2; World Health Organization).[31] Details with regard to the number and intra-esophageal location of biopsies, or information on the indication for performing an endoscopic procedure are not uniformly registered. Each pathology report can, however, be traced to an individual patient with a unique identifier, allowing histological follow-up of individuals, irrespective of where subsequent biopsies were taken or resections were performed.[32] For each report, gender, date of birth, date of pathology review, summary text and diagnostic codes were made available. It was not, however, possible to access additional clinical data. The present study was based on data recorded in the PALGA database between 1991 and 2007.

Data collection

Patients with between 1991 and 2006 a first histologically confirmed diagnosis of BE with no dysplasia (ND) or at most low-grade dysplasia (LGD) were identified in the database. Codes that were used to classify a lesion as BE are described in the Appendix. Patients with a baseline diagnosis of high-grade dysplasia in BE were excluded, as were patients with either gastric or esophageal surgery or malignancy registered prior to or simultaneously with the first diagnosis of BE. For each patient, all pathology excerpts concerning colorectal biopsies from the first diagnosis of BE to the end of the study period (November 2007) were retrieved. Follow-up excerpts were scrutinized for codes indicating the presence of CRC.

Statistical analysis

Person-years of follow-up were calculated for each member of the cohort with censoring either on the date of diagnosis CRC or EAC, or on the date of death. However, date of death of patients registered in the PALGA database is not uniformly recorded, unless an autopsy has been performed, reports of which are also registered in the system. Censoring was therefore imputed to evaluate the number of person-years at risk for BE patients that did not develop CRC or EAC during follow-up. Survival data from the general Dutch population were collected, starting from age and gender of the patients, and calendar year (Dutch Cancer Registry 2007). A dataset with an approximately unbiased number of person years at-risk was subsequently created, by drawing from a binomial distribution for every year, as was done previously.[33, 34] Multiple imputation did not significantly change results. Finally, imputed survival estimates were corrected for an assumed 16% risk of excess mortality in BE patients, as was previously reported.[35]

The CRC incidence of the BE cohort was calculated as the total number of CRCs registered in the PALGA database within the cohort divided by the total number of person-years at risk. Calculation of the expected number of CRCs was based on age- and gender specific CRC incidence rates in the general Dutch population, as registered in the PALGA database from 1991 until 2007, and the midyear Dutch population.[36] Relative risks (RR) with 95% confidence intervals (CI) were calculated by a Poisson model, corrected for age categories, gender and calendar year. As some patients may be diagnosed with CRC shortly after a diagnosis of BE as a result of medical work-up, diagnostic bias may have influenced assessment of CRC risk in BE around the time of diagnosis. Therefore, CRC risks were calculated separately for the first year of follow-up, the second to fifth year of follow-up and the period subsequent to the fifth year of follow-up after the initial diagnosis of BE. Statistical analyses were conducted using S-PLUS (S-PLUS 6.0 Insightful Corp, Seattle, WA, USA) and SPSS software (SPSS 16.0, Chicago, IL, USA).

RESULTS

Patients

The study cohort consisted of 42,207 patients with a first diagnosis of BE registered between 1991 and 2006, with a 1.6/1.0 male to female ratio (Table 1). Males were significantly younger at diagnosis of BE as compared to women (mean age: 59±15 vs. 65±14 yrs, $p<0.001$).

Colorectal cancer risk in Barrett's esophagus

The cohort was followed for a mean of 5.6 years (SD 4), with a total of 234,821 follow-up years. During follow-up, CRC was diagnosed in 713 patients (1.7%). These cancers were pre-

Table 1. Baseline characteristics of the study cohort (42,207 patients).

Characteristic	Number (%)
Gender	
Male	25,890 (61)
Female	16,317 (39)
Age group (years)	
<40	3,616 (9)
40-49	6,050 (14)
50-59	9,396 (22)
60-69	9,803 (23)
70-79	9,192 (22)
>80	4,150 (10)

Table 2. Risk of colorectal cancer in Barrett's esophagus as compared to the general Dutch population aged above 40 years.

Variable	Person-years of follow-up	No. of CRCs	RR	95%CI
Overall	213,558	713	1.70	(1.58-1.83)
Gender				
Male	123,204	425	1.65	(1.50-1.81)
Female	90,354	298	1.79	(1.59-2.01)
Histology				
No dysplasia	194,208	651	1.73	(1.60-1.86)
Male	111,532	390	1.69	(1.53-1.86)
Female	82,676	261	1.79	(1.58-2.02)
Low-grade dysplasia	19,350	62	1.50	(1.17-1.93)
Male	11,761	35	1.33	(0.96-1.86)
Female	7,679	27	1.80	(1.23-2.62)
Age group (years)				
40-49	35,604	27	1.74	(0.92-3.31)
50-59	50,628	95	2.09	(1.64-2.65)
60-69	57,752	198	1.81	(1.55-2.12)
70-79	52,229	267	1.61	(1.43-1.82)
>80	17,344	126	1.65	(1.43-1.89)

CRC: colorectal cancer, RR: relative risk, CI: confidence interval.

dominantly found in the colon (79%) and the remainder in the rectum. All CRCs occurred in BE patients aged above 40 years, and the majority (96%) in those over 50 years of age. Mean age at CRC development was 73.7 years (SD 10), with a 1.5:1.0 male to female ratio. In addition, male BE patients were significantly younger at diagnosis of CRC as compared to women (72±10 vs. 77±9 yrs, $p<0.001$).

Crude incidence rates for CRC in male and female BE patients older than 40 years were 3.5 (95%CI: 3.1-3.8) and 3.2 (95%CI: 2.9-3.6) per 1000 person-years, respectively. The incidence of CRC increased with age, from 0.7 (95%CI: 0.3-1.8) in aged 40-49 to 5.8 (95%CI: 4.6-7.3) in aged>70. In Table 2, risk of CRC is stratified by age, gender, and baseline status of dysplasia. As compared to the general population aged above 40 years, the risk of CRC development was significantly elevated in both male (RR 1.65 (95%CI: 1.50-1.81)) and female BE patients (RR 1.79 (95%CI: 1.59-2.01)). However, no clear increasing trend could be demonstrated among the different age groups (p>0.05). In patients with ND at baseline and those with baseline LGD, RRs were respectively 1.73 (95%CI: 1.60-1.86) and 1.50 (95%CI: 1.17-1.93).

Exploration of diagnostic bias

Figure 1 shows the risk of CRC for specified periods of follow-up after initial BE diagnosis. For all patients with BE, the risk of CRC was 1.70 (95%CI: 1.58-1.83), as compared to the gen-

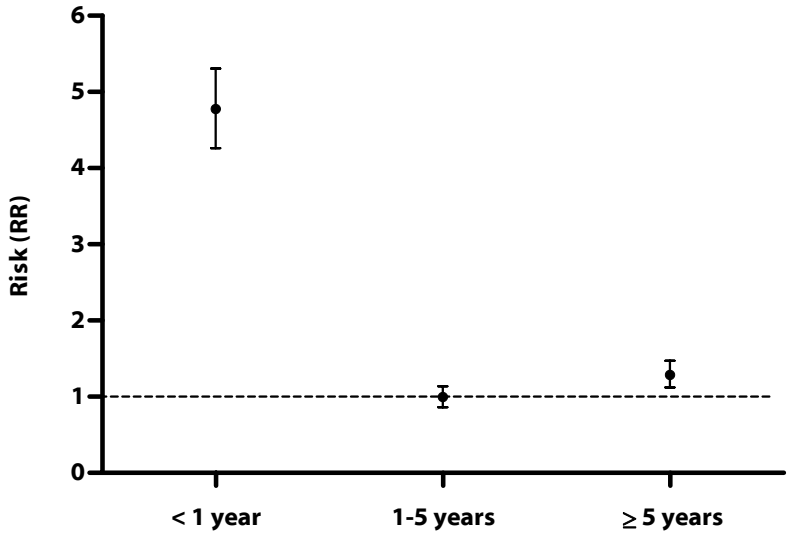

Figure 1. Relative risks (RR) of colorectal cancer in Barrett's esophagus for specified follow- up intervals, as compared to the general Dutch population aged above 40 years.

eral Dutch population aged over 40 years. However, the CRC risk was significantly higher within the first year of follow-up after BE diagnosis (RR: 4.76 (95%CI: 4.26-5.31), as compared to the risk in the period of one to five years of follow-up after BE diagnosis (RR: 0.99 (95%CI: 0.86-1.14) or more than five years of follow-up after BE diagnosis (RR: 1.28 (95%CI: 1.11-1.47) (p<0.001). The overall increased risk of CRC in BE patients after 5 years of follow-up was mainly due to an increased risk in males (RR 1.38 (95%CI: 1.17-1.64)), as CRC risk was not significantly increased in female BE patients as compared to the Dutch general population (RR 1.11 (95%CI: 0.88-1.40)).

DISCUSSION

In the absence of consistent supportive data, the association between BE and CRC has remained controversial. Should a true association exist, this would provide a basis for seeking shared environmental or common genetic markers. In addition, a definite establishment of a strong association between BE and CRC could indicate that BE patients should receive targeted CRC screening and surveillance, which might then well be more effective in cancer prevention than surveillance endoscopy of BE.[37]

This large population-based study indeed found an overall significant RR of 1.7 for the development of CRC in patients with BE, in comparison to the Dutch general population. This increased risk was significantly higher within the first year of diagnosis, as compared to one to five years of follow-up or more than five years of follow-up. These findings suggest that the overall increased risk of CRC in BE can in part be explained by the presence of diagnostic bias, *i.e.,* once having become a patient of a gastroenterology service, the chance of undergoing other gastroenterological procedures is greatly increased. Nevertheless, in patients with more than five years of follow-up, the risk of CRC was again significantly elevated as compared to that of the general population, in particular in male BE patients. Therefore, screening colonoscopy could be recommended for these patients, in case they do not already participate in other CRC screening programs. These findings also call into question whether colonoscopy would be a more worthwhile cancer screening procedure in BE than repeated surveillance esophagoscopy. The total number of CRCs (*n*=713, 1.7%) detected during follow-up of this cohort of BE patients was somewhat higher than the number of cases with either esophageal high-grade dysplasia (HGD) or adenocarcinoma (EAC) detected during follow-up (*n*=666, 1.6%). However, after exclusion of both cancers occurring within one year after initial BE diagnosis, the number of detected HGD/EAC cases (*n*=454, 1.1%) was now slightly higher than that of the detected CRCs (*n*=397, 0.9%), which suggests surveillance colonoscopy not to be a more rewarding approach than surveillance esophagoscopy in BE patients.

Our findings are in line with another population-based study[38] in which the incidence of CRC in BE patients was compared to that in the general population of Northern Ireland. The authors demonstrated an overall standardized incidence ratio of 1.46 (95%CI: 1.00-1.92), with declining RRs for cancers diagnosed on exclusion of the first month and 3 months after diagnosis of BE (1.09 and 0.94, respectively), suggesting that the increased risk of CRC in BE can be explained by diagnostic bias. However, the authors did not study CRC risk according to specified follow-up periods, as was done in our study. In addition, their results could not be interpreted unequivocally, as the relative short total length of follow-up was likely to have influenced the results. In addition, of all 39 patients that were diagnosed with CRC during follow-up, 16 (41%) had a known previous history of CRC, biasing the detected risk if these CRCs were treated as recurrences. Another study[39], in which incidence rates of esophageal, extra-esophageal, and colorectal cancers as well as cataract were studied in BE, esophagitis and reflux cohorts, identified within a General Practice Research Database, were compared to the general population, could not confirm an increased CRC risk in either of the cohorts. Unfortunately, the authors did not study CRC risk in relation to different periods of follow-up, to explore the temporal relationship between BE and CRC.

In addition to these population-based studies, the risk of CRC in BE has been the subject of many other smaller studies.[10-21, 38, 40] Most of these studies were either based on low patient numbers,[15, 17, 18] did not include a proper control group,[10, 20] or lacked histological confirmation of suspected BE.[11, 16, 38] A recent case-control study, which avoided these pitfalls, showed

veterans with BE, independent of their use of proton pump inhibitors (PPIs) or non-steroidal anti-inflammatory drugs (NSAIDs), to be at an increased risk of developing colorectal neoplasia.[19] However, the number of CRCs (1% of cases) was too small to allow a sub-analysis of CRC risk in BE patients. Other epidemiological approaches exploring the relationship between BE and CRC have involved the use of cancer registry data. One study, comparing the risk of developing CRC between patients with either EAC or esophageal squamous cell carcinoma, did demonstrate an increased risk of CRC among male EAC patients, whereas a reduced risk in women was found.[21] Another study, however, failed to confirm this common link between EAC and CRC, with neither increased EAC risks nor important gender differences being found among Swedish patients with CRC.[13]

As compared to the aforementioned studies, our present study has a number of specific strengths. Firstly, it was based on a nationwide sample of over 42,000 unselected BE patients, thus avoiding selection bias, which was a specific drawback of small referral-based cohorts. The interplay of differential admission rates from an underlying population to a particular study group can result in an artificial associations, also known as Berkson's fallacy.[41] Therefore, in tertiary and referral hospitals, both BE and colonic neoplasms are likely to occur together by chance in the same patient, as many patients have BE and many patients are likely to have colonic neoplasms.[42] Secondly, as this is, to our knowledge, by far the largest reported cohort of BE patients, CRC risk in BE could be estimated with greater accuracy than has previously been possible, with stratification of CRC risks according to age, gender and baseline dysplasia status of BE patients. In addition, its size enabled us to explore the temporal relationship between BE and CRC by performing risk analyses for different follow-up periods. Moreover, both by exclusively including histologically confirmed BE patients and by excluding patients who were known with CRC prior to or simultaneously with the first diagnosis of BE, we were able to provide more reliable risk estimates.

This study is not, however, without limitations. Firstly, owing to the absence of data about possible confounding variables such as PPI and NSAID use, no adjustments for these factors could be made within the multivariate analyses. Use of PPIs is known to raise serum gastrin levels, potentially increasing colorectal mucosa proliferation and promoting adenoma progression.[43-46] However, three recent large studies failed to confirm this association.[47-49] In addition, there is evidence that the use of NSAIDs has no influence on the association between BE and CRC.[19] Secondly, we imputed survival estimates of the BE population at risk, based on survival data for the general Dutch population. This method provides valid data, but ignores the alleged excess mortality of BE patients and may as such have led to an overestimation of number of patients years of follow-up and consequently, to an underestimation of the CRC risk. However, to overcome this issue, the imputed survival estimates were corrected for a hypothetical 16% risk of excess mortality, as was previously reported.[35]

In conclusion, this population-based study shows an overall increased risk of CRC in patients with BE as compared to the Dutch general population, which cannot be solely attributed to

the presence of diagnostic bias, as CRC incidence in male BE patients was still significantly increased after more than five years of follow-up. Based on these findings, we suggest that screening colonoscopy could be recommended for male BE patients aged above 50 years, in case they do not already participate in other CRC screening programs. The magnitude of the association between BE and CRC does, however, not merit a more extensive CRC screening strategy in BE patients than has currently been recommended for the general population.

APPENDIX

PALGA diagnosis codes used in the analysis:
Barrett's esophagus: T62310M73330, M73320
Low-grade dysplasia: M74000, M74006, M74007
Colon cancer: A T-code of format T67… combined with an M-code with format: M8…3, M8…9, M9…3, M8…9 and the first 4 digits of the M-code in the range: 8000–8004, 8010–8012, 8020–8022, 8030–8035, 8140, 8144, 8200–8201, 8210–8211, 8220–8221,8230–8231, 8240–8246, 8260–8263, 8480–8481, 8490, 8800, 8890–8891, 8894–8896, 9140, 9590–9593, 9595, 9670–9673, 9675, 9677, 9680–9682, 9684–9688, 9690–9691, 9693–9695,9697–9698, 9702–9705, 9711–9716, 9723, 9750, 9990.
Rectal cancer: A T-code of format T68… combined with an M-code with format: M8…3, M8…9, M9…3, M8…9 and the first 4 digits of the M-code in the range: 8000–8004, 8010–8012, 8020–8022, 8030–8035, 8050–8052, 8070–8075, 8140, 8144, 8200–8201, 8210–8211, 8220–8221, 8230–8231, 8240–8246, 8260–8263, 8480–8481, 8490, 8560, 8570–8573, 8720–8722, 8730, 8743, 8770–8772, 8775, 8800, 8890–8891, 8894–8896, 9140, 9590–9593, 9595, 9670–9673, 9675, 9677, 9680–9682, 9684–9688, 9690–9691, 9693–9695, 9697–9698, 9702–711–9716, 9723, 9750, 9990.

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Chapter 9

Proton pump inhibitors in gastroesophageal reflux disease decreases the esophageal immune response but does not reduce the formation of DNA adducts

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ABSTRACT

Background

Chronic esophageal inflammation and related oxidative stress are important in the pathogenesis of erosive esophagitis (EE) and its malignant progression.

Aim

To study the effect of proton pump inhibitors (PPI) on esophageal cellular immune response and oxidative damage in EE patients.

Methods

Forty GERD patients (NERD: 15, EE: 25) were included, after 7-days off anti-suppressive drugs. EE patients were randomized to 20 mg rabeprazole once daily for either 4 or 8 weeks with baseline and follow-up endoscopy with distal esophageal biopsies. T-lymphocytes, macrophages, and mast cells were quantified by immunohistochemistry. DNA adducts were measured by analysis of 8-oxo-deoxyguanosine (dG) levels.

Results

EE patients had more T-lymphocytes and CD8+ T-lymphocytes in squamous epithelium than NERD patients (p=0.001, respectively p=0.002). Levels of DNA adducts between both groups were, however, not different (p=0.99). Four- and eight-week rabeprazole treatment in EE patients resulted in significant decrease in number of T-lymphocytes and CD8+ T-lymphocytes (all p<0.05). PPIs did not, however, affect levels of DNA adducts.

Conclusion

Short-term PPI therapy in EE patients reduces the esophageal cellular immune response, but does not change oxidative damage. PPI therapy may therefore not be effective in reducing the risk of esophageal cancer in GERD patients.

INTRODUCTION

Gastro-esophageal reflux disease (GERD) is characterized by symptomatic reflux of gastroduodenal contents into the esophagus, which can result in mild-to-severe injury of the esophageal mucosa.[1] Patients with GERD can be categorized into those with non-erosive reflux disease (NERD), those with erosive esophagitis (EE), and those with complicated GERD, such as peptic stricture and Barrett's esophagus (BE).[2] Chronic GERD and BE are well-established risk factors for the development of esophageal adenocarcinoma (EAC),[3-5] which usually portrays a poor prognosis.[6] The incidence of EAC has increased dramatically since the 1970s, at a rate faster than any other type of cancer.[7] A parallel increase in incidence of detected BE in the general population predicts an even further increase in the incidence of EAC.[8] As a consequence, there is growing interest in potential chemopreventive strategies that can effectively reduce the risk of developing EAC.

Profound acid suppression with proton pump inhibitors (PPIs) has been suggested to be important in the prevention of EAC. Whether PPI therapy in GERD patients can prevent the development of intestinal metaplasia of the distal esophagus as a first step in the cascade that can lead to EAC is still unclear. Some cohort studies of patients with GERD demonstrated that long-term PPI therapy cannot prevent the development of BE;[9, 10] other studies indicate that acid suppression does slow down and possibly prevents progression of this process.[11-13]

Insight into mechanisms leading to esophageal injury is important to elucidate the suggested chemopreventive action of PPI therapy. Although these mechanisms are still poorly understood, an important role in the progression of GERD has been attributed to the esophageal immunoregulatory environment and response to oxidative stress. Several studies suggest that differences exist in the cytokine profile within the esophageal mucosa of patients with BE and those with EE,[14-16] *i.e.,* BE is characterized by Th-2 anti-inflammatory cytokines (IL-10 and IL-4), and EE by an increase in pro-inflammatory cytokines (IL1β, IL-8 and IFN-γ).[15] These observations are substantiated by others demonstrating that the inflammatory response is shifted from a cellular immune response towards a more pronounced humoral immune response when EE progresses to BE.[14] This immune response is thought to facilitate the progression towards neoplasia, as a humoral environment promotes angiogenesis, and may contribute to the malignant propensity of BE.[17, 18]

As a consequence of chronic esophageal inflammation, reactive oxygen species (ROS) are generated, which in the presence of an imbalance between pro-oxidants and anti-oxidants promote oxidative stress.[19] Indeed, increased levels of the pro-oxidant myeloperoxidase and decreased levels of glutathione, a potent antioxidant, have been observed in patients with EE and BE.[20] Furthermore, oxidative stress may result in formation of DNA adducts, which can initiate and promote carcinogenesis. Increased levels of DNA adducts have been revealed along the metaplasia-dysplasia-adenocarcinoma sequence in Barrett'sepithelium.[20-22]

So far, in spite of the existence of a strong link between chronic inflammation and oxidative stress, the inflammatory profiles and oxidative stress in GERD have not been studied simultaneously. Moreover, little is known about the effect of profound acid suppression on these processes. Therefore, in this study, we characterized the esophageal cellular-mediated immune response and levels of DNA adducts in GERD patients in parallel, and assessed to what extent these processes are affected by PPI therapy *in vivo*.

METHODS

Patients and study design

Between November 2005 and September 2007, all consecutive patients with typical GERD symptoms in the Erasmus MC, Ikazia Hospital, and Sint Franciscus Gasthuis, Rotterdam, were invited to participate in this study. Typical symptoms of GERD were defined as the presence of heartburn and/or acid regurgitation. The GERD-HRQL questionnaire was used to evaluate the frequency and intensity of symptoms and their impact on patient's quality of life. Only patients with a score ≥ 12 were included in this study.[23, 24] Patients presenting with predominantly atypical GERD symptoms, such as chronic cough and noncardiac chest pain, were excluded. In addition, patients with a history of upper GI surgery, peptic stricture, duodenal or gastric ulcer, bleeding diathesis or coagulopathy, stroke or ischemic attack, significant GI bleed within the past 6 months, or the presence of esophageal varices were excluded as well.

All patients underwent upper GI endoscopy with biopsies and subsequent wireless 48-hr pH monitoring, in order to characterize them as nonreflux or GERD patients with either NERD or EE. The latter two patient categories were compared with regard to the number of inflammatory cells and levels of local oxidative damage in the distal esophagus. To assess the effect of PPI therapy on these processes, patients with EE were randomized to either rabeprazole treatment (20 mg once daily) either for 4 weeks or for 8 weeks, and underwent a follow-up endoscopy with biopsy specimens at the end of treatment. All participants signed an informed consent form. The study was approved by the Institutional Review Boards of the Erasmus MC, Ikazia Hospital and Sint Franciscus Gasthuis, Rotterdam.

Endoscopy

Baseline upper GI endoscopy was performed after at least a seven-day period off anti-suppressant medication. During endoscopy, the squamo-columnar junction (SCJ) was identified and the distance between the incisors and SCJ was measured. The distal part of the esophagus was evaluated to determine the absence or presence of mucosal injury and a sliding hiatus hernia. The extent of mucosal inflammation was determined using the Los Angeles (LA) Classification System.[25] Subsequently, 2-4 biopsy specimens were obtained from the distal esophagus 1 cm above the SCJ. In EE patients, care was taken to sample these biopsies from

non-eroded sites. In addition, two biopsies from the antrum and 2 biopsies from the corpus of the stomach were taken for assessment of *Helicobacter pylori* gastritis.

In patients from the Erasmus MC, additional biopsies from the distal esophagus 1 cm above the SCJ were obtained for determination of DNA adducts. Biopsies for histology and immunohistochemistry were fixed in 10% buffered formalin solution. Biopsies for determination of DNA adducts were immediately frozen and stored at -80°C.

Wireless pH monitoring

After the upper GI endoscopy was completed, a BRAVO pH delivery system (Medtronic, Minneapolis, MN, USA) was introduced orally, and the pH capsule was positioned 6 cm proximal to the SCJ according to standard procedures. Proper functioning of the BRAVO probe was confirmed by a reading of pH >4 immediately after placement.

All studies were intended to be performed for 2 days (off PPI), during which time pH data were received and stored in the receiver. Patients were encouraged to engage in their usual activities and were asked to keep a diary documenting food intake, periods of sleep, and occurrence of symptoms.

Acid exposure time (AET) was defined as pathologic if the proportion of time with a pH <4 exceeded 4.4%.[26] Patients were considered to have NERD when a positive symptom association probability (SAP> 95%) was found on 48-hr pH monitoring.[27] Those NERD patients with an abnormal AET (% time pH<4 exceeding 4.4%) and a positive SAP were further defined as NERD pH positive, while those without abnormal AET but with a positive SAP were defined as NERD pH negative.

Histology and immunohistochemistry

Biopsy specimens obtained during the baseline and follow-up endoscopy were serially sectioned at 4 µm, mounted on adhesive slides, dried overnight at 37°C and deparaffinized with xylene. The first of these serially sectioned slides was stained with haematoxylin and eosin (H&E), and evaluated by an experienced pathologist (HvD) for presence of microscopic esophagitis according to established criteria.[28, 29] Using these criteria, the following lesions were considered compatible with reflux disease of increasing severity: (1) basal layer hyperplasia, (2) elongation of papillae, (3) dilation of papillary vascular spaces, (4) intraepithelial inflammatory infiltration, (5) mucosal erosion and (6) granulation tissue. For practical purposes, inflammation was graded as chronic (*i.e.,* esophagitis grade 1, in the presence of criteria 1–3), chronic active (*i.e.,* esophagitis grade 2, in the presence of criteria 4 with or without criteria 1–3), or eroding ulcerating (*i.e.,* esophagitis grade 3, in the presence of criteria 5 or 6).[30]

For immunohistochemistry, antigen retrieval was performed by boiling the deparaffinized samples in either 10 mM monocitric acid buffer (pH 6.0) for 10 min or in 10mM/1mM TRIS/EDTA (pH 9.0) for 10 min. Then, samples were slowly cooled down to room temperature (RT). Prior to staining, endogenous peroxidase activity was blocked by either incubating the slides

in a 0.3% solution of H_2O_2 in methanol or in a 40mM/150mM citrate-phosphate buffer for 20 min at RT. The samples were blocked with 10% rabbit non-immune serum and 10% normal human plasma in PBS for 30 min at RT. Sections were stained using antibodies specific for cells representing the cellular-mediated immune response: T-lymphocytes (CD-3, Dako, Glostrup, Denmark), CD8+ T cells (CD-8, Dako), macrophages (CD-68, Dako) and mast cells (anti-tryptase, Dako). For T-lymphocytes and CD8+ T-cells, biotin-labelled rabbit-anti-mouse antibody (Dako) was used as a second antibody, followed by the addition of a streptavidin-horseradish peroxidase complex (Dako) using 3-amino-9-ethylcarbazole as substrate. For visualisation of macrophages and mast cells, biotin-labelled rabbit-anti-mouse antibody (Dako) was used as a second antibody, followed by the addition of a streptavidin-alkaline phosphatase complex (Dako) using new-fuchsin as substrate. Reactive lymph nodes were used as positive controls.

Following the recommendations of Wang *et al.*[31], the number of immune cells was quantified in the most densely populated field in the squamous epithelium of biopsy specimens, using HPF examination (magnification 400x). Counting was performed by two independent investigators (PdJ, KvZ), blinded to patient characteristics, treatment regimen and endoscopic findings. In total, six HPFs were counted and means and standard deviations (SD) were subsequently calculated.

Detection of oxidative DNA damage in esophageal mucosa

Oxidative DNA damage was quantified by measurement of 8-hydroxy-2-deoxyguanosine (8-OHdG), a known pro-mutagenic DNA adduct. Biopsy specimens were homogenized with a potter (1000 rpm) in 1% SDS/1mM EDTA/10 mM TEMPO and solutions thus obtained were incubated overnight at 37°C with 0.5mg Proteinase K. After the incubation period, DNA was isolated in the presence of 8-hydroxyquinoline to prevent artificial 8-oxo-dG, by means of repetitive extraction with phenol/chloroform/isoamyl alcohol (25:24:1) and chloroform/isoamyl alcohol (24:1). Subsequently, DNA was precipitated with two volumes of 100% cold ethanol and 1/30 volume of 3M sodium acetate (pH 5.3). Precipitated DNA was rinsed with 70% ethanol, dissolved in 2mM Tris (pH 7.4) and stored at -20°C until analysis. HPLC-ECD analysis of 8-oxo-dG was performed as described previously.[32] Briefly, after extraction, DNA was digested into deoxyribonucleosides by treatment with nuclease P1 [0.02U/ml] and alkaline phosphatase [0.014U/ml]. The digest was then injected into a Gynkotek 480 isocratic pump (Gynkotek, Bremen, Germany) coupled with a Midas injector (Spark Holland, Hendrik Ido Ambacht, the Netherlands) and connected to a Allsphere ODS-2 5i column (250 x 4.6 mm) (Altech) and an electrochemical detector (Antec, Leiden, the Netherlands). The mobile phase consisted of 10% aqueous methanol containing 94mM KH2PO4, 13mM K2HPO4, 26mM NaCl and 0.5mM EDTA. Elution was performed at a flow rate of 1.0 ml/min with a lower detection limit of 40 fmol absolute for 8-OHdG, or 1.5 residues/10^6 2'-deoxyguanosine (dG). dG was

simultaneously monitored at 260 nm. The 8-oxo-dG concentration was expressed as the ratio of 8-oxo-dG/10^6 dG.

Statistical analysis

Continuous data were compared using the Wilcoxon rank-sum test and the Kruskal-Wallis test for independent samples, and the Wilcoxon signed-rank test for paired samples. Categorical data were analyzed using the chi-squared test and Fisher exact test. Correlations between variables were evaluated by Pearson correlation analysis. Two-sided statistical significance was set at $p<0.05$. Statistical analyses were conducted using SPSS software (10.1, Chicago, IL, USA).

RESULTS

Patients

Fifty-eight of 89 eligible patients with typical GERD symptoms were included in this study (66%), the remaining were not included for reasons of unwillingness to undergo pH monitoring ($n=7$), unwillingness to undergo a follow-up endoscopy ($n=18$) or other reasons ($n=6$). Of those included, 9 patients (16%) were not eligible for final analysis either because their histological specimens were inadequate ($n=4$) such as lacking squamous epithelium in biopsies taken across the SCJ, or because the pH studies were incomplete ($n=5$) because of early dislodgement of the pH capsule. Nine patients (16%) were withdrawn from the study because of non-adherence to the study protocol.

Of the remaining 40 GERD patients, 25 had EE (Grade A: 13, B: 8, C: 4) confirmed by endoscopy, and 15 patients presented with NERD. Of those NERD patients, 5 presented AET within the normal range and were defined NERD pH-negative; 10 patients presented a pathological AET and were defined as NERD pH-positive. Baseline characteristics of the GERD patient groups are shown in Table 1. No differences were detected in age and gender. Patients with EE, however, had a significantly higher BMI than NERD pH- patients. Although GERD-HRQL scores were similar between the three groups, EE and NERD pH+ patients had significantly higher levels of intra-esophageal acid exposure as compared to NERD pH- patients (Table 2).

Effect of PPI therapy on the cellular immune response

At histology, 20 of 25 (80%) EE patients had esophagitis grade 1, three (12%) esophagitis grade 2 and one (4%) patient grade 3. Of all NERD patients, grade 1 esophagitis was found by histology in 12 (80%), whereas grade 2 esophagitis was present in 2 (13%) patients (Table 2). No statistically significant difference was found in prevalence of *H. pylori* gastritis between NERD and EE patients (13% vs. 16%, $p=0.819$).

Table 1. Demographic characteristics of patients.

Variable	NERD pH- (*n*=5)	NERD pH+ (*n*=10)	EE (*n*=25)	*p*-value*
Age (mean ± SD)	34 (13)	49 (13)	46 (15)	0.187
Gender (% female)	4 (80)	7 (70)	13 (52)	0.384
Smoking (%)	2 (40)	3 (30)	10 (40)	0.852
Alcohol consumption (%)	3 (60)	6 (60)	20 (80)	0.390
BMI (mean ± SD)	24 (1)	25 (3)	28 (5)	0.042
GERD-HRQL-score (mean ± SD)	20 (6)	21 (8)	22 (8)	0.678

NERD: non-erosive reflux disease, EE: erosive esophagitis, BMI: body mass index, GERD-HRQL: gastroesophageal reflux disease health-related quality of life.

*p-value from Kruskal-Wallis tests/χ^2-tests

Table 2. Endoscopic and histologic characteristics of patients.

Variable	NERD pH- (*n*=5)	NERD pH+ (*n*=10)	EE (*n*=25)	*p*-value
Mean % time acid exposure (mean ± SD)	1.7 (2)	8.7 (5)	9.5 (6)	0.006
Endoscopy (%)				
Normal	5 (100)	10 (100)	--	--
Grade A	--		13 (52)	
Grade B	--		9 (36)	
Grade C	--		3 (12)	
Grade D	--		0 (0)	
Histology (%)				
Normal	0 (0)	0 (0)	1 (4)	0.947
Grade 1	4 (80)	8 (80)	20 (80)	
Grade 2	1 (20)	1 (10)	3 (12)	
Grade 3	0 (0)	1 (10)	1 (4)	
Helicobacter pylori infection (%)	1 (20)	1 (10)	4 (16)	0.855

NERD: non-erosive reflux disease, EE: erosive esophagitis.

*p-value from Kruskal-Wallis tests/χ^2-tests

Results of immunohistochemically stained sections of esophageal biopsies from NERD and EE patients are shown in Figure 1. The inflammatory infiltrate in esophageal squamous epithelium of GERD patient groups mainly consisted of T-lymphocytes. Patients with EE had significantly higher numbers of T-lymphocytes in the squamous epithelium than NERD pH- patients (29±2 vs. 10±1, p<0.001), and NERD pH+ patients (29±2 vs. 22±4, p=0.02). The mean number of T-lymphocytes was also higher in patients with NERD pH+ compared to NERD pH- patients, although not statistically significant (p=0.08). CD8+ T-cells were the predominant

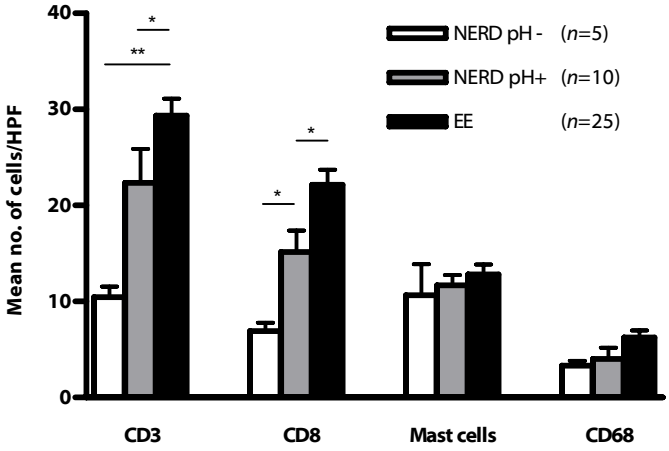

Figure 1. Graphic representation of mean number T-lymphocytes (CD3), CD8+ T-lymphocytes, mast cells and macrophages (CD68) in distal esophageal biopsy specimens from GERD patients (*p<0.05, **p<0.01).

subpopulation of T-lymphocytes in all three GERD groups, with an increasing CD8+/CD3+ ratio from NERD pH- to EE (NERD pH-: 0.67±0.06, NERD pH+: 0.68±0.02, EE: 0.75±0.02).

The number of T-lymphocytes correlated significantly with the endoscopic grade of esophagitis (r=0.42, p=0.002), but did not correlate with the grade of microscopic esophagitis (r=0.15, p=0.37) nor with levels of intra-esophageal acid exposure (r=0.18, p=0.28). Patients with EE, NERD pH+ and NERD pH- patients did not differ with regard to the number of mast cells, nor were any differences detected in the mean number of macrophages in the squamous epithelium.

In 10 patients with EE (Grade A: 5, B: 3, C: 2), randomized to a 4-week treatment with 20 mg rabeprazole once daily, a significant reduction in the cellular-mediated immune response in the distal esophagus was seen (Figure 2A). PPI therapy led to a significant decrease in the number of T-lymphocytes and of CD8+ T-lymphocytes in the squamous epithelium (28±2 to 11±1, p=0.005, and 22±2 to 7±1, p=0.005, respectively). In addition, the number of mast cells and macrophages in the squamous epithelium also decreased upon rabeprazole treatment (13±2 vs. 9±1, p=0.074, and 5±0.4 vs. 3±1, p=0.005, respectively). Mean post-treatment levels of these cell types were similar to those determined in NERD pH- patients at baseline. A similar response was seen in 10 EE patients (Grade A: 6, B: 2, C: 2) randomized to a 8 week treatment regimen (Figure 2B) (all p<0.05). Although the mean decrease in number of immune cells was larger in the group treated for 8 weeks, the difference between 4 and 8 weeks treatment was statistically not significant. In addition, the presence of *H. pylori* gastritis in EE patients did not influence the change in esophageal immunological response during PPI therapy (data not shown).

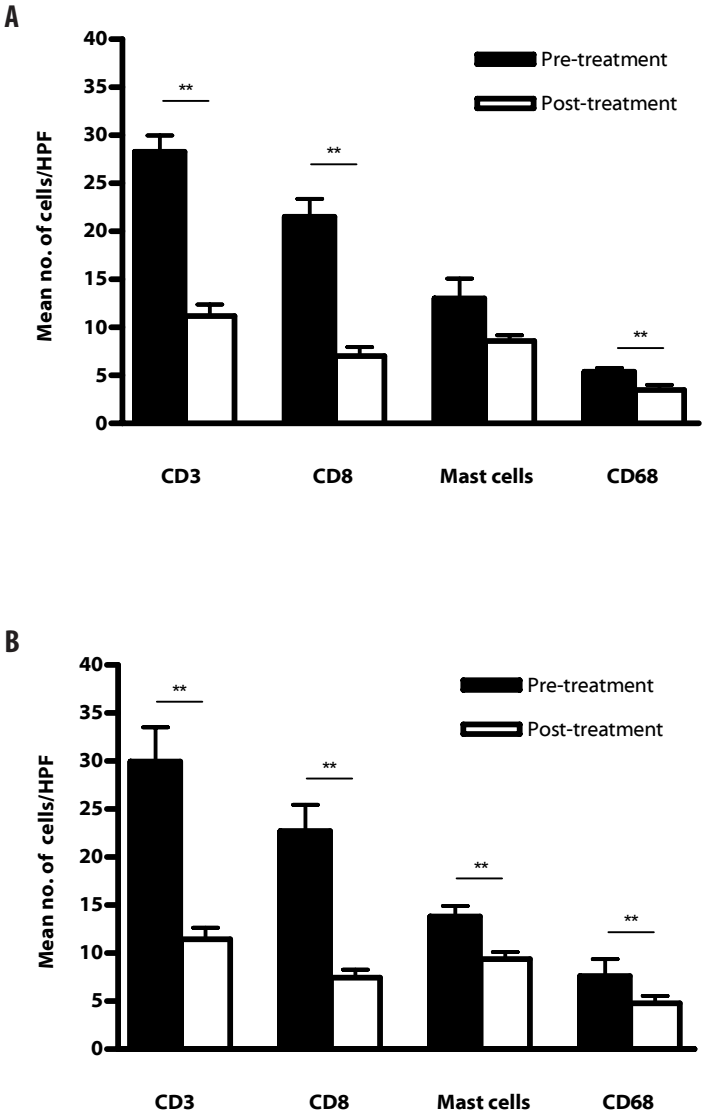

Figure 2. Effect of PPI therapy on the esophageal immune response. Mean number of T-lymphocytes (CD3), CD8+ T-lymphocytes, mast cells and macrophages (CD68) in the distal esophagus in (a) patients with erosive esophagitis (EE), before and 4 weeks after treatment with rabeprazole (20 mg once daily), and in (b) patients with EE, before and 8 weeks after treatment with rabeprazole (20 mg once daily); **p<0.01.

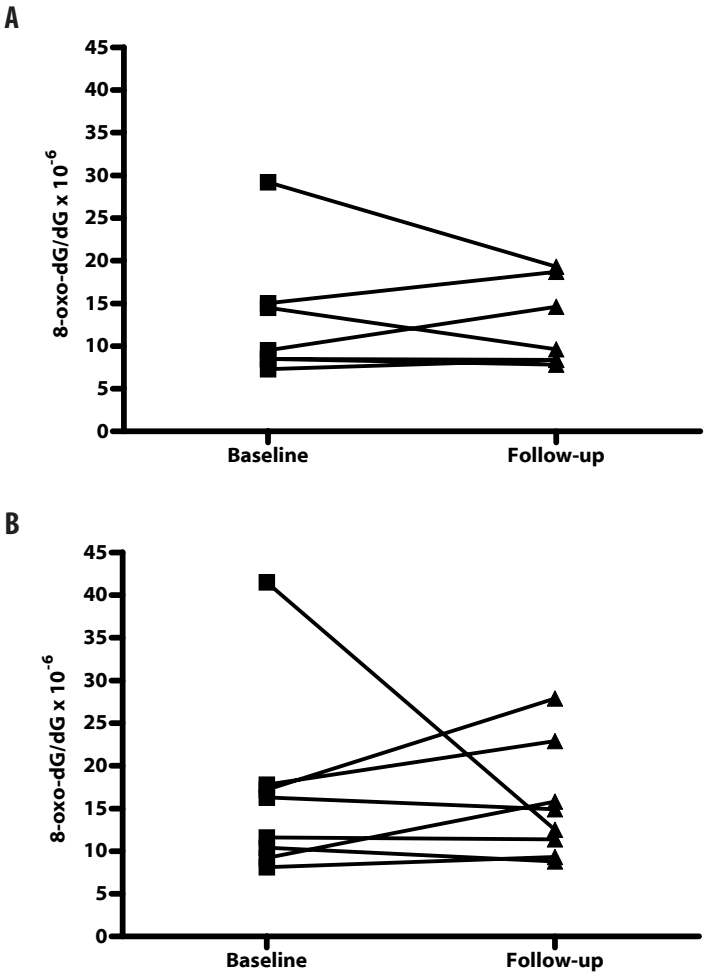

Figure 3. Effect of PPI therapy on esophageal oxidative DNA damage. 8-oxo-dG levels (as a measure of oxidative DNA damage) in (a) the distal esophagus in patients with erosive esophagitis (EE), before and 4 weeks after treatment with rabeprazole (20 mg once daily), and in (b) the distal esophagus in patients with EE, before and 8 weeks after treatment with rabeprazole (20 mg once daily).

Effect of PPI therapy on oxidative DNA damage

In a subset of GERD patients (11 NERD, 17 EE), we determined the level of DNA adducts in the distal esophagus. The stepwise increase in the number of immune cells within the GERD spectrum, detected at immunohistochemistry, did not, however, reflect in an increased formation of DNA adducts in NERD pH+ and EE patients, as compared to NERD pH- patients (NERD pH-: 15±4, NERD-pH+: 14±3, EE: 14±2, p=0.97).

In 7 patients with EE, randomized to a 4 week treatment with 20 mg rabeprazole once daily, no differences in the amount of DNA adducts between pre-treatment biopsies and post-treatment biopsies were detected (13±3 vs. 12±2, p=0.94) (Figure 3A). Rabeprazole treatment for a period of 8 weeks also did not result in decreased formation of DNA adducts in EE patients (n=8) (17±4 vs. 15±2, p=0.84) (Figure 3B).

DISCUSSION

To our knowledge, this is the first study in GERD patients where the esophageal inflammatory infiltrate was characterized by and correlated to levels of local oxidative damage before and after PPI therapy. We found an increased cellular-mediated inflammatory infiltrate in the esophageal epithelium of patients with EE and NERD pH+ compared to NERD pH- patients. This infiltrate mainly consisted of CD8+ T-lymphocytes, whereas macrophages and mast cells represented only a minor proportion of the inflammatory infiltrate. Despite the stepwise increase in the number of immune cells demonstrated within the GERD spectrum, similar amounts of DNA adducts were found in EE and NERD patients. Acid suppression with PPI therapy resulted in a significant drop in the number of inflammatory cells within the distal esophageal mucosa, especially esophageal T-lymphocytes and their CD8+ T-cell subpopulation; however, levels of DNA adducts remained unaffected.

The role of profound acid suppression with PPIs in the prevention of BE and EAC in patients with GERD is still controversial. Evidence exists that PPI therapy in BE reduces esophageal acid exposure,[33] decreases mucosal cell proliferation and increases differentiation,[13] and possibly reduces the length of Barrett's segment and dysplasia incidence.[11, 12, 34] This seems to be in contrast with cohort studies on long-term PPI therapy, which indicate that this therapy cannot prevent the development of BE.[9, 10] Furthermore, another cohort study including 417 patients with BE did not find any effect of 4 years omeprazole treatment on the incidence of EAC.[35] To elucidate the possible chemopreventive properties of PPIs, understanding of the mechanisms important in the pathophysiology of EE and its progression towards BE and EAC is necessary. The distinct inflammatory environment within the GERD spectrum has been proposed to be an important factor in the pathophysiology of esophageal mucosal injury, and may be critical in carcinogenesis.[14, 15] We previously showed that when EE progresses to BE, the inflammatory response is shifted from a cellular-mediated immune response towards a more pronounced humoral immune response.[14] Inhibition of the mechanisms responsible for this shift has been suggested to play an important role in the prevention of carcinogenesis in GERD.

In this study, PPI therapy resulted in a substantial decrease in the cellular-mediated immune response in the distal esophagus, both in EE patients treated for 4 weeks and in those treated for 8 weeks (Figure 2). Esophageal T-lymphocytes and their CD8+ T-cell subpopula-

tion were predominantly affected by acid suppression. Mean post-treatment levels of these immune cells were similar to those determined in NERD pH- patients at baseline.

The finding of increased numbers of esophageal T-lymphocytes in pre-treatment biopsies is in agreement with other studies.[31, 36-40] Similar to the findings of Geboes *et al.*[36], the majority of esophageal T-lymphocytes possessed the CD-8 suppressor T-cell phenotype, probably including a subset of cells with cytotoxic potential. The significance of this lymphocytic infiltration in GERD is yet unclear, although several hypotheses have been proposed. One hypothesis that could account for the increased number of T-lymphocytes seen in esophagitis is that gastric acid and bile acids may modulate epithelial surface antigens, which are subsequently recognized by the immune system as foreign. Alternatively, the presence of T-lymphocytes in GERD may be related to the general inflammatory reaction, resulting in the release of cytokines known to induce nonspecific activation of cytotoxic T-lymphocytes.[41] However, others have shown that T-lymphocytes follow infiltration by acute inflammatory cells, particularly at the site of metaplastic foci.[42] Furthermore, T-cell infiltrates are predominantly seen in persistent areas of BE following endoscopic ablation therapy, suggesting that lymphocytes may be important in the maintenance of the metaplastic tissue.[43, 44] It is thus likely that T-lymphocytes play an important role in the pathogenesis of BE. Therefore, the sharp drop of particularly esophageal T-lymphocytes in EE patients observed after PPI therapy, suggests that profound acid suppression in GERD may be capable of inhibiting the immunological shift along the EE-BE sequence and may be important in the prevention of carcinogenesis in GERD.

One of the consequences of chronic esophageal inflammation is the induction of oxidative stress by production of ROS.[45] Irritation of epithelial cells by gastroduodenal contents followed by excess production of ROS by inflammatory cells drawn to this area has been shown to contribute to EE and to the development of BE.[19] Furthermore, excess production of ROS has been shown to result in formation of DNA adducts, which play a major role in the induction of spontaneous mutations, a prerequisite for carcinogenesis in GERD.[46] As effective acid control decreased the amount of reflux, DNA damage as a result of chronic esophageal inflammation may be reduced. In this study, however, we showed that PPI therapy did not affect the formation of DNA adducts in EE, either in those patients treated for 4 weeks, or in those treated for 8 weeks (Figure 3). In addition, despite the stepwise increase in the number of immune cells from NERD to EE patients, no differences were detected in the level of DNA adducts between both patient groups at baseline. The latter finding is in agreement with a study, in which mean DNA adduct levels were similar between reflux patients with and without endoscopic esophagitis, but significantly higher than those in healthy controls without symptoms.[20] Levels of DNA adducts determined in our GERD patient groups were equivalent to those determined in another recent study in BE patients with dysplasia and patients with EAC. Moreover, these levels were again significantly higher as compared to those determined in controls.[47] This suggests that oxidative DNA damage plays an important role in the pathogenesis of GERD, in the development of BE and its progression towards EAC and is in line with

the reported inverse link between intake of anti-oxidants and risk of EAC development.[48] In addition, as the amount of DNA base changes related to oxidative stress depends on DNA repair mechanisms,[49] it may well be that key genes involved in DNA repair are malfunctioning within the GERD spectrum.

Although we cannot rule out completely that we failed to detect any effect of PPI therapy on esophageal mucosa DNA-damage either because the PPI dosage used was too low or the treatment follow-up period (8 weeks) was too short, this is unlikely as the cellular-mediated immune response in EE patients was already substantially decreased after 4 weeks of PPI treatment. This decrease is probably accompanied by a reduction in local ROS production, but apparently without much effect on DNA damage. Furthermore, a recent study showed that fundoplication, which can be very effective in normalizing reflux, was not capable of decreasing DNA damage in the distal esophagus of GERD patients, after a follow-up for 6 months.[50] This suggests that in GERD a subpopulation of transformed cells with defective mechanisms against DNA-damage is present, which could be responsible for constant presence of DNA adducts despite acid suppression. This supports the finding that even long-lasting acid suppression therapy does not alter malignant transformation of BE, and may partially explain why EAC is found even after successful medical and surgical therapies for GERD.[51]

Some limitations of our study warrant consideration. First, the number of patients with NERD included in this study was relatively small. However, statistically significant changes between and within GERD patient groups could be found and results obtained are in agreement with others. Second, we did not include healthy controls without symptoms and without evidence of esophageal injury. Third, we did not perform pH monitoring after 4 or 8 weeks of PPI treatment and therefore we do not exactly know the amount of esophageal acid exposure at these time points. However, the significant decrease in the cellular-mediated immune response detected after PPI therapy indicates that acid-suppression was effective in our patients, in the absence of additional medical interventions.

Strengths of our study include its prospective randomized study design and systematic data collection. In addition, GERD patients were uniformly classified with both endoscopy and wireless pH monitoring. Moreover, for the first time, the cellular-mediated immune response and amount of oxidative DNA damage were studied simultaneously and the effect of acid suppression on these processes was evaluated prospectively.

In conclusion, we have shown that PPI therapy in GERD patients reduces the esophageal cellular-mediated immune response, thereby possibly inhibiting the progression of GERD towards BE. However, short-term PPI therapy is insufficient to reverse DNA damage caused by gastroesophageal reflux, suggesting that PPI therapy is perhaps not effective in the prevention of carcinogenesis because of persisting DNA damage. The relative contribution of these contrasting effects of PPI therapy to the prevention of EAC warrants further study with long-term follow-up.

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Chapter 10

General discussion and conclusion

INTRODUCTION

With the rising incidence and overall poor prognosis of esophageal adenocarcinoma (EAC),[1, 2] Barrett's esophagus (BE) remains an area of great interest as it is the principal precursor lesion, found in the vast majority of EAC cases.[3, 4] The cascade of gastroesophageal reflux disease (GERD) to BE and ultimately EAC seems to offer potentially attractive targets for screening programs in order to decrease mortality and improve survival related to EAC. Endoscopic screening of subjects with chronic GERD symptoms has been proposed as a method to detect BE and early cancer.[5] Patients with BE are then typically enrolled into surveillance programs in order to detect neoplastic lesions at an early, curable stage. However, the efficacy of both screening and surveillance of BE remains a strongly debated issue, as there is insufficient evidence to show that these strategies improve survival and are cost-effective. Epidemiological dilemmas with regard to screening for BE are mainly that the at-risk group is too broadly characterized, and that too many cancers occur outside of this risk pool. With regard to surveillance endoscopy, the natural history of BE is largely unknown, and the ability to predict who is at the highest risk for progression remains poor. Other problems with current screening and surveillance techniques include test invasiveness, high costs, and sampling errors. Nevertheless, despite these shortcomings, surveillance endoscopy in BE is widely practiced. Improved identification of groups at risk for BE and EAC, as well as the development of more accurate and less invasive methods of diagnosis could improve the effectiveness of screening and surveillance in BE patients, and achieve the ultimate goal of reducing EAC mortality.

In this thesis, we aimed to reassess the yield of screening for and surveillance of BE in the prevention of EAC, by exploring the natural course of BE, by investigating various risk factors involved in the progression of chronic GERD to BE and finally to HGD or EAC, and by examining the value of non-invasive techniques in the identification of high risk groups. In this chapter, the main findings of this research and their clinical implications in the context of ongoing research are discussed, and directions for future research are highlighted.

MAIN FINDINGS

Epidemiology of Barrett's esophagus

As the ascertainment of BE in the general population is low, true population-based estimates of the prevalence and incidence of BE are difficult to obtain.[6, 7] Nevertheless, increases in the incidence and prevalence of BE have been reported, although it has often remained unclear whether this reflected a true increase, a greater awareness of BE, increased detection of short segment BE (SSBE), or increased use of esophagogastroduodenoscopy (EGD).[8-10] Exploring the factors which continue to drive the increased incidence of this disease is essential with regard to improved BE and EAC prevention. Recently, a study based on data from a gen-

eral practitioner database reported on an increase in BE incidence in the Netherlands, which could not be explained by a rise in the number of performed EGDs.[11] However, other explanations remained unproven. In order to achieve greater certainty about the existence of a secular rise in the incidence of BE and to explore its causes, we analyzed data from a nationwide registry of pathology reports (PALGA) recorded between 1991 and 2006 **(Chapter 2)**. The constancy of the age and gender specific esophageal biopsy rates was investigated, and age-period-cohort analyses were performed. This study showed a substantial increase in the number of new patients diagnosed annually with BE in the Netherlands during the 16-year study period, predominantly affecting males. The annual increase in BE incidence significantly exceeded the annual increase in number of patients with a first esophageal biopsy, pointing to a true increase in BE incidence. The increase in BE may anticipate a further increase of approximately 35% in males and 13% in females in the incidence of EAC in the coming decade. Birth cohort effects were demonstrated for both genders, indicating that this rise in BE incidence could not be solely attributed to an increased awareness of BE by endoscopists, but was for the larger part explained by altered circumstances for the general population after World War II. This was also reported by van Soest *et al.*[11], who showed that the increase in incidence was most pronounced among males under 60 years of age. Although the increasing prevalence of obesity, the decreasing occurrence of *Helicobacter pylori,* changes in the use of medications that cause GERD, and greater use of nitrogenous fertilizers, might all be factors that have contributed to the increasing incidence of BE and EAC, the sex distribution of these factors does not match the male predominance of both conditions. However, abdominal obesity, especially visceral obesity, explains a number of epidemiological features of BE, as it more common in men, and has been associated with GERD symptoms in Caucasians, but not in African Americans or Asians.[12] Others have shown abdominal diameter measured as waist circumference to be a risk factor of BE, independent of BMI.[13, 14] In addition, visceral adipose tissues are strongly associated with increased serum levels of interleukin 6 (IL-6), tumor necrosis factor α (TNF-α) and leptin,[15, 16] which have been been shown to stimulate cell proliferation and inhibit apoptosis in Barrett's-derived EAC cells.[17] In aggregate such factors tend to augment both inflammation and malignant transformation in patients with GERD. These findings, combined with the increased prevalence of visceral obesity in men, suggest that obesity is one of the major factors that continues to drive the increased incidence of BE and EAC in the general population, particularly in males, and partly explains the overall male predominance in BE and EAC.[18-20]

Risk factors for esophageal and gastric cardia adenocarcinoma

The dramatic increase in the incidence of EAC in the Western world has coincided with a rise in the incidence of gastric cardia adenocarcinoma (GCA),[2] which suggests that common risk factors account for both disorders. So far, the close proximity of EAC to GCA around the gastroesophageal junction, the identical staging and treatment protocols for both malignancies

and the fact that tumor location is not an independent risk factor with regard to prognosis have made that these cancers are regarded as one disease entity.[21-23] More recent epidemiological data from the U.S. and the Netherlands investigating time trends with regard to these two malignancies, however, showed a declining incidence rate of GCA during the last decade,[24, 25] suggesting differences in risk factor profiles between EAC and GCA patients. We conducted a study in which we investigated the distributions of environmental risk factors among patients with EAC, GCA, using patients with esophageal squamous cell carcinoma as the control group **(Chapter 3)**. The majority of the evaluated risk factors did not show differences between EAC and GCA patients. Patients were similar with regard to male predominance and age at the time of diagnosis. In addition, no differences were found in alcohol intake and smoking, use of fruits and vegetables, body posture and occupational activities. Nonetheless, patients with EAC more often experienced symptoms of heartburn and had more often longstanding symptoms of heartburn compared with GCA patients. Our findings are in line with those from other studies, showing an association between the history of reflux symptoms and GCA, but that this association is much weaker than that between reflux symptoms and EAC.[3, 26] This would therefore suggest that only a relatively small proportion of cardia cancers might be attributed to gastroesophageal reflux. A recent case-control study investigated the association between adenocarcinoma of the different regions of the upper gastrointestinal tract and atrophic gastritis and GERD symptoms.[27] The authors showed that cardia cancer was positively associated with both severe gastric atrophy and with frequent GERD symptoms, although the latter was only apparent in the intestinal type of cardia cancer. These findings indicate that intestinal subtype tumors with non-atrophic gastric mucosa and frequent GERD symptoms are highly likely to be of esophageal origin, whereas intestinal subtype tumors with atrophic gastric mucosa and less frequent GERD symptoms are likely to be gastric in origin. A more precise assessment of GERD and a more accurate determination of the presence of gastric atrophy may result in a better distinction between gastric and esophageal origins of cardia cancers.

Role of non-invasive screening techniques for Barrett's esophagus

Apart from the difficulties in identifying a target population, as mentioned previously, widespread screening for BE is hampered by the fact that endoscopy is expensive, invasive and associated with a small, but significant risk of complications. Alternative preferably non-invasive methods could potentially enhance the acceptability of BE screening and improve compliance. Esophageal capsule endoscopy (CE) has attracted interest in its use for BE screening, as it is less invasive than conventional EGD, can be performed without sedation and has a low complication rate. Studies reporting on the application of CE for esophageal disorders, however, have shown conflicting results.[28-31] Poor visualization of the Z-line and inconvenience of capsule ingestion for the patient in the supine position were suggested as explanations for these variations. In **chapter 4**, we evaluated the operating characteristics of CE for the

detection of esophageal mucosal disorders using a new ingestion protocol. CE identified the presence of erosive esophagitis and BE with a high degree of accuracy, and had a high negative predictive value. The new ingestion protocol significantly improved visualization of the Z-line. Furthermore, nearly all patients preferred CE over EGD, and experienced less discomfort and pain during CE as compared to EGD. These findings indicate that CE is an accurate method for detecting esophageal mucosal abnormalities, and is a well tolerated procedure. Nonetheless, CE was much more expensive than EGD, which was mainly due to the high purchase costs of the capsule. Recently, two studies analyzing the cost-effectiveness of CE for BE screening concluded that EGD with biopsies was superior and also less costly than CE when BE detection was the end-point.[32, 33] These findings indicate that the application of CE in the setting of screening can only be considered in case its associated costs are substantially lowered. One solution to this dilemma could be the application of string CE, which allows multiple uses after disinfection. Recently, Ramirez *et al.*[34] validated the use of this technique for screening for BE. String CE had an acceptable sensitivity and specificity for the visual diagnosis of BE and compared favorably when histology was used as the criterion standard. In addition, the calculated cost of CE in their hands ranged from $16.07 and $20.45 per procedure, based on an average of 25 uses per capsule. Intuitively, string CE may prove to be a cost-effective strategy for BE screening, and could potentially replace conventional EGD as a first screening method.

Cancer risk and risk stratification in Barrett's esophagus

Despite current practice guidelines, there are no data from randomized trials that demonstrate the value of surveillance. Clarification of the precise incidence of EAC in patients with BE is urgently needed, as cancer risk and risk of cancer-specific mortality are major determinants of the efficacy of BE surveillance. Wide variation in cancer risk has been observed, ranging from 0 percent to 3 percent per annum.[35] In addition, EAC-specific mortality rates in BE patients differ considerably between studies.[36, 37]

A meta-analysis on both the risk of cancer and cancer-related deaths in BE provides an alternative to clarify the value of surveillance, in the absence of randomized trials. In **chapter 5**, we calculated a pooled estimate for EAC incidence of 6.2/1,000 person-years (95%CI: 4.9-7.8) based on 51 studies including 13,777 patients followed up for a total of 60,688 person-years. Eighteen studies reported data on mortality due to EAC, and included 6,274 patients followed up for 30,407 person-years, with 75 deaths due to EAC and 1,173 deaths due to other causes. The pooled incidence of fatal EAC was only 2.9/1,000 person-years (95%CI: 2.2-3.7). In addition, as has been shown previously,[35] there was evidence of publication bias in surveillance studies from the USA. The selective publication of small studies with high cancer risks could result in an overestimation of cancer risk in BE. Study size is an important determinant of reported cancer risks, as was also demonstrated by another systematic review on the incidence of EAC in BE, in which cancer incidence was much lower in large than in small studies:

4.4 per 1,000 person-years in studies with 500 person-years or more, compared with 11.6 per 1,000 person-years in smaller studies.

Large scale and long-term follow-up studies of unselected BE patients could provide more reliable risk estimates for malignant progression, but are unfortunately very scarce. We therefore estimated the progression rate of BE to high-grade dysplasia (HGD) and EAC in a nationwide cohort of BE patients in the Netherlands, and assessed the value of the factors age, sex and initial histology as predictors of malignant progression in BE **(Chapter 6)**. In this, to our knowledge, largest reported cohort of BE patients, the overall annual risk of cancer in BE was 0.4% (95%CI: 0.3-0.6), and the annual risk of cancer and HGD combined only 0.6% (95%CI: 0.5-0.7). These figures are lower than previously published estimates.[38] In fact, the annual cancer risk decreased to 0.14%, in case cancer risk for all BE patients was analyzed, regardless of whether any follow-up was performed. Against this background, male gender, older age and LGD at initial BE diagnosis were identified as independent predictors for malignant progression. Overall, none of the previous cost-effectiveness models have shown a substantial benefit from BE surveillance. The overall annual cancer risk obtained from our study is even lower than those incorporated in these mathematical models, especially with regard to the annual cancer risk of the whole cohort of BE patients (0.14%). Our findings therefore indicate that both quality of life benefit and cost-effectiveness of Barrett's surveillance is highly questionable unless it can be targeted at those BE patients who are at the highest risk of cancer.

Although age and sex seem to enable tailoring of surveillance programs towards a better selection of high-risk patients, currently these factors have not been routinely included in planning BE surveillance programs. The development of further risk stratification is urgently needed. Although flow cytometry and molecular typing techniques might be able to distinguish between high-risk and low-risk groups,[39, 40] identification of easily applicable and simple epidemiological factors could be of help in daily clinical practice, and supplement histological findings. In **chapter 7**, we report on risk factors that are associated with an increased risk of developing EAC in BE patients. In this case-control study, tobacco smoking, a BMI above 25, working in a stooped posture, and male gender were such risk factors. Our study is one of the few case-control studies reported in literature so far in which patients with BE instead of healthy control subjects were selected as controls.[41-43] In comparison to these studies, we investigated a considerably larger number of cases and controls, and collected data on several potential factors that could increase or decrease the risk of EAC development in BE. Validation of these markers in new large-scale clinical trials is needed to determine whether these factors have a potential role in identifying patients at greatest risk of progression. Ideally, the development of simple prediction models based on clinical risk factors may aid in the decision-making on whether surveillance in a patient with BE should be performed, and which surveillance intervals are appropriate.

Besides the acknowledged risk of EAC, BE has also been associated with an increased risk of colorectal cancer (CRC), although this association remains controversial.[44] Regarding the

shortcomings of BE surveillance strategies, a definite establishment of a strong association between BE and CRC could indicate that BE patients should receive targeted CRC screening and surveillance, which might then well be more effective in cancer prevention than surveillance esophagoscopy in BE. In **chapter 8**, we demonstrate that there is an overall increased risk of CRC in patients with BE as compared to the Dutch general population, which cannot be solely attributed to the presence of diagnostic bias, as CRC incidence in male BE patients was still significantly increased after more than five years of follow-up. The magnitude of the association between BE and CRC does not, however, merit a more extensive CRC screening strategy in BE patients than has currently been recommended for the general population. In addition, intuitively, surveillance colonoscopy does not seem to be a more rewarding approach than surveillance esophagoscopy in BE patients, as the number of detected incident HGD/EAC cases within our BE cohort was slightly higher than that of detected CRCs. Our findings are in line with a recent case-control study, which showed that veterans with BE are at increased risk of developing colorectal neoplasia, independent of the use of PPIs or aspirin. The association between BE and CRC can probably be explained by other shared risk factors, of which obesity is the most promising. Several studies have shown that increased BMI is associated with an increased risk of CRC, especially in men.[45-47] As mentioned previously, adipose tissue is a source of TNF-α, IL-6, adiponectin, and also of growth factors such as insulin-like growth factor-1 (IGF-1).[15] These substances are known to cause insulin resistance syndrome, particular in obesity, in which levels of insulin and IGF-1 are elevated. Insulin is an important growth factor for colonic mucosal cells and colonic carcinoma cells *in vitro* and IGF-1 inhibits apoptosis and promotes cell cycle progression, potentially leading to development of colorectal cancer.[48] Unfortunately, in our study no data on BMI were available, which made it impossible to stratify CRC risk according to BMI levels. Based on our findings, we suggest that screening colonoscopy could be recommended for male BE patients aged above 50 years, in case they do not already participate in other CRC screening programs.

Role of acid suppression in preventing carcinogenesis in Barrett's esophagus

Chronic esophageal acid exposure is a well-established risk factor for the development of BE and EAC, and is also thought to initiate progression along the dysplasia-adenocarcinoma sequence. Therefore, acid suppression could be a biologically plausible mechanism for chemoprevention in BE. Indeed, evidence exists that PPI therapy in BE reduces esophageal acid exposure, decreases mucosal cell proliferation and increases differentiation, and possibly reduces the length of Barrett's segment and dysplasia incidence.[49-53] However, the advent of potent inhibitors of acid production in the past 20 years has done nothing to slow the rising incidence of BE and EAC in the general population. To elucidate the possible chemopreventive properties of PPIs, we studied the effect of short term PPI therapy in GERD patients on inflammatory profiles and oxidative stress *in vivo* **(Chapter 9)**. PPI therapy resulted in a substantial decrease in the cellular-mediated immune response in the distal esophagus,

both in patients with erosive esophagitis (EE) treated for 4 weeks and in those treated for 8 weeks. However, it did not affect the formation of DNA adducts in EE, either in those patients treated for 4 weeks or in those treated for 8 weeks. Levels of DNA adducts determined in our GERD patient groups were equivalent to those determined in another recent study in BE patients with dysplasia and patients with EAC, and these levels were significantly higher than those determined in healthy controls.[54] This suggests that oxidative DNA damage plays an important role in the pathogenesis of GERD, BE and its progression towards EAC, and is in line with the reported inverse link between intake of anti-oxidants and risk of EAC.[55] Our findings show that PPI therapy in GERD patients reduces the esophageal cellular-mediated immune response, but is insufficient to reverse DNA damage caused by gastroesophageal reflux. This suggests that PPI therapy is perhaps not effective in the prevention of carcinogenesis because of persisting DNA damage, which is already present in the early phases of the GERD spectrum.

Combinatory chemoprevention with PPIs and nutrients that target specifically on inhibition of oxidative damage might be more promising than PPI therapy alone. Intake of selenium, α-tocopherol, and β-carotene have been suggested to reduce total and cancer mortality in a Chinese population.[56] Combinations of these nutritional elements and high dose PPIs are required to be studied in a randomized, controlled trial, in order to evaluate whether this appears to be a promising approach in reducing cancer risk and mortality from EAC.

CONCLUSIONS AND FUTURE DIRECTIONS

Esophageal adenocarcinoma is the most rapidly increasing cancer in the Western world, and has a poor prognosis. The majority if EACs arise from BE in the course of a metaplasia-dysplasia-carcinoma sequence. Current recommended screening and surveillance strategies for BE are controversial, as there is insufficient evidence for both strategies on survival benefit and cost-effectiveness. Improved identification of groups at risk for BE and EAC, as well as the use of novel endoscopic techniques could improve the effectiveness of screening and surveillance in BE patients, and achieve the ultimate goal of reducing EAC mortality.

This thesis shows that the incidence of BE in the Netherlands is still on the rise, which has significant implications for health resource utilization and costs, and heralds a further increase in the number of new cases of EAC in the coming decade. Changes in the prevalence of risk factors in the second half of the 20th century have for the greater part driven this increase, of which the increased prevalence of abdominal obesity seems to be the most important contributor. Although more research is needed to explore the factors which continue to drive the increased incidence of BE, interventions that lower BMI could be a promising approach in reducing EAC risk in high risk populations. In addition, less invasive screening technology may soon lead to cheaper, safer, and acceptable screening for BE. Esophageal

capsule endoscopy is an accurate and well tolerated method for the detection of esophageal mucosal disorders, and could replace EGD as a first screening method, though at present is has not proven to be an inexpensive screening tool. Further research is necessary to evaluate the cost-effectiveness of this alternative procedure, as the possible benefits of screening should not exceed the costs and inconveniences for patients and health care systems.

Although widely practiced, no clinical randomized trials are available which demonstrate the value of surveillance. Although the precise incidence of EAC in BE remains unknown, it has previously been estimated to be approximately 0.5% per year. The studies described in this thesis show even lower cancer risk estimates and also considerable low risks of mortality due to EAC, which further indicate that endoscopic surveillance of all BE patients, in general, will not be cost-effective. Against this background male gender, age, increased BMI, smoking, working in a stooped posture and low-grade dysplasia are all independent predictors of malignant progression in patients with BE. At present, except for grade of dysplasia, none of these factors have been routinely included in planning BE surveillance programs. Future large studies from other unselected populations should be performed to develop further risk stratification, preferably a multicenter trial with randomization of patients to varying surveillance intervals based on the aforementioned predictors of progression. This thesis also shows that BE patients are at increased risk of developing CRC, especially male patients. Whether screening colonoscopy in BE patients would be a more cost-effective approach than surveillance esophagoscopy in the future needs further study.

Apart from improving strategies that focus on the detection of BE and cancer, new research should focus on strategies that could have a direct impact on the risk of cancer development, such as the identification of targets of drug activity for development of new potential chemopreventive agents, and the destruction of non-dysplastic BE with advanced endoscopic ablation and resection techniques.

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Chapter 11A

Summary

Samenvatting

SUMMARY

Cancer of the esophagus is a highly aggressive malignancy, of which the incidence has increased tremendously during the last decades, and still continues to rise at present. Barrett's esophagus (BE) is the only recognized precursor lesion and is associated with the majority, if not all, of cases with esophageal adenocarcinoma (EAC). Unfortunately, the efficacy of screening and surveillance of BE remains a strongly debated issue, as there are many unresolved epidemiological dilemmas, of which the inability to predict who has BE prior to endoscopy, and the lack of data on the natural history of BE are the major ones. Improved risk stratification could improve the effectiveness of screening and surveillance in BE patients, and achieve the ultimate goal of reducing EAC mortality.

The general aims and outline of this thesis are described in **chapter 1**.

In **chapter 2,** we analyzed data from a nationwide registry of pathology reports (PALGA) recorded between 1991 and 2006, in order to study the existence of a secular rise in the incidence of BE and to explore its causes. This study shows an ongoing substantial rise in the incidence of histologically confirmed BE in the Dutch population over the past 16 years, affecting males even more than females. Period and cohort phenomena for BE were demonstrated for both genders, the former likely to have been explained by both an increasing awareness of BE among endoscopists and improved endoscopic techniques, the latter by changes in prevalences of environmental risk factors after World War II, such as the increasing prevalence of obesity and the declining prevalence of *Helicobacter pylori* infection. The increasing BE incidence is a harbinger of a further rise in the number of EACs of nearly 35% in males and 13% in females within the coming decade, in case the yield of strategies that have a direct impact on cancer risk is disregarded.

Chapter 3 describes a study in which we investigated the distributions of environmental risk factors among patients with EAC, gastric cardia adenocarcinoma (GCA), and patients with esophageal squamous cell carcinoma. The majority of the evaluated risk factors did not show differences between EAC and GCA patients. Patients were similar with regard to male predominance and age at the time of diagnosis. In addition, no differences were found in alcohol intake and smoking, use of fruits and vegetables, body posture and occupational activities. Nonetheless, patients with EAC more often experienced symptoms of heartburn and had more often longstanding symptoms of heartburn compared with GCA patients. This would therefore suggest that only a relatively small proportion of cardia cancers might be attributed to gastroesophageal reflux, and that symptoms of gastroesophageal reflux can aid in the distinction between esophageal and gastric origins of cardia cancers.

In **chapter 4**, we evaluated the operating characteristics of capsule endoscopy (CE) for the detection of esophageal mucosal disorders using a new ingestion protocol. CE identified the presence of erosive esophagitis and BE with a high degree of accuracy, and had a high negative predictive value. The new ingestion protocol significantly improved visualization of

the Z-line. Furthermore, nearly all patients preferred CE over esophagogastroduodenoscopy (EGD), and experienced less discomfort and pain during CE as compared to EGD. These findings indicate that CE is an accurate method for detecting esophageal mucosal abnormalities, and is a well tolerated procedure. Nonetheless, CE was much more expensive than EGD, which was mainly due to the high purchase costs of the capsule.

In **chapter 5**, we calculated a pooled estimate for EAC incidence of 6.2/1,000 person-years (95%CI: 4.9-7.8) based on 51 studies including 13,777 patients followed up for a total of 60,688 person-years. Eighteen studies reported data on mortality due to EAC, and included 6,274 patients followed up for 30,407 person-years, with 75 deaths due to EAC and 1,173 deaths due to other causes. The pooled incidence of fatal EAC was only 2.9/1,000 person-years (95%CI: 2.2-3.7). In addition, there was evidence of publication bias in surveillance studies from the USA.

As study size is an important determinant of reported cancer risks, we estimated the progression rate of BE to high-grade dysplasia (HGD) and EAC in a nationwide cohort of BE patients in the Netherlands, and assessed the value of the factors age, sex and initial histology as predictors of malignant progression in BE **(Chapter 6)**. In this, to our knowledge, largest reported cohort of BE patients, the overall annual risk of cancer in BE was 0.4% (95%CI: 0.3-0.6), and the annual risk of cancer and HGD combined only 0.6% (95%CI: 0.5-0.7). These figures are lower than previously published estimates. In fact, the annual cancer risk decreased to 0.14%, in case cancer risk for all BE patients was analyzed, regardless of whether any follow-up was performed. Against this background, male gender, older age and LGD at initial BE diagnosis were identified as independent predictors of malignant progression in BE.

In **chapter 7**, we report on risk factors that are associated with an increased risk of developing EAC in BE patients. A hospital-based case-control study was performed in which 91 cases with EAC and 244 controls with histologically confirmed BE with no dysplasia or low-grade dysplasia were included. Tobacco smoking, a BMI above 25, working in a stooped posture, and male gender were such risk factors.

In **chapter 8**, we demonstrate that there is an overall increased risk of CRC in patients with BE as compared to the Dutch general population, which cannot be solely attributed to the presence of diagnostic bias, as CRC incidence in male BE patients was still significantly increased after more than five years of follow-up. Based on our findings, we suggest that screening colonoscopy could be recommended for male BE patients aged above 50 years, in case they do not already participate in other CRC screening programs.

Chronic inflammation and oxidative DNA damage play an important role in the pathogenesis of GERD, BE and its progression towards EAC. In **chapter 9,** we studied the effect of short term proton pump inhibitor (PPI) therapy in GERD patients on inflammatory profiles and oxidative stress *in vivo*. PPI therapy resulted in a substantial decrease in the cellular-mediated immune response in the distal esophagus, both in patients with erosive esophagitis (EE) treated for 4 weeks and in those treated for 8 weeks. However, it did not affect the formation

of DNA adducts in EE, either in those patients treated for 4 weeks or in those treated for 8 weeks. Our findings show that PPI therapy in GERD patients reduces the esophageal cellular-mediated immune response, but is insufficient to reverse DNA damage caused by gastroesophageal reflux. This suggests that PPI therapy is perhaps not effective in the prevention of carcinogenesis because of persisting DNA damage, which is already present in the early phases of the GERD spectrum.

The main findings of this thesis and directions for future research are discussed in **Chapter 10**.

SAMENVATTING

Slokdarmkanker is een bijzonder aggressieve vorm van kanker, waarvan de incidentie in de afgelopen decennia wereldwijd sterk is gestegen, en hedendaags nog steeds stijgt. In het meerendeel van de gevallen, en waarschijnlijk zelfs in alle gevallen, wordt het adenocarcinoom van de slokdarm voorafgegaan door Barrett slokdarm. De detectie van Barrett slokdarm biedt de mogelijkheid tot endoscopische surveillance en vroegtijdige interventie ter preventie van slokdarmkanker. Echter, de effectiviteit van screening en surveillance van patiënten met Barrett slokdarm is omstreden, met name door het bestaan van verschillende epidemiologische dilemma's. Belangrijke belemmeringen zijn onder andere het onvermogen om binnen de algehele populatie te voorspellen wie een Barrett slokdarm heeft voorafgaand aan het uitvoeren van een endoscopie van de slokdarm, en het relatieve gebrek aan gegevens over het natuurlijk beloop van de Barrett slokdarm. Het identificeren van Barrett patiënten met een verhoogd risico op het ontwikkelen van slokdarmkanker middels betrouwbare risico markers zou de effectiviteit van screening en surveillance kunnen optimaliseren, teneinde de sterfte ten gevolge van slokdarmkanker te verminderen.

De algemene doelen en achtergrond van dit proefschrift worden beschreven in **hoofdstuk 1**.

In **hoofdstuk 2** worden met behulp van gegevens van de landelijke pathologie database PALGA de stijging van de incidentie van Barrett slokdarm van 1991 tot en met 2006 in Nederland en de mogelijke oorzaken hiervan onderzocht. Deze studie toont een aanhoudende stijging van de incidentie van Barrett slokdarm in Nederland gedurende de 16-jarige onderzoeksperiode, waarbij de stijging bij mannen sterker aanwezig is dan bij vrouwen. Periode en cohort effecten werden aangetoond voor zowel mannen als vrouwen. Het periode effect kan worden verklaard door een toegenomen interesse in Barrett slokdarm bij endoscopisten en door nieuwe endoscopische detectie technieken. Het cohort effect wordt verklaard door veranderingen in de prevalentie van risicofactoren bij cohorten geboren na de Tweede Wereldoorlog, met name de stijging van de prevalentie van obesitas en de afname van de prevalentie van *Helicobacter pylori* infectie in de algehele bevolking. De stijgende incidentie van Barrett slokdarm voorspelt een toename van het aantal adenocarcinomen van de slokdarm van bijna 35% bij mannen en 13% bij vrouwen binnen de komende 10 jaar, indien het effect van screening en surveillance strategieën buiten beschouwing wordt gelaten.

Hoofdstuk 3 beschrijft een studie waarin risicofactoren bij patiënten met een adenocarcinoom van de slokdarm, een adenocarcinoom van de maagcardia, en plaveiselcelcarcinomen van de slokdarm worden vergeleken. Het meerendeel van de bestudeerde risicofactoren verschilde niet significant tussen patiënten met een adenocarcinoom van de slokdarm of cardia. De geslachtsverdeling en leeftijd ten tijde van diagnose bij deze carcinomen waren vergelijkbaar. Tevens werden er geen verschillen gevonden in het gebruik van alcohol en roken, consumptie van groente en fruit, lichaamsbouw en fysieke werkzaamheden. Echter,

patiënten met een adenocarcinoom van de slokdarm hadden vaker last van zuurbranden en ook vaker langer bestaande klachten, in vergelijking tot patiënten met een adenocarcinoom van de cardia. Dit suggereert dat slechts een klein gedeelte van de cardia carcinomen ontstaat ten gevolge van gastro-oesofageale refluxziekte, en dat bij het onderscheid tussen cardia carcinomen van maag of slokdarm origine symptomen ten gevolge van refluxziekte van aanvullende waarde kunnen zijn.

In **hoofdstuk 4** werd de waarde van capsule endoscopie voor de detectie van slokdarmafwijkingen bestudeerd, waarbij gebruik werd gemaakt van een nieuw ingestie protocol. Capsule endoscopie stelde de aanwezigheid van reflux oesofagitis en Barrett slokdarm met een hoge nauwkeurigheid vast, met tevens een hoge negatief voorspellende waarde. Het nieuwe ingestie protocol verbeterde de visualisatie van de Z-lijn aanzienlijk. Bovendien prefereerden bijna alle patiënten capsule endoscopie boven de conventionele gastroscopie, en ondervonden zij ook significant minder discomfort en pijn ten gevolge van capsule endoscopie, in vergelijking tot een gastroscopie. Deze bevindingen impliceren dat capsule endoscopie een accurate methode is voor de detectie van slokdarmafwijkingen, en dat deze techniek een comfortabele procedure is voor de patiënt. Capsule endoscopie bleek echter een duurdere procedure te zijn dan gastroscopie, met name door de hoge aanschafkosten van de capsule.

In **hoofdstuk 5** werd met behulp van een meta-analyse het risico op een adenocarcinoom van de slokdarm bij patiënten met Barrett slokdarm in 51 studies met in totaal 13.777 patiënten en 60.688 persoonsjaren bestudeerd. Het risico op adenocarcinoom van de slokdarm op basis van deze gegevens betrof 6,2/1000 persoonsjaren (95% betrouwbaarheidsinterval (BI) 4,9-7,8). Achttien van deze studies rapporteerden data over de mortaliteit ten gevolge van adenocarcinomen van de slokdarm, en includeerden in totaal 6.274 patiënten met follow-up gedurende 30.407 persoonsjaren, met 75 sterftegevallen ten gevolge van adenocarcinoom van de slokdarm en 1.173 sterftegevallen ten gevolge van andere oorzaken. De samengestelde incidentie op basis van deze studies van fatale gevallen van adenocarcinomen van de slokdarm was slechts 2,9/1000 persoonsjaren (95% BI 2,2-3,7). Bovendien werd een publicatie bias aangetoond in surveillance studies die afkomstig waren uit de Verenigde Staten.

Aangezien studie grootte een belangrijke determinant is van gerapporteerde kanker risico's, werd het risico op progressie van Barrett slokdarm naar hoog-gradige dysplasie en adenocarcinoom van de slokdarm onderzocht in een landelijk cohort van Barrett patiënten, en werd de voorspellende waarde van de factoren leeftijd, geslacht en initiële histologische diagnose op de progressie kans in Barrett slokdarm bestudeerd (**Hoofdstuk 6**). Binnen dit grote cohort van patiënten met Barrett slokdarm betrof het jaarlijkse risico op het ontstaan van adenocarcinoom van de slokdarm 0,4% (95% BI 0,3-0,6), en het jaarlijkse risico op de gecombineerde uitkomst van slokdarmkanker danwel hoog-gradige dysplasie slechts 0,6% (95% BI 0,5-0,7). Deze waarden zijn lager dan eerder gepubliceerde schattingen. Het jaarlijkse risico daalde zelfs tot 0,14%, indien het kanker risico voor alle patiënten met Barrett slokdarm werd berekend, ongeacht het wel of niet uitvoeren van surveillance endoscopie.

Uit deze gegevens bleek dat het mannelijk geslacht, oudere leeftijd en de aanwezigheid van laag-gradige dysplasie ten tijde van de initiële diagnose van Barrett slokdarm onafhankelijke voorspellers zijn voor maligne ontaarding.

In **hoofdstuk 7** werden risicofactoren bestudeerd die geassocieerd zijn met een toegenomen risico op adenocarcinoom van de slokdarm bij patiënten met Barrett slokdarm. Een case-control studie werd uitgevoerd bij 91 patiënten met adenocarcinoom van de slokdarm en 244 controle patiënten met een histologisch aangetoonde Barrett slokdarm, zonder dysplasie of met laag-gradige dysplasie. Het roken van tabak, een BMI groter dan 25, een voorovergebogen houding tijdens het werk, en het mannelijk geslacht werden als onafhankelijke voorspellers voor maligne ontaarding in Barrett slokdarm geïdentificeerd.

In **hoofdstuk 8** werd aangetoond dat het risico op darmkanker bij patiënten met Barrett slokdarm verhoogd is ten opzichte van de algehele populatie. Deze resultaten kunnen niet alleen worden toegeschreven aan de aanwezigheid van een diagnostische bias, aangezien het risico op darmkanker bij mannelijke patiënten met Barrett slokdarm na 5 jaar follow-up nog steeds verhoogd was. Gebaseerd op deze bevindingen wordt een screenings coloscopie geadviseerd aan mannelijke patiënten met Barrett slokdarm ouder dan 50 jaar, indien zij niet reeds deelnemen aan andere darmkanker screeningsprogramma's.

Chronische ontsteking en DNA schade spelen een belangrijke rol bij het onstaan van gastro-oesofageale refluxziekte, Barrett slokdarm en de progressie naar adenocarcinoom van de slokdarm. In **hoofdstuk 9** werd het effect van kortdurend gebruik van proton pomp remmers bestudeerd bij patiënten met gastro-oesofageale refluxziekte op de ontstekingsreactie en oxidatieve stress in slokdarmweefsel. Behandeling met proton pomp remmers resulteerde in een substantiële afname van de cellulaire immuun respons in de distale slokdarm, zowel bij patiënten met erosieve oesofagitis die gedurende 4 weken werden behandeld, alsmede bij patiënten met een behandeling gedurende 8 weken. Echter, bij beide patiëntgroepen veranderde deze behandeling de vorming van DNA adducten in de slokdarm niet. Deze bevindingen tonen dat kortdurende behandeling met proton pomp remmers de cellulaire immuun respons in de slokdarm vermindert, maar dat deze behandeling onvoldoende is om DNA schade ten gevolge van gastro-oesfageale reflux te herstellen. Dit suggereert dat behandeling met proton pomp remmers wellicht niet effectief is in de preventie van slokdarmkanker door aanhoudende DNA schade, welke al vroeg aanwezig is in het spectrum van gastro-oesofageale refluxziekte.

De belangrijkste bevindingen van dit proefschrift en aanwijzingen voor toekomstig onderzoek worden besproken in **hoofdstuk 10**.

Chapter 11B

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Chapter 11C

Dankwoord

DANKWOORD

Een proefschrift schrijf je niet alleen, het betreft een collectieve prestatie. Graag wil ik dan ook de volgende personen hartelijk danken voor hun bijdrage en steun.

Allereerst mijn promotoren prof.dr. E.J. Kuipers en prof.dr. P.D. Siersema. Beste Ernst, jouw energieke colleges tijdens het thema "Voeding, Metabolisme en Hormonale Regulatie" hebben voor het eerst mijn interesse gewekt voor de gastroenterologie. Ik ben je erg dankbaar dat je mij de mogelijkheid hebt gegeven om in dit veld promotie-onderzoek te verrichten, voorafgaand aan mijn co-schappen. Door jouw aanstekelijke drive, enthousiasme, en scherpzinnigheid heb ik een zeer plezierige begeleiding ondervonden, waarbij, ondanks je volle agenda, de kenmerkende laagdrempeligheid voor overleg niet ongenoemd mag blijven. Ik prijs me daarom ook gelukkig dat ik mij de afgelopen jaren onder jouw hoede op wetenschappelijk niveau heb kunnen ontwikkelen. Ik hoop van harte dat onze samenwerking nog een tijd zal voortduren.

Beste Peter, onder jouw supervisie maakte ik voor het eerst echt kennis met wetenschappelijk onderzoek. Jouw enthousiasme overtuigde me om mijn afstudeeronderzoek voort te zetten in een promotietraject. De wekelijkse werkbesprekingen waren stimulerend, zeer efficiënt, maar bovenal gemoedelijk. Ik waardeer in het bijzonder je buitengewone betrokkenheid en behulpzaamheid, zowel binnen als buiten het werk. In Utrecht ben je een nieuwe uitdaging aangegaan, wat vanzelfsprekend een succes zal worden. Ik dank je voor de uitstekende begeleiding en kijk terug op een uiterst prettige samenwerking.

Graag wil ik alle leden bedanken voor hun bereidheid om plaats te nemen in de promotiecommissie. Beste Wendy Holleman, veel dank voor alle administratieve werkzaamheden in de aanloop naar deze promotie.

Beste Mark van Blankenstein, ook na de afronding van je eigen proefschrift is je passie voor de Barrett epidemiologie blijven voortbestaan. Ik ben je erg dankbaar voor alle waardevolle adviezen, je scherpe commentaar, en ben zeer verheugd dat je zitting wilt nemen in de commissie. Ik kijk uit naar nieuwe samenwerkingen.

Zonder de inzet van endoscopie-verpleegkundigen, arts-assistenten, en stafleden van de afdeling Maag-, Darm- en Leverziekten van het Erasmus MC, het IJsselland Ziekenhuis, het Sint Franciscus Gasthuis en het Ikazia Ziekenhuis zou dit proefschrift niet tot stand zijn gekomen. In het bijzonder wil ik Leonieke Wolters, Pieter Honkoop, Brechje van Eijck, Manon Spaander, Monique van Leerdam, Frank Bekkering, Han Geldof, Yvonne Leeuwenburgh, Rob Ouwendijk, Dirk Jan Bac, en Corry Leunis hartelijk danken voor een zeer plezierige en vruchtbare samenwerking. Dank gaat tevens uit naar pathologen Gerrit Meijer en Herman van Dekken,

en naar Mariël Casparie voor het herhaaldelijk uitvoeren van de zoekopdrachten in de PALGA database.

Geen betrouwbare resultaten zonder degelijke statistiek. Een amateur statisticus strandt al snel in complexe mathematiek, waarbij hulp van experts onontbeerlijk is. Beste Ewout Steyerberg en Caspar Looman, dank voor jullie altijd snelle "output" en oplossingen om een complex vraagstuk tot een behapbaar probleem te reduceren.

De opleiding geneeskunde kent in het huidige curriculum helaas nog maar weinig ruimte voor basale leerstof. Hans Kusters en Arnoud van Vliet ben ik erkentelijk voor het wegwijs maken in het land van gelletjes, epjes en pipetten. Daarnaast ook mijn dank aan Simone van Breda en Katinka van Zoest voor de ondersteuning bij de laboratorium analyses.

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Voor alle ontspannende momenten buiten het ziekenhuis dank ik verder de leden van de "Master groep"; er staan nog een fiks aantal promotiefeestjes in de planning voor de komende jaren! Mark E., dat zal wel weer een cummetje worden?! Mijn jaargenoten Claire, Antoinette, Vincent, Arthur, Frans en Marco voor de altijd zeer "ontspannen" diners, in de toekomst onder toezicht van de wedergeboren Leen alias Roy. Mijn mede-co-assistenten voor de gezellige borrels, en met name voor de prachtige discussies tijdens het RPG onderwijs.

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Mijn familie dank ik voor alle steun en belangstelling. Mijn schoonfamilie dank ik voor alle interesse en goede adviezen. Een gastvrijere (en grotere) schoonfamilie kun je je niet wensen.

Lieve pap en mam, jullie hebben me geleerd ambitieus te zijn, daarmee staan jullie aan de basis van dit proefschrift. Dank voor jullie onvoorwaardelijke steun en interesse. Lieve Krista, ik ben trots op je, en je hebt het getroffen met Tim!

Lieve Annemarie, wat een geluk dat ik jou heb getroffen! Je staat altijd weer voor me klaar, je bent onmisbaar! Vanaf nu hebben we extra tijd voor andere leuke dingen. Ik kijk ontzettend uit naar wat de toekomst ons zal brengen. Eén plus eén is in ieder geval drie!

Chapter 11D

Curriculum vitae auctoris

CURRICULUM VITAE AUCTORIS

De auteur van dit proefschrift werd geboren op 28 februari 1983 te Oud-Beijerland. Na het behalen van zijn Gymnasium diploma aan de Rijksscholengemeenschap Hoeksche Waard te Oud-Beijerland in 2001, werd in datzelfde jaar gestart met de studie Geneeskunde aan de Erasmus Universiteit Rotterdam. Na het behalen van de propedeutische fase met judicium cum laude, werd in 2002 tevens gestart met de MSc. opleiding Clinical Epidemiology. In het kader van deze studie werd onder meer deelgenomen aan de Summer School van Harvard University te Boston. In juli 2005 behaalde hij het doctoraalexamen en rondde hij gelijktijdig de studie Clinical Epidemiology af. Aansluitend werkte hij tot en met december 2007 als promovendus op de afdeling Maag-, Darm- en Leverziekten van het Erasmus MC. Onder leiding van zijn promotoren Prof.dr. E.J. Kuipers en Prof.dr. P.D. Siersema startte hij hier zijn onderzoek naar de epidemiologische aspecten met betrekking tot patienten met Barrett's oesofagus. Op 7 januari 2008 is hij gestart met zijn co-schappen. Het arts-examen zal naar verwachting in december 2009 behaald worden.

Chapter 11E

Portfolio

PORTFOLIO

Conferences:

2009 Risk of colorectal cancer in patients with Barrett's esophagus: a Dutch population-based study.
Digestive Disease Week, Chicago, USA. (Poster)

Risk of malignant progression in patients with Barrett's esophagus: a Dutch nationwide cohort study.
Dutch Society of Gastroenterology, Veldhoven, the Netherlands. (Oral)

2008 Trends in the epidemiology of Barrett's esophagus in the Netherlands 1991-2006.
Digestive Disease Week, San Diego, USA. (Oral)

Risk of malignant progression in patients with Barrett's esophagus: a long-term nationwide study in the Netherlands.
Digestive Disease Week, San Diego, USA. (Poster of distinction)

2007 Esophageal capsule endoscopy in patients with gastro-esophageal reflux disease and Barrett's esophagus: improvement of diagnostic accuracy.
Digestive Disease Week, Washington, USA. (Poster)
Dutch Society of Gastroenterology, Veldhoven, the Netherlands. (Oral)

Proton pump inhibitor therapy in patients with symptomatic gastro-esophageal reflux disease reduces the cellular immune response in the distal esophagus.
Digestive Disease Week, Washington, USA. (Poster)
UEGW, Paris, France. (Poster)

Wireless 48-hr pH monitoring is not superior to 24-hr pH monitoring combined with upper endoscopy for the detection of gastro-esophageal reflux disease.
Digestive Disease Week, Washington, USA. (Poster)
UEGW, Paris, France. (Poster)

Day-to-day variation in acid reflux patterns in patients with non-erosive reflux disease and erosive reflux disease: clinically relevant or trivial?
Dutch Society of Gastroenterology, Veldhoven, the Netherlands. (Oral)

2006 Environmental risk factors for the development of esophagus-cardia adenocarcinoma: a cross-sectional study in a Dutch cohort.

Digestive Disease Week, Los Angeles, USA. (Poster)
European Society of Esophagology, Leuven, Belgium. (Poster)

De 48-uurs pH meting met BRAVO capsule.
Dutch Society of Gastroenterology, Veldhoven, the Netherlands. (Oral)

2005 Risk factors in Barrett's esophagus for the development of adenocarcinoma.
Digestive Disease Week, Chicago, USA. (Poster)
Dutch Society of Gastroenterology, Veldhoven, the Netherlands. (Oral)

Memberships:

2005 Member of the Dutch Society of Gastroenterology (NVGE)

Peer reviewer activities:

2008 Digestive and Liver Disease
American Journal of Clinical Nutrition

2007 Scandinavian Journal of Gastroenterology

2006 American Journal of Gastroenterology