Optimizing Surveillance in Barrett's Esophagus

From Chemoprevention and Biomarkers
to Cost-effectiveness and Survival

Florine Kastelein

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Optimaliseren van surveillance voor Barrett slokdarm
Van chemopreventie en biomarkers tot kosteneffectiviteit en overleving

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Chapter 1

Introduction and outline of the thesis

F. Kastelein

Barrett's esophagus

Barrett's esophagus (BE) is a condition in which the squamous epithelium of the distal esophagus is replaced by columnar epithelium containing goblet cells, which can be recognized during endoscopy by red discoloration of the normally vale pink mucosa.[1] BE is thought to be a complication of chronic gastro-esophageal reflux disease and approximately 10% of patients with symptomatic reflux will eventually develop BE.[2] In Western countries, the prevalence of BE is estimated at 1-2% of the general population, with white males over 60 years predominantly affected.[2-4] BE patients have an increased risk of developing esophageal adenocarcinoma (EAC) with an estimated incidence of 0.1-0.5% per year, which makes it the single most important risk factor for EAC.[5-7] The development of EAC in BE is thought to be a gradual process, in which metaplastic BE epithelium evolves to low-grade dysplasia, high-grade dysplasia and eventually EAC under the influence of chronic esophageal acid exposure (Figure 1).[8, 9] Once a patient has developed EAC the prognosis is poor with a 5 year survival of less than 20%.[10] Surveillance is therefore recommended for patients with BE to detect EAC at an early stage when curative treatment is still feasible, and to reduce mortality due to EAC.[11-13]
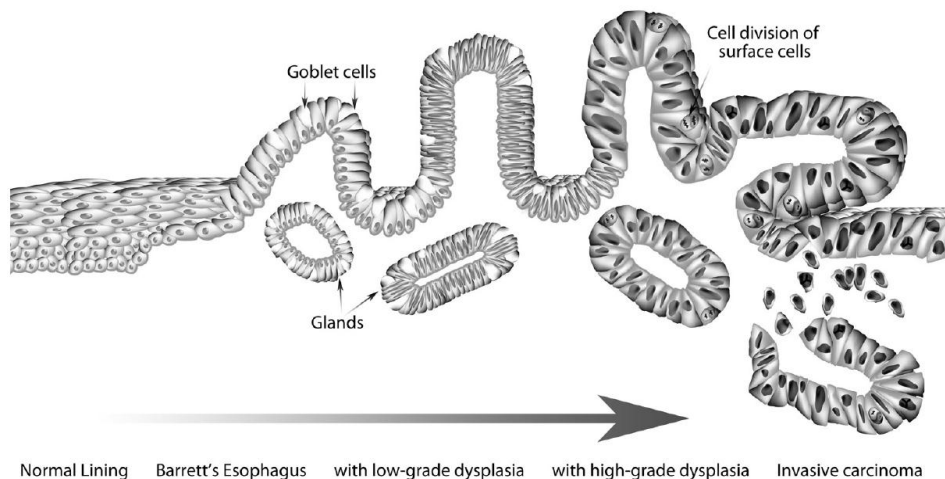

Figure 1. The development of normal squamous epithelium to columnar Barrett's epithelium containing goblet cells, low-grade dysplasia, high-grade dysplasia and eventually esophageal adenocarcinoma.[14]

Surveillance

Recommendations for BE surveillance are based on guidelines of the American College of Gastroenterology. These guidelines recommend surveillance every 3 to 5 years in patients without dysplasia, surveillance, every 6 to 12 months in patients with low-grade dysplasia, and (endoscopic) treatment or intensive surveillance in patients with high-grade dysplasia. At each endoscopy targeted biopsies are taken from mucosal abnormalities and quadrant biopsies are taken every 2 centimeters from the gastroesophageal junction to the most proximal BE margin to obtain a histological diagnosis and grading of dysplasia.[11-13, 15] To date histological diagnosis of dysplasia is the only accepted predictor for neoplastic progression in BE and therefore used for defining surveillance intervals. However, histology is subject to sample error and considerable interobserver variation which limits its predictive value.[16, 17] Identification of other predictors for neoplastic progression could improve risk stratification and hence the effectiveness of BE surveillance.

Chemoprevention

Multiple studies have given support to the use of chemoprevention to reduce the risk of developing cancer, including EAC. Since esophageal acid exposure plays an import role in the initiation of BE and its progression to EAC, chemoprevention with acid suppressants seems a promising prevention strategy. As a result use of proton pump inhibitors has become a mainstay in the treatment of BE patients. However, although proton pump inhibitors are effective in relieving reflux symptoms, healing esophagitis, and decreasing proliferation of BE epithelium, it is unknown whether they truly prevent neoplastic progression in BE.[9, 18-22] Current guidelines do therefore not provide strong recommendations for the use of proton pump inhibitors in BE.

In addition, studies have given support to chemoprevention with nonsteroidal anti-inflammatory drugs and statins. Nonsteroidal anti-inflammatory drugs inhibit cyclooxygenase enzymes, which results in decreased cell growth, proliferation and angiogenesis in human tissue.[23] Statins not only inhibit the biosynthesis of cholesterol, but also decrease the activation of intracellular proteins through prenylation, which results in reduced cell proliferation and induced apoptosis.[24] Use of nonsteroidal anti-inflammatory drugs and statins may therefore reduce the risk of neoplastic progression in BE.[25]

Biomarkers

Application of biomarkers in addition to histology may contribute to the identification of patients at high risk for neoplastic progression. Many biomarkers have been investigated for risk stratification in BE, of which immunohistochemical staining of p53 appears to be one of the most promising. Moreover, this immunohistochemical staining is widely established and available in most pathology labs. TP53 is a tumor suppressor gene that plays an important role in regulation of the cell cycle and apoptosis. Mutations in the p53 protein may allow progression of abnormal cells and thereby development of cancer.[26] Antibodies used for p53 immunohistochemistry not only stain protein derived from mutant TP53 but also from wild-type TP53. Nevertheless, p53 expression is considered indicative for the presence of mutant TP53, because the latter has a longer half-life than wild-type p53 and is not degraded in the normal way. This results in accumulation of p53 in the cell nucleus, which is detectable by immunohistochemistry. Truncating mutations, loss of the TP53 gene and epigenetic silencing may result in absence of p53 in the cell nucleus and thereby a negative immunohistochemical staining. Although less common, loss of p53 expression is also detectable because it sharply contrasts with the surrounding tissue.[27, 28] Although the first studies have shown promising results, the value of p53 immunohistochemistry for predicting neoplastic progression in BE has not been validated in large prospective studies.

Another potential biomarker for risk stratification is BE is Alpha-Methylacyl-CoA Racemase (AMACR). AMACR is a cytoplasmic enzyme that plays an essential role in the β-oxidation of branched-chain fatty acids and is an established biomarker for prostate cancer. AMACR is expressed in colon adenomas and adenocarcinomas but not in normal colon epithelium, which suggests that it may play a role in the development of gastrointestinal malignancies.[29, 30] Although little is known about the value of AMACR in BE patients, the first small studies have shown promising results.

Cost-effectiveness and survival

Although surveillance of BE patients seems reasonable and is incorporated in guidelines, there is little scientific evidence that BE surveillance is actually beneficial. BE patients have a much higher risk of developing EAC compared to the general population, but the absolute risk of neoplastic progression is low

and most patients never develop EAC. Which patients have the highest risk of neoplastic progression remains unknown. To date no randomized controlled trials have been performed and as a result the value of BE surveillance is under discussion. Key questions in this discussion are whether BE surveillance is cost-effective and reduces mortality due to EAC. In addition, over the past decade there has been a major shift in treatment modalities for patients with neoplastic progression. While in the previous century almost all patients underwent esophagectomy, nowadays endoscopic treatment with endoscopic mucosal resection and radiofrequency ablation is frequently used.[11-13] Application of these endoscopic treatment modalities may improve the cost-effectiveness of BE surveillance and reduce mortality due to EAC.

Aim of this thesis

The aim of this thesis is to evaluate whether chemoprevention and use of biomarkers can contribute to risk stratification in BE patients in order to optimize surveillance. In addition the effect of BE surveillance was evaluated in terms of cost-effectiveness and survival.

Outline of this thesis

In **chapter 2** the existing literature is reviewed regarding the role of esophageal acid exposure in the development of BE and its progression to EAC. In **chapter 3** and **chapter 4** we investigated whether chemoprevention with proton pump inhibitors, non-steroidal anti-inflammatory drugs and statins reduces the risk of neoplastic progression in BE patients. In **chapter 5** and **chapter 6** the value of two biomarkers, p53 and AMACR, is assessed for predicting neoplastic progression in BE. In **chapter 7** the cost-effectiveness of different surveillance intervals and treatment strategies is evaluated. In **chapter 8** the survival of BE patients detected with HGD or EAC in a surveillance program is explored and compared to the survival of patients with EAC in the general population. In **chapter 9** and **chapter 10** the results of this thesis are discussed and summarized.

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Chapter 2

The role of acid suppression in the development and progression of dysplasia in patients with Barrett's esophagus

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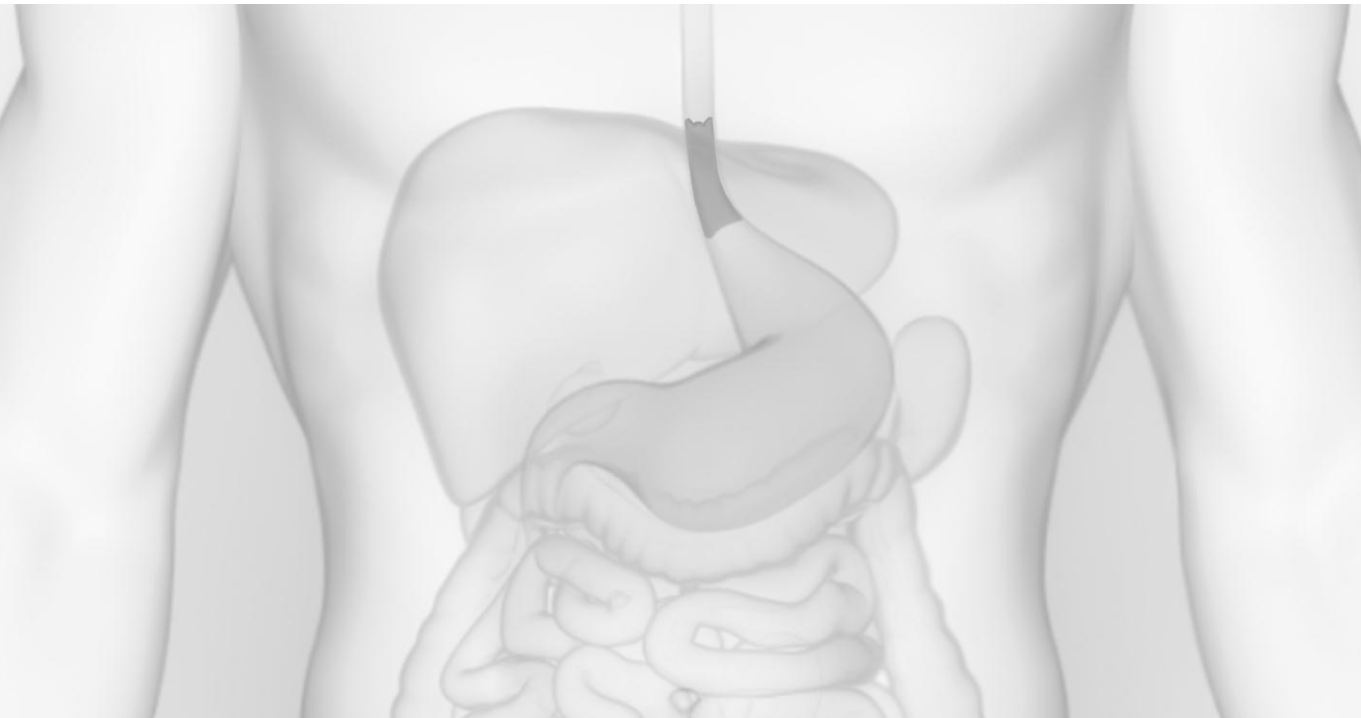
Abstract

Barrett's esophagus (BE) usually develops in patients with gastroesophageal reflux disease and therefore it has been suggested that esophageal acid exposure plays an import role in the initiation of Barrett's esophagus and it's progression toward esophageal adenocarcinoma (EAC). The mechanisms whereby acid exposure causes Barrett's esophagus are not completely revealed and the potential role of esophageal acid exposure in carcinogenesis is unclear as well. Since acid exposure is thought to play an important role in the progression of Barrett's esophagus, therapies aimed at preventing the development of EAC have primarily focused on pharmalogical and surgical acid suppression. In clinical practice, acid suppression is effective in relieving reflux symptoms and decreases esophageal acid exposure in most patients. However, in some individuals pathological acid exposure persists and these patients continue to be at risk for developing dysplasia or EAC. To date, published trials suggest that acid suppression is able to prevent the development and progression of dysplasia in patients with Barrett's esophagus, but definite and compelling proof is still lacking. This article reviews the mechanisms of acid induced carcinogenesis in Barrett's esophagus and the role of acid suppression in the prevention of neoplastic progression.

Introduction

Barrett's esophagus (BE) is an important premalignant condition in which the squamous epithelium of the distal esophagus is replaced by columnar epithelium containing goblet cells.[1] BE is thought to be a complication of chronic gastroesophageal reflux disease (GERD) and approximately 10% of patients with GERD will eventually develop BE.[2] The prevalence of BE in the general population is estimated at 1-2%, with white males over 60 years predominantly affected.[2-5] BE is the single most important risk factor for the development of esophageal adenocarcinoma (EAC) with a yearly incidence of approximately 0.5%.[6,7] The incidence of EAC has increased over the last decades at a rate exceeding that of any other cancer.[8,9] This rise may be due to an increase in the prevalence of BE.[10] The majority of carcinomas arising in a BE present at an advanced stage and have a poor prognosis with a 5 year survival rate less than 20%.[11] The development of EAC in BE is a gradual process in which important biological processes become disrupted which in a step-wise progression model may lead from low-grade dysplasia (LGD), via high-grade dysplasia (HGD), to early adenocarcinoma and eventually invasive adenocarcinoma.[1,12,13] However, most individuals with BE will not develop dysplasia or EAC during their lifetime. It is not yet possible to predict which patients are at high risk for neoplastic progression. Even though GERD is the major risk factor for the development of BE, only few patients with symptoms of chronic GERD will eventually develop BE. Besides, some patients with BE never experienced any reflux symptoms.[14] Therefore the role of esophageal acid exposure in the development and progression of BE is not completely understood. Whether pharmalogical or surgical acid suppression prevents the development and progression of dysplasia in BE is unclear as well.

This review focuses on the mechanisms whereby acid exposure may contribute to the development and progression of dysplasia in BE and the potential role of acid suppression in the prevention of neoplastic progression in patients with BE.

Pathophysiology

BE usually develops in patients with symptoms of chronic GERD and therefore it has been suggested that esophageal acid exposure plays an important etiological role in the development of BE. Several studies have shown that acid exposure is greater in patients with BE compared to individuals with esophagitis

and healthy controls. In BE patients the percentage of time at pH < 4 is increased compared to individuals with esophagitis. The number of reflux episodes lasting longer than 5 minutes is increased as well.[15,16] Patients with BE may be predisposed to greater acid exposure because of reduced pressure of the lower esophageal sphincter and impaired motility of the distal esophageal body.[15] The ability to clear refluxate from the esophagus is reduced as a result of these alterations. Some studies suggest that duration of acid exposure is correlated to BE length.[17] Besides, ex vivo studies have shown that the pattern of acid exposure is important as well. The pattern of acid exposure has effects on the differentiation of the epithelium. Continuous acid exposure induces differentiation, whereas intermittent acid exposure increases proliferation.[18]

Chronic esophageal acid exposure may lead to esophagitis with inflammation and increased proliferation of esophageal squamous cells. In the majority of patients the esophagus heals with regeneration of new esophageal squamous cells. However, in some patients the esophagus heals though a process of metaplasia in which the squamous epithelium is replaced by BE epithelium.[19] Considerable evidence suggests that the basal layer of the esophageal epithelium contains pluripotent stem cells.[20,21] Stimulation of these esophageal stem cells for instance by acid exposure may lead to metaplastic changes. The transcription factor CDX2 seems to play a key role in the differentiation towards columnar epithelium. CDX2 is overexpressed in BE and can be induced by acid exposure. Animal studies have shown that inducing CDX2 can elicit transformation into columnar epithelium containing intestinal metaplasia.[22] The prevailing hypothesis is that BE occurs via abnormal differentiation of esophageal epithelial stem cells exposed to acid in individuals with GERD.

Carcinogenesis

While GERD appears to play a central role in the initiation of BE, the role of reflux in carcinogenesis is less clear. The development of EAC in BE is a multistep process in which metaplastic epithelium evolves into LGD, HGD and eventually EAC. The development of dysplasia is characterized by changes in cell cycling, such as increased cyclin D1 expression and accumulation of p16 and p53 mutations. The effect of cell cycling abnormalities may be increased by elevated activity of stimulators of cell proliferation, like TGF-α and EGF. During neoplastic progression changes in intracellular adhesion arises as well, such as loss of APC, reduced cadherin expression and catenin phosphorylation.[23]

Although, much knowledge about molecular defects in carcinogenesis has been acquired, there are still major questions. The mechanisms by which acid exposure may promote neoplastic progression in BE has not been fully elucidated.[19] Chronic esophageal acid exposure can cause direct damage of the BE epithelium and may directly trigger proliferative factors and suppress apoptosis in this way. On the other hand, chronic esophageal acid exposure can cause inflammation of the distal esophagus. Inflammation can damage cellular components, such as proteins, lipids and DNA and may promote proliferation and mutagenesis indirectly.

Direct effects of acid exposure

The direct effect of acid exposure on cell proliferation has been investigated in several in vitro studies. Continuous acid exposure decreases proliferation of BE epithelium by delaying cell cycle progression, whereas intermittent acid exposure increases proliferation and cell survival.[19,24] Several studies suggest that pulsatile acid exposure has hyperproliferative effects by activation of the Na^+-H^+ exchanger and MAPK pathway.

Na^+-H^+ exchanger
Pulsatile acid exposure stimulates the Na^+-H^+ exchanger in BE cells. As long as the pH remains low, cells are arrested in the cell cycle and are unable to proliferate. However, as soon as the external pH is normalised, the activation of the Na^+-H^+ exchanger leads to a temporarily alkalinisation of the cytoplasm. This rise in pH may be sufficient to drive cells from the resting G0/G1 phase to the DNA replicative S phase of the cell cycle and results in hyperproliferation.[25]

MAPK pathways
The mitogen-activated protein kinase (MAPK) pathways are known to transmit extracellular, growth-regulating signals to effector genes in the nucleus. Activation of the MAPK pathways may result in increased proliferation or decreased apoptosis. The MAPK components ERK and p38 can be activated by acid exposure in BE epithelium. Acid induced activation of ERK enhances cell survival, whereas activation of p38 increases cell proliferation.[26] Several studies have shown that acid exposure activates the ERK and p38 pathways, which results in an increase in cyclooxygenase 2 (COX-2) expression.[27] COX-2 catalyzes the conversion of arachidonic acid into prostaglandins, which in turn

induce proliferation of BE epithelium.[28] Apparently, chronic induction of prostaglandins may lead to accumulation of cells with replicative errors and therefore promote carcinogenesis.

Indirect effects of acid exposure

Chronic esophageal acid exposure may lead to inflammation and can injure proteins, lipids and DNA in this way. Such injuries can result in the activation of oncogenes and the inactivation of tumor suppressor genes, facilitating carcinogenesis. Inflammation of BE epithelium results in the production of cytokines from infiltrating inflammatory cells. Besides, during inflammation reactive oxygen and nitrogen species are generated. In this way acid exposure may predispose BE patients to the development of EAC.[29,30]

Cytokine production
In biopsy samples from patients with reflux esophagitis, elevated levels of pro-inflammatory cytokines have been detected. In addition, biopsies of BE with histological evidence of inflammation, express IL-1β, IL-8 and NF-κB. IL-8 has been shown to promote cell proliferation and angiogenesis, NF-κB is known to activate pro-proliferative and anti-apoptotic genes and IL-1β also has been implicated in carcinogenesis, although the mechanism is not yet known. An increase in the expression of these cytokines is seen in EAC biopsy samples as well.[31,32] These data suggest that acid-induced increases in pro-inflammatory cytokine expression may facilitate neoplastic progression of BE.

Reactive oxygen species
Acid induced inflammation can lead to oxidative stress due to the production of reactive oxygen species (ROS). ROS can cause damage to DNA, including double-strand DNA breaks, leading to activation of oncogenes and inactivation of tumor suppressor genes. In biopsy samples from BE patients with esophagitis increased levels of ROS have been detected, measured by lipid peroxidation.[33] When the concentration of ROS exceeds the concentration of antioxidants and genomic repair capacities, the probability of inducing DNA damage is increased. Reduced levels of glutathione and vitamin C are an indication that antioxidant defences are compromised.[34]

Reactive nitrogen species

Nitric oxide (NO) can be derived from dietary nitrate, which is primarily found in green leafy vegetables. A part of the ingested nitrate is concentrated by the salivary glands and secreted in the mouth where bacteria reduce nitrate to nitrite. In the esophagus nitrite encounters gastric acid and is converted to NO. Generation of NO in the esophagus can be genotoxic and potentially carcinogenic. Physiological luminal concentrations of NO can cause DNA damage in the form of double-strand DNA breaks, without loss of cell survival. In addition, gastric acid can increase the levels of intra-epithelial cell NO by regulating inducible nitric oxide synthase (iNOS).[35]

Acid suppression

GERD has been identified as the major risk factor for both BE and EAC.[36] Therefore, therapies aimed at preventing EAC have focused primarily on suppressing acid exposure of the distal esophagus. Esophageal acid exposure can be reduced by pharmalogical or surgical treatment. Pharmalogical treatment mainly involves the use of proton pump inhibitors (PPIs). PPIs suppress the gastric acid production, which in turn reduces gastroesophageal reflux. Surgical treatment most often involves open or laparoscopic fundoplication. By means of fundoplication the normal lower esophageal sphincter function is restored, resulting in reduced reflux of gastric acid. Where PPIs only decrease the reflux of gastric acid, surgery has the ability to prevent any type of reflux. Protection against the damaging effects of acid may only be achieved if acid suppression is relatively complete. Intermittent pulses of gastric reflux into the esophagus may result in enhanced BE cell proliferation, increasing the risk of dysplasia or EAC. It may be important to completely eliminate reflux to reduce the likelihood of developing dysplasia or EAC.[18,37]

Pharmalogical acid suppression

Pharmacological treatment with PPIs is highly effective in relieving reflux symptoms in patients with BE and provides superior relief compared to H_2-receptor antagonists. Furthermore pharmalogical acid suppression heals concomitant esophagitis and ulcers and may prevent stricture formation. Studies in patients with severe GERD have found that aggressive acid suppression also dramatically decreases reflux.[38-41] However, some patients show persistent pathologic acid reflux during PPI treatment. Apparently

complete symptom eradication does not guarantee normalisation of the esophageal pH. This could be explained by decreased sensitivity to acid exposure of BE patients.[42-45] Several investigators have performed 24 hour ambulatory pH monitoring in asymptomatic BE patients receiving omeprazole or lansoprazole. These studies show, that approximately 20% of asymptomatic patients continue to have pathological reflux, despite PPI treatment. Nocturnal acid reflux is the most common finding.[45-49] The persistence of pathological reflux in BE patients despite PPI treatment, may explain why acid suppression therapy fails to completely prevent neoplastic progression.[50] These findings also indicate that pH-monitoring is needed to confirm acid suppression to physiological levels, even when a patient is asymptomatic.[51] With the use of pH monitoring and titrating acid inhibitory medication, it seems possible to achieve complete acid suppression. Several studies have shown that normalisation of the esophageal pH with PPI treatment decreases cell proliferation and stimulates cell differentiation.[37]

Although, there is much literature indicating that PPI treatment may decrease damage by acid exposure, there is also a suggestion that pharmalogical acid suppression may actually increase the generation of BE.[36] Treatment with PPIs can cause secondary hypergastrinemia in some patients, by inhibiting acid secretion and increasing pH. Gastrin is a growth factor for gastrointestinal cell types that express the cholecystokinin-2/gastrin receptor (CCK2R). Several studies have suggested that BE epithelium has increased expression of CCK2R and that gastrin is able to stimulate proliferation in BE. Although PPI therapy and hypergastrinemia seems to increase proliferation in the short term, there is no evidence of long-term harm due to pharmalogical acid suppression.[52-54] Furthermore, an ex vivo study has demonstrated that intermittent acid exposure has anti-proliferative effects on an in vitro nonneoplastic immortalized BE cell line. These findings contradict the results of previous in vitro studies showing that pulsatile acid exposure increases proliferation. The results of this study suggest that acid suppressive therapy in dosages beyond those required to heal reflux esophagitis and reflux symptoms might be unfavourable.[24]

Surgical acid suppression

Anti-reflux surgery is highly effective in relieving reflux symptoms in patients with BE as well. However, the effect of anti-reflux surgery on symptom control in BE patients is inferior to the results achieved in patients with uncomplicated GERD. In approximately 20% of patients with BE, reflux symptoms recur after

surgery. Recurrent symptoms are least common in patients undergoing transthoracic anti-reflux surgery. Although most patients are free of symptoms after anti-reflux surgery, pathological reflux may persist. Anti-reflux surgery decreases the esophageal acid exposure in most patients, but at least 20% of BE patients continue to have abnormal acid exposure. In about 10% of BE patients recurrence of anatomic abnormalities is seen after surgery.[55-57]

Regression of Barrett's esophagus

Regression of BE epithelium is considered to be an important endpoint in the treatment of BE patients, because the risk of neoplastic progression is dependent on the BE length.[58,59] The greater the BE length, the higher the number of cells at risk for the development of dysplasia and EAC. Therefore, regression of BE epithelium should reduce the risk of developing dysplasia and EAC. Acid suppression may produce partial regression of BE by shortening of the columnar epithelium. However, measurement of the surface and length of the BE epithelium is prone to inter- and intra-observer variability. A number of studies investigating regression of BE epithelium during PPI treatment have been published with conflicting results. Several studies reported a decrease in the BE length in patients treated with high dose omeprazole, nevertheless other studies did not report evidence of regression. Most patients treated with PPIs develop islands of squamous epithelium within the BE. These islands may reflect true regression, however in more than one third of biopsy samples microscopic intestinal metaplasia is found.[60-62] The importance of these islands is therefore not clear. In conclusion, there is no convincing evidence that pharmalogical acid suppression results in regression of BE.[63-65] Regression of BE epithelium may also occur after anti-reflux surgery. Published studies show inconsistent results, but most studies have reported regression rates in patients with short segment BE varying between 4% and 20%.[55,66]

Development and progression of dysplasia

Exposure of the esophagus to gastric acid is not only a risk factor for the development of BE, but is also thought to initiate the development and progression of dysplasia. BE patients with HGD or EAC seem to have more frequent episodes of reflux and longer duration of acid exposure compared to BE patients without dysplasia.[67] Long term clinical studies demonstrated that

consistent acid suppression in patients with BE decreases cell proliferation and increases cell differentiation.[37] However, whether acid suppression can truly prevent the development and progression of dysplasia is not clear.

Effect of proton pump inhibitors

Five recent studies have investigated the effect of PPI treatment on the risk of progression to HGD or EAC in BE patients.[68-72] The results of these studies are shown in table 1. Four studies examined the association between PPI use and the risk of the development of HGD or EAC. Three studies reported a strong inverse relationship between PPI use and neoplastic progression in BE (HR 0.39, OR 0.09 and HR 0.12 respectively).[69,70,72] In the fourth study most patients (>90%) had used a PPI. As a result, the association between PPI use and the development of HGD or EAC could not be evaluated.[68] In addition, three studies investigated whether the risk of neoplastic progression was influenced by the duration of PPI use. These studies demonstrated that a longer duration of PPI treatment was associated with a reduced risk of developing dysplasia or cancer.[69,70,72] Furthermore, in one study the time between BE diagnosis and start of PPI use was investigated. This study reported an increased risk of neoplastic progression when PPI therapy was started more than 2 years after the initial BE diagnosis.[71] Altogether the results of these studies suggest that use of PPIs may prevent the development and progression of dysplasia in BE patients. However, PPIs are not able to completely eliminate the risk of neoplastic progression.

Effect of anti-reflux surgery

The development of dysplasia after anti-reflux surgery has been investigated as well. Six uncontrolled studies have evaluated the risk of neoplastic progression after anti-reflux surgery without PPI treatment.[55,56,73-76] The results of these studies are shown in table 2. The published studies show almost uniformly a low incidence of progression to HGD or EAC after fundoplication. However, the number of included patients is low, since only a minority of patients is referred for fundoplication. Large studies will be needed to demonstrate a clinically relevant effect of anti-reflux surgery.

Table 1. Studies evaluating the effect of proton pump inhibitors

Study	Design	Sample size		Follow-up	Effect on neoplastic progression
Nguyen[68]	Case control	116 696	EAC BE		PPI use in 95% versus 94% ÌDR 1.50, p=0.67, ever use
Nguyen[69]	Cohort	33 251	HGD/EAC BE	7.6 years	PPI use in 52% versus 69% HR 0.39 (0.19 - 0.80), ever use HR 0.38 (0.18 - 0.80), use > 3 y
de Jonge[70]	Case control	91 244	EAC BE		PPI use in 47% versus 93% OR 0.09 (0.05 - 0.2), ever use OR 0.05 (0.02 - 0.1), use > 0.5 y
Hillman[71]	Cohort	11 339	HGD/EAC BE	4.7 years	PPI use in 91% HR 21 p=0.003, start after > 2 y
El-Serag[72]	Cohort	14 180	HGD BE	5.0 years	PPI use in 66% HR 0.12, p=0.002, ever use Duration 0 versus 2 y, p=0.006

BE, Barrett's esophagus; HGD, high-grade dysplasia; EAC, esophageal adenocarcinoma; PPI, proton pump inhibitor, IDR, incidence density ratio; HR, hazard ratio; OR, odds ratio; y, years

Table 2. Studies evaluating the effect of anti-reflux surgery

Study	Design	Sample size		Follow-up	Effect on neoplastic progression
Biertho[74]	Cohort	92	BE	4.2 years	None developed HGD or EAC
Abbas[73]	Cohort	49	BE	2.4 years	1 patient developed EAC
O'Riodan[76]	Cohort	58	BE	4.9 years	2 patients developed EAC
Desai[75]	Cohort	68	BE	2.5 years	None developed HGD or EAC
Oelschlager[56]	Cohort	106	BE	3.3 years	1 patient developed EAC
Hofstetter[55]	Cohort	97	BE	5 years	None developed HGD or EAC

BE, Barrett's esophagus; HGD, high-grade dysplasia; EAC, esophageal adenocarcinoma

Effect of proton pump inhibitors compared to anti-reflux surgery
At last few studies have compared the ability of pharmalogical and surgical acid suppression to prevent the development and progression of dysplasia in BE patients.[57,77-79] The results of these studies are shown in table 3. In one study a trend was seen toward anti-reflux surgery being more protective, but not enough patients were included in the surgery arm to reach statistical

significance. However, this study did not control for many selection factors, which may have led to confounding.[78] Two small randomized controlled trials showed that there was no difference between pharmalogical and surgical acid suppression with respect to preventing neoplastic progression in BE. Although in these studies successful anti-reflux surgery seemed to be more efficient than pharmalogical treatment.[57,79] At last a meta-analysis has been published, evaluating the incidence of EAC in patients with anti-reflux surgery compared to patients treated with PPIs. The reported incidence of EAC was 3.8/1000 patient-years after anti-reflux surgery and 5.3/1000 patient-years with pharmalogical acid suppression. Apparently, there is no difference between anti-reflux surgery and PPI treatment, with respect to prevention of the development and progression of dysplasia in BE.[77]

Table 3. Studies comparing proton pump inhibitors and anti-reflux surgery

Study	Design	Sample size		Follow-up	Effect on neoplastic progression
Gatenby[78]	Cohort	532	PPI	5 years	HGD/EAC in 30 versus 0 patients
		41	Surgery		p=0.13
Corey[77]	Meta-analysis		PPI	4906 py	Incidence EAC 0.53% versus 0.38%
			Surgery	4678 py	p=0.29
Parrilla[79]	RCT	43	PPI	5 years	HGD in 2 versus 2 patients
		58	Surgery		Not significant
Ortiz[57]	RCT	27	PPI	4.5 years	HGD in 1 versus 1 patient
		32	Surgery		Not significant

PPI, proton pump inhibitor; HGD, high-grade dysplasia; EAC, esophageal adenocarcinoma; py, person-years; RCT, randomized controlled trial

Conclusions

Acid exposure appears to play a central role in the development of BE. Besides, direct and indirect acid exposure seems to be important in carcinogenesis. Even though acid suppression may not heal intestinal metaplasia in patients with BE, the available studies strongly suggests that pharmalogical as well as surgical acid suppression prevents the development and progression of dysplasia in patients with BE. However, the evidence is limited and large (randomized) controlled trials are needed to draw any definitive conclusions.

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Chapter 3

Proton pump inhibitors reduce the risk of neoplastic progression in patients with Barrett's esophagus

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Abstract

Introduction: Acid exposure plays an import role in the initiation of Barrett's esophagus and its progression toward esophageal adenocarcinoma. Acid suppressants are therefore frequently used in patients with Barrett's esophagus, but it is unclear whether this is truly an effective prevention strategy. We investigated therefore whether acid suppression reduces the risk of neoplastic progression in patients with Barrett's esophagus.

Methods: 540 patients with Barrett's esophagus were included and followed in a multicenter prospective cohort study. Information on medication use was collected with each surveillance visit, and was cross-checked with pharmacy records. Patients also completed a questionnaire on their use of over-the-counter medication. Incident cases of high-grade dysplasia and esophageal adenocarcinoma were identified during follow-up. Time-dependent Cox-regression models were used to investigate the effect of acid suppression on the risk of neoplastic progression.

Results: 40 (7%) patients developed high-grade dysplasia or esophageal adenocarcinoma during a median follow-up period of 5.2 years. Use of histamine-2 receptor antagonists did not affect the risk of neoplastic progression. However, use of proton pump inhibitors (PPIs) at inclusion was associated with a trend toward a protective effect (HR 0.47; 95% CI 0.19-1.18) and PPI use during follow-up was associated with a significantly reduced risk of neoplastic progression (HR 0.21; 95% CI 0.07-0.66). Prolonged PPI use and good adherence were associated with an additional protective effect. The prevalence of esophagitis decreased during PPI use, but Barrett's esophagus length was not affected.

Conclusion: PPI use is associated with a reduced risk of neoplastic progression in patients with Barrett's esophagus.

Introduction

In Barrett's esophagus (BE) the squamous epithelium of the distal esophagus is replaced by metaplastic columnar epithelium containing goblet cells as a result of chronic esophageal acid exposure.[1] BE patients have an increased risk of developing esophageal adenocarcinoma (EAC) with an incidence of 0.5% per year.[2, 3] Strategies to prevent the development of EAC have focused primarily on acid suppression and early detection of EAC during surveillance. However, despite these prevention strategies the incidence of EAC has risen rapidly.[4]

Because esophageal acid exposure plays an import role in the initiation of BE and its progression toward EAC, acid suppression with proton pump inhibitors (PPIs) and to a lesser extent histamine-2 receptor antagonists (H_2RAs) has become a mainstay in the treatment of BE patients. However, in the absence of data from long-term prospective clinical trials current guidelines do not provide strong recommendations for the use of acid suppressants in BE patients.[5-7] PPIs are effective in relieving reflux symptoms, healing esophagitis, and decreasing proliferation, but may also cause secondary hypergastrinemia, which induces proliferation and perhaps expansion of metaplasia.[8] To justify prolonged PPI use in BE patients it is therefore essential to provide scientific evidence that PPIs truly prevent neoplastic progression. Some observational studies investigated the effect of acid suppressants on the risk of neoplastic progression, but were unable to draw definite conclusions, as only small numbers of patients were included or clinical information was unavailable.[9-13] The aim of this prospective cohort study was therefore to investigate whether acid suppression reduces the risk of neoplastic progression in patients with BE.

Methods

Study design

We conducted a multicenter prospective cohort study in 3 academic and 12 regional hospitals in the Netherlands. Between November 2003 and December 2004 756 patients were included with known or newly diagnosed BE. The endoscopic diagnosis was confirmed by the presence of intestinal metaplasia. We excluded patients with BE shorter than 2 cm, patients who had anti-reflux surgery and patients with a history of high-grade dysplasia (HGD) or EAC. Incident cases of HGD or EAC were identified during follow-up. Two hundred sixteen patients dropped out of the study, because of severe co-morbidity

(n=30), death unrelated to BE (n=18), refusal of participation (n=89), migration (n=10), no follow-up endoscopy (n=66), or neoplastic progression within 9 months after inclusion (n=3). Patients who dropped out were older than those still participating in surveillance (median 67 years versus 61 years), but there were no differences in gender, time of BE diagnosis, BE length, esophagitis, histology or medication use.

Endoscopic surveillance

Surveillance was performed according to the guidelines of the American College of Gastroenterology. Patients without dysplasia underwent gastroscopy with biopsy sampling every 3 years and patients with low-grade dysplasia (LGD) every year.[7] Patients who developed HGD or EAC were considered to have reached an endpoint and received appropriate treatment. All endoscopic procedures were performed by experienced gastroenterologists, according to a standardized protocol. Endoscopic landmarks such as the diaphragm, gastroesophageal junction and squamocolumnar junction were noted. In addition, we graded the presence of esophagitis according to the Los Angeles Classification, and reported abnormalities including nodules, ulcers and erosions.[14] Targeted biopsies were taken from mucosal abnormalities and four-quadrant biopsies were taken every 2 cm from the most distal to the most proximal part of the BE epithelium. At each surveillance visit, patients completed a questionnaire on demographic factors, height, weight, smoking habits, alcohol use, symptoms, and medication use. Data were prospectively recorded and processed in a central database.

Histological examination

Biopsy specimens were fixed with formalin and embedded in paraffin. Four-micrometer sections were cut and stained with haematoxylin-eosin. The slides were first graded by a local pathologist and then by an expert gastrointestinal pathologist for second opinion. When the pathologists disagreed on the grade of dysplasia, slides were reviewed by a second expert pathologist. Pathologists were blinded to the diagnosis of each other. A final diagnosis was made only if at least 2 pathologists agreed on the grade of dysplasia. If there was disagreement, a panel of expert pathologists reviewed the slides and a final diagnosis was made based on consensus agreement.

Medication use

Information on medication use was collected with each surveillance visit and patients were asked to complete a questionnaire on their use of over-the-counter medication. All collected information was cross-checked with pharmacy records, which contain information on delivered medications including dose and time of prescription and including over-the-counter medication. Using the pharmacy records, we recorded filled prescriptions for H_2RAs (cimetidine, famotidine, nizatidine, ranitidine) and PPIs (omeprazole, esomeprazole, rabeprazole, pantoprazole, lansoprazole) from the time of BE diagnosis to the most recent endoscopy. At baseline patients were classified as current user of H_2RAs or PPIs when they used these drugs at that time for at least 1 month, as former user when they used these drugs for at least 1 month but not at the time of inclusion, and as non-user when they used these drugs for less than 1 month. During follow-up patients were classified as user of H_2RAs or PPIs according to their exact start and stop dates. The duration of H_2RA and PPI use was calculated by adding the duration of individual prescriptions starting from the time of inclusion. Adherence was calculated by dividing the duration of medication use by the duration of follow-up. To compare the dose of various medicines we calculated a standardized dose by dividing the Prescribed Daily Dose (PDD) by the Defined Daily Dose (DDD) as described by the World Health Organization.[15]

Ethics

The study protocol was approved by the Institutional Review Boards of the Erasmus University Medical Centre, as well as those of all participating hospitals. Before the first endoscopy, written informed consent was obtained from all patients. Patients also gave written informed consent to obtain their pharmacy records.

Statistical analysis

The effect of acid suppressants on the risk of neoplastic progression was estimated in Cox-regression models. Neoplastic progression was defined as the development of HGD or EAC at least 9 months after inclusion to avoid inclusion of patients in whom HGD or EAC was missed at index endoscopy. Follow-up time was defined as the time from 9 months after inclusion to the most recent surveillance endoscopy or the endoscopy that resulted in a diagnosis of neoplastic progression. The effect of H_2RA and PPI use during follow-up was

estimated in time-dependent models, in which patients switched between groups according to the exact start and stop dates of their medication use. Kaplan Meier curves were constructed using modulated renewal processes.[16] In multivariable Cox-regression models, we adjusted for age, gender, time of BE diagnosis, BE length, esophagitis, baseline histology, and use of aspirin, NSAIDs, and statins. To investigate a duration and dose-response relationship we evaluated several durations, levels of adherence, and doses of PPI use. In addition, we evaluated changes in BE length and prevalence of esophagitis among PPI-users using the Friedman test. Two sided P-values <0.05 were considered to be statistically significant. Data were analysed using SPSS Statistics (version 19.0, Chicago, Illinois, USA).

Results

Table1. Baseline characteristics and the risk of neoplastic progression in BE

		Cohort n = 540	Progression n = 40	HR (95%CI)
Age	Median, years	61 (53-68)	66 (57-72)	1.04 (1.01-1.07)
Sex	Male	386 (71%)	33 (82%)	1.86 (0.82-4.21)
BMI	≤ 25 kg/m^2	151 (28%)	15 (38%)	Reference
	> 25-30 kg/m^2	273 (51%)	17 (42%)	0.62 (0.31-1.25)
	> 30 kg/m^2	105 (19%)	8 (20%)	0.77 (0.32-1.81)
Smoking	Current	103 (19%)	8 (20%)	1.05 (0.49-2.28)
Alcohol use	Current	418 (77%)	32 (80%)	1.04 (0.48-2.25)
Reflux	Current	155 (29%)	15 (38%)	1.46 (0.77-2.76)
H$_2$RA use	No	469 (87%)	35 (87%)	Reference
	Former	57 (10%)	4 (10%)	1.00 (0.36-2.81)
	Current	14 (3%)	1 (3%)	0.83 (0.11-6.03)
PPI use	No	68 (13%)	10 (25%)	Reference
	Former	10 (2%)	2 (5%)	1.32 (0.29-6.04)
	Current	462 (85%)	28 (70%)	0.43 (0.21-0.88)
Diagnosis	At inclusion	80 (15%)	8 (20%)	1.39 (0.64-3.01)
BE length	Median, cm	4 (2-5)	5 (4-7)	1.17 (1.06-1.29)
Esophagitis	Current	48 (9%)	9 (23%)	2.99 (1.42-6.27)
Histology	LGD	76 (14%)	17 (43%)	4.98 (2.66-9.32)

BE, Barrett's esophagus; HR, hazard ratio; CI, confidence interval; BMI, body mass index; H$_2$RA, histamine-2 receptor antagonist; PPI, proton pump inhibitor; LGD, low-grade dysplasia

Patient characteristics

A total of 540 BE patients were included in this study and followed for a median duration of 5.2 years (interquartile range (IQR) 3.5-5.7). Patients had a median age of 61 years and 386 (71%) patients were male. During follow-up 28 patients developed HGD and another 12 patients developed EAC. The annual incidence of HGD and EAC was 1.6% (95% confidence interval (CI) 1.1-2.1) and the incidence of EAC alone was 0.5% (95% CI 0.2-0.8). The risk of neoplastic progression increased with age, BE length, esophagitis, and LGD (Table 1).

Effect of histamine-2 receptor antagonists

At inclusion in the study 14 (3%) patients used an H_2RA for a median duration of 2.0 years and 20 (4%) patients used an H_2RA during follow-up for a median duration of 2.9 years. All H_2RA-users also used PPIs except for 1 patient. H_2RA use did not affect the risk of neoplastic progression (hazard ratio (HR) 0.83; 95% CI 0.11-6.03).

Effect of proton pump inhibitors

At inclusion in the study 462 (85%) patients used a PPI for a median duration of 4.0 years. PPI use at inclusion was associated with a reduced risk of neoplastic progression (HR 0.43; 95% CI 0.21-0.88) and remained associated with trend toward a protective effect after adjusting for age, gender, time of BE diagnosis, BE length, esophagitis, histology and use of other medications (HR 0.47; 95% CI 0.19-1.18). PPI-users were less likely to have reflux or esophagitis, but were more likely to be diagnosed with BE before inclusion than non-users (Table 2).

During follow-up 532 (99%) patients used a PPI for a median duration of 5.1 years. In a time-dependent analysis PPI use during follow-up was associated with a reduced risk of neoplastic progression (HR 0.15; 0.06-0.40) and remained associated with a protective effect after adjusting for age, gender, BE length, histology, baseline PPI use and use of other medications (HR 0.21; 0.07-0.66) (Table 3). Patients who used a PPI during follow-up were younger and reported fewer reflux symptoms than non-users. The cumulative incidence of neoplastic progression is shown in Figure 1 and was lower in PPI-users than in non-users (log rank p=.002). There was no significant difference between the effect of various PPIs (log rank p=.075). However, the risk of neoplastic progression decreased with prolonged PPI use (p<.001) indicating a duration response relationship. In addition, PPI use for at least 90% of the follow-up time was associated with a lower risk of neoplastic progression than PPI use for less

than 90% of the follow-up time (HR 0.24; 95% CI 0.08-0.71). PPI dose did not affect the risk of progression (HR 1.27; 95% CI 0.64-2.49). As a sensitivity analysis, we investigated the effect of PPIs in case that the diagnosis of HGD or EAC was made 3 months earlier and found no difference in the magnitude or direction of the effect.

Table 2. Baseline characteristics of patients using proton pump inhibitors

		Baseline			Follow-up		
		PPI-user n = 462	Non-user n = 78	P	PPI-user n = 514	Non-user n = 26	P
Age	Median, years	60 (53-67)	61 (53-70)	.377	60 (53-67)	68 (59-72)	.015
Sex	Male	328 (71%)	58 (74%)	.543	365 (71%)	21 (81%)	.282
BMI	≤ 25 kg/m^2	126 (28%)	25 (33%)	.364	139 (28%)	12 (46%)	.076
	> 25-30 kg/m^2	239 (53%)	34 (44%)		261 (52%)	12 (46%)	
	> 30 kg/m^2	87 (19%)	18 (23%)		103 (20%)	2 (8%)	
Smoking	Current	84 (19%)	19 (25%)	.205	97 (19%)	6 (23%)	.627
Alcohol use	Current	357 (79%)	61 (79%)	.935	398 (79%)	20 (77%)	.803
Medication	Aspirin	76 (17%)	6 (8%)	.046	80 (16%)	2 (8%)	.403
	NSAID	26 (6%)	2 (3%)	.406	27 (5%)	1 (4%)	.999
	Statin	91 (20%)	11 (14%)	.243	97 (19%)	5 (19%)	.999
Reflux	Current	121 (27%)	34 (44%)	.002	143 (28%)	12 (46%)	.050
Diagnosis	At inclusion	31 (7%)	49 (63%)	<.001	75 (15%)	5 (19%)	.568
BE length	Median, cm	4 (2-5)	4 (3-6)	.254	4 (2-5)	4 (2-6)	.881
Esophagitis	Current	27 (6%)	21 (27%)	<.001	44 (9%)	4 (15%)	.278
Histology	LGD	63 (14%)	13 (17%)	.477	69 (13%)	7 (27%)	.076

BE, Barrett's esophagus; BMI, body mass index; PPI, proton pump inhibitor; NSAID, nonsteroidal anti-inflammatory drug, LGD, low-grade dysplasia

Proton pump inhibitor use and Barrett's esophagus length
At inclusion in the study, patients had a median BE length of 4 cm (IQR 2-5). There was no difference in BE length between PPI-users and non-users (p=.420). PPI-users had a median BE length of 4 cm (IQR 2-5) at inclusion in the study, 4 cm (IQR 2-5) after 2 years follow-up, and 4 cm (IQR 2-5) after 4 years follow-up. None of the patients showed complete regression of Barrett epithelium during PPI use. Thus BE length did not change during prolonged PPI use (Friedman p=.179).

Table 3. Use of proton pump inhibitors and the risk of progression

		Cohort 2543 py	Progression 104 py	HR (95% CI)	HR (95% CI)[a]
Use during follow-up[1]	No	55 (2%)	12 (12%)	Reference	Reference
	Yes	2488 (98%)	92 (88%)	0.15 (0.06-0.40)	0.21 (0.07-0.66)
Type[1]	Omeprazole	832 (33%)	37 (35%)	0.17 (0.06-0.48)	0.29 (0.08-1.10)
	Esomeprazole	572 (23%)	22 (21%)	0.20 (0.07-0.60)	0.30 (0.08-1.12)
	Rabeprazole	533 (21%)	8 (8%)	0.08 (0.02-0.30)	0.10 (0.02-0.49)
	Pantoprazole	416 (16%)	13 (12%)	0.08 (0.02-0.33)	0.12 (0.02-0.65)
	Lansoprazole	135 (5%)	12 (12%)	0.39 (0.11-1.35)	1.01 (0.21-4.86)
Duration[2]	> 0 to 2 years	668 (26%)	42 (40%)	2.12 (0.54-8.32)	3.46 (0.78-15.37)
	≥ 2 to 4 years	1004 (40%)	32 (31%)	0.30 (0.06-1.38)	0.44 (0.09-2.29)
	≥ 4 years	816 (32%)	18 (17%)	0.06 (0.02-0.18)	0.07 (0.02-0.26)
Adherence	≥ 90%	2448 (97%)	87 (90%)	0.22 (0.08-0.61)	0.24 (0.08-0.71)
Dose	Once a day	1862 (73%)	76 (73%)	Reference	Reference
	Twice a day	626 (25%)	16 (15%)	1.03 (0.48-2.20)	1.31 (0.60-2.83)
	PDD/DDD ≤ 1	1353 (53%)	50 (48%)	Reference	Reference
	PDD/DDD > 1	1135 (45%)	42 (40%)	1.12 (0.58-2.19)	1.27 (0.64-2.49)

HR, hazard ratio; CI, confidence interval; PDD/DDD, prescribed/defined daily dose; py, person-years; [a] Adjusted for age, gender, BE length, esophagitis, histology, medication, baseline[1], duration[2]

Figure 1. Cumulative incidence of high-grade dysplasia (HGD) and esophageal adenocarcinoma (EAC), stratified by proton pump inhibitor (PPI) use.
━ No PPI use, ― PPI use, Log rank P=.002
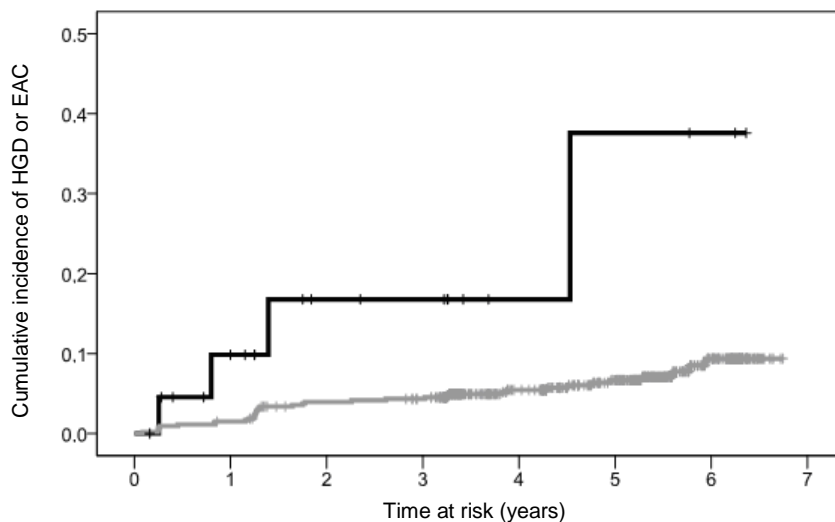
Proton pump inhibitor use and esophagitis

Esophagitis was diagnosed in 48 (9%) patients at inclusion in the study and was reported less often in PPI-users than in non-users (6% versus 27% p=<.001). Of all PPI-users, 44 (9%) patients were diagnosed with esophagitis at inclusion, 17 (3%) after 2 years follow-up, and 12 (2%) after 4 years follow-up. Of all patients with baseline esophagitis 85% showed complete healing with PPI use. Thus the prevalence of esophagitis significantly decreased with prolonged PPI use (Friedman p=<.001) (Figure 2).

Figure 2. Prevalence of esophagitis in patients using proton pump inhibitors

Grade A, Grade B, Grade C, Grade D, Friedman P=<.001
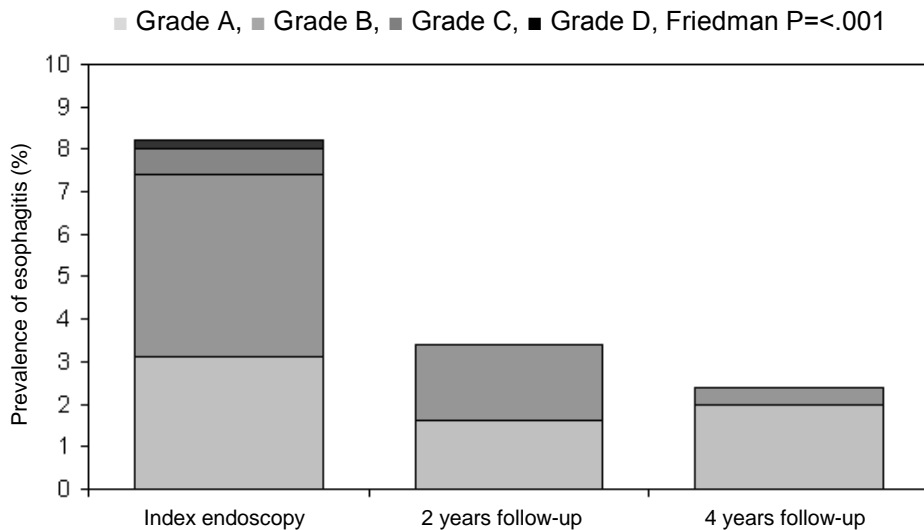
Discussion

In this large prospective cohort study, PPI use was associated with 75% reduction in the risk of neoplastic progression in patients with BE, independent of age, gender, BE length, esophagitis, histology, and use of other medications. H_2RA use did not affect the risk of neoplastic progression.

To our knowledge this is the first methodological sound prospective study which shows that PPIs strongly reduce the risk of neoplastic progression in BE. The protective effect of PPIs increased with prolonged use and good adherence, supporting a causal relationship. In addition, use of all PPIs was associated with a reduced risk of neoplastic progression, indicating that the protective effect is a class effect, and likely related to the acid suppressive mechanism. However,

none of the PPIs could completely prevent neoplastic progression. Previous studies have shown that 20% of BE patients continue to have pathological reflux despite PPI use.[17] Taken into account that intermittent esophageal acid exposure enhances proliferation, this may explain why BE patients remain at risk for neoplastic progression during PPI use.[18] Since long segment BE and esophagitis are risk factors for neoplastic progression, healing of esophagitis and potentially regression of BE are considered important clinical endpoints.[19] In our study, the prevalence of esophagitis decreased during PPI use, but BE length was not affected, indicating that PPIs not induce regression of BE.

Three previous cohort studies and 2 case-control studies investigated the association between PPI use and risk of neoplastic progression in BE, but were not able to provide definite conclusions as only small numbers of patients were included or essential clinical information was unavailable. Moreover, these studies were not able to perform time-dependent analyses. The results of our study are consistent with those of previous studies, which all reported an inverse relationship between PPI use and neoplastic progression in BE and a decreased risk with prolonged PPI use. One case control study was not able to evaluate the association between PPI use and the risk of cancer development because of the small number of patients with no PPI use.[9, 10, 12] Previous studies also reported that PPIs are effective in healing esophagitis, but showed conflicting results with regard to the effect on BE length.[20-24]

In contrast to studies in BE, studies in the general population and patients with reflux disease have suggested that PPI use is in fact associated with an increased risk of developing EAC.[25, 26] However, these results are probably subject to confounding by indication, whereby the underlying indication for PPI use actually is the risk factor for developing EAC rather than the PPI use itself.

Although prolonged PPI use is accompanied with considerable costs, it is an effective strategy to prevent neoplastic progression in BE, which obviates the need for expensive endoscopic mucosal resection, ablation therapies, and surgical resection. In addition, PPIs are effective in relieving reflux symptoms and consequently patients are exceptionally medication compliant, which is corroborated by the overall adherence of 98% in our study. Prolonged PPI use is therefore justified and feasible in BE patients and should be strongly recommended, in particular in guidelines.

This study has several strengths, including the large sample size and long follow-up time. Since we included all consecutive BE patients in three academic and twelve regional hospitals, our cohort should be representative for the BE

population in Western countries. This is corroborated by the observation that the incidence of EAC in our study equals the incidence estimated in most studies.[3] We excluded patients with neoplastic progression within the first 9 months after inclusion, since HGD or EAC may be missed at index endoscopy in these patients. Although this cut-off point is somewhat arbitrary, the results stayed the same when we excluded all patients with neoplastic progression within the first 6 months, the first year or even the first 2 years after inclusion. In contrast to most observational studies we used a standardized endoscopy and biopsy protocol and all histological diagnoses were made based on consensus. Because users of acid suppressants may differ from non-users in other ways than use of these drugs, we collected information on possible confounding factors such as age, sex, time of BE diagnosis, BE length, histology and use of other medications such as aspirin, NSAIDs, and statins and we adjusted for these factors in multivariable models.[27, 28] In addition, we composed a complete picture of the medication use, including over-the-counter medication.

This study also has some limitations. Despite the large sample size, only 8 (2%) patients never used a PPI and 18 (3%) patients used a PPI during a part of their follow-up period. Although this reflects clinical practice in Western countries and is representative for a disease in which patients seek to avoid reflux symptoms, it limits the options for investigating the effect of PPIs. Because not all patients used PPIs throughout their entire follow-up period, we were able to perform time-dependent analyses. Secondly, only patients with BE of at least 2 cm were included in this study and therefore we are not sure whether these results also apply to patients with short BE. However, since the risk of neoplastic progression increases with BE length, the protective effect of PPIs is also most relevant in patients with long BE. Thirdly, since this is an observational study we cannot exclude uncontrolled confounding despite our efforts to consider this as much as possible. We did not have information on socioeconomic factors and although esophagitis was included as a covariate in the multivariable model, the question remains whether concomitant reflux esophagitis plays an additive, synergistic or dominant role in the development of EAC. A randomized controlled trial would be the ideal way to investigate the effect of PPIs without the risk of confounding. Although not impossible, it will be difficult to perform such a trial, since many BE patients suffer from reflux symptoms without the use of a PPI. Finally, patients were informed about changes seen during endoscopy or histology. This may have influenced their medication use or lifestyle.

In conclusion, this large prospective cohort study shows that PPI use is associated with a strongly reduced risk of neoplastic progression in BE patients, and that this protective effect increases with prolonged PPI use and good adherence.

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Chapter 4

Nonsteroidal anti-inflammatory drugs (NSAIDs) and statins have chemopreventive effects in patients with Barrett's esophagus

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Abstract

Introduction: The incidence rate of both Barrett's esophagus and esophageal adenocarcinoma has increased despite surveillance of patients with Barrett's esophagus. Limited data suggest that use of nonsteroidal anti-inflammatory drugs (NSAIDs) and statins may reduce the risk of neoplastic progression. The aim of this study was therefore to investigate whether use of NSAIDs or statins reduces the risk of neoplastic progression in patients with Barrett's esophagus.

Methods: 570 patients with Barrett's esophagus were included and followed in a prospective cohort study in 3 academic and 12 regional hospitals throughout the Netherlands. Information on medication use was collected in patient interviews at each surveillance visit and cross-checked with pharmacy records. Patients also completed a questionnaire on use of over-the-counter medication. Incident cases of high-grade dysplasia and adenocarcinoma were identified during follow-up.

Results: During a median follow-up of 4.5 years, 38 patients (7%) developed high-grade dysplasia or adenocarcinoma. After Barrett's esophagus diagnosis, 318 (56%) patients used NSAIDs for a median duration of 2 months, 161 (28%) used aspirin for a median duration of 5 years, 209 (37%) used statins for a median duration of 5 years and 107 (19%) used both NSAIDs and statins. NSAID and statin use were associated with a reduced risk of neoplastic progression (HR 0.47; P=.030 and HR 0.46; P=.048 respectively). Use of both NSAIDs and statins was associated with an additive protective effect (HR 0.22; P=.028).

Conclusion: Use of NSAIDs or statins is associated with a reduced risk of neoplastic progression in patients with Barrett's esophagus. Use of both NSAIDs and statins appears to have an additive protective effect.

Introduction

Barrett's esophagus (BE) is a premalignant condition in which the normal squamous epithelium of the distal esophagus is replaced by metaplastic columnar epithelium containing goblet cells.[1] It is a relatively common condition with an estimated prevalence of 1–2% in Western countries.[2-4] Chronic gastroesophageal reflux disease (GERD) appears to play a central role in the development of BE epithelium and approximately 10% of patients with GERD will eventually develop BE.[5] BE patients have an 30 to 125-fold increased risk for developing esophageal adenocarcinoma (EAC) with an annual incidence of approximately 0.5%.[6-7] Unfortunately, it is not yet possible to predict which patients have the highest risk of neoplastic progression and as a result endoscopic follow-up is recommended in all BE patients.[8-9] Strategies to prevent the development of EAC in BE have focused primarily on reversal of BE epithelium and early detection of EAC during surveillance. However, despite surveillance the incidence of EAC has been rising rapidly.[10-11] Therefore, new strategies are needed to prevent the development of EAC. Multiple studies have given support to the use of chemoprevention in the treatment of several cancers including esophageal cancer.[12] Observational studies have suggested that use of nonsteroidal anti-inflammatory drugs (NSAIDs) and statins may also reduce the risk of neoplastic progression in BE patients.[13-15] Chemoprevention with a combination of NSAIDs and statins might provide an even stronger risk reduction.[16-17] However only limited studies have investigated the effect of NSAID and statin use on the development of high-grade dysplasia (HGD) or EAC in BE. Most studies included only small numbers of patients and lacked clinical information. To our knowledge no large prospective cohort studies have been published investigating the combination of NSAID and statin use. The aim of this study was therefore to investigate whether use of NSAIDs and statins reduces the risk of neoplastic progression in BE patients.

Methods

Study design
We conducted a multicenter prospective cohort study in 3 university medical centers and 12 regional hospitals throughout the Netherlands. Between November 2003 and December 2004, 786 patients were included, presenting at the endoscopy unit with known or newly diagnosed BE. We excluded patients

with BE shorter than 2 cm, patients younger than 18 years, and patients with HGD or EAC in the past or at index endoscopy. There were no restrictions regarding medication use. The endoscopic BE diagnosis was confirmed in all patients by the presence of intestinal metaplasia. Incident cases of HGD or EAC were identified during follow-up. Two hundred sixteen patients dropped out of the study, because of severe co-morbidity (n=30), death unrelated to BE (n=18), migration (n=10), refusal of participation (n=155), or neoplastic progression within 9 months after inclusion (n=3). Patients who dropped out, were older than those still participating in surveillance (median 66 years versus 60 years), but there were no differences in gender, BE length, baseline histology and medication use.

Endoscopic surveillance
A central trial coordinator controlled the follow-up of all BE patients participating in the study. Surveillance was performed according to the ACG guidelines: patients without dysplasia received surveillance every 3 years and patients with low-grade dysplasia (LGD) every year.[18] Patients who developed HGD or EAC during follow-up were considered to have reached an endpoint, and received appropriate endoscopic or surgical treatment. Endoscopies were performed by experienced gastroenterologists at the 15 participating hospitals, according to a standardized protocol. During upper endoscopy, endoscopic landmarks such as the diaphragm, gastroesophageal junction and squamocolumnar junction were determined, the presence and grade of esophagitis was documented according to the Los Angeles Classification and mucosal abnormalities were reported including nodules, ulcers and erosions.[19] Targeted biopsies were taken from mucosal abnormalities and in addition four-quadrant biopsies were taken every 2 cm from the most distal to the most proximal part of the BE epithelium. At each surveillance visit, patients filled out a questionnaire on demographic factors, length, weight, former and current smoking habits, former and current alcohol use, time of BE diagnosis, relatives with BE or EAC, symptoms such as reflux, regurgitation and dysphagia, and medication use. The endoscopic findings and clinical data were prospectively recorded in individual case-record forms, and processed in a central database by the trial coordinator.

Histological examination
Biopsy specimens were fixed with 10% formalin and embedded in paraffin. Four-micrometer serial sections were cut and stained with haematoxylin-eosin.

The histological slides were first examined by a local pathologist at the participating hospital. Dysplasia was graded according to the consensus criteria of 1988, with adjustments as proposed in 2001.[20-21] Previous studies have shown high interobserver variability in the interpretation of dysplasia in BE.[22] All biopsy specimens were therefore reviewed by 1 or 2 expert GI-pathologists. These expert pathologists were blinded to the diagnosis of the local pathologist. A final diagnosis was made only if at least 2 pathologists agreed on the grade of dysplasia. If there was disagreement between the pathologists, a panel of expert pathologists reviewed the slides as well, and the final diagnosis was made based on consensus agreement.

Medication use

Information on medication use after BE diagnosis, was collected in patient interviews with each surveillance visit. In addition, patients filled out a questionnaire on their use of over-the-counter medication. This questionnaire included items on NSAID use without prescription, such as medication name, dosage and frequency of use. The information collected in both the patient interviews and questionnaire was cross-checked using pharmacy records. All patients gave written consent for requesting their complete pharmacy records. The pharmacy records contain information on all delivered medications including dose, time of prescription and over-the-counter medication. The pharmacies are legally required to keep these pharmacy records for at least 15 years. Using the pharmacy records, we recorded filled prescriptions for PPIs (omeprazole, lansoprazole, rabeprazole, pantoprazole, esomeprazole), non-selective NSAIDs (nsNSAIDs) (acetylsalicylic acid > 325 mg per day, carbasalate calcium > 325 mg per day, diclofenac, flurbiprofen, ibuprofen, indomethacin, ketoprofen, nabumetone, naproxen, aceclofenac, dexibrufen, dexketoprofen, fenylbutazon, piroxicam, sulindac, tolmetin), cyclooxygenase-2 (COX-2) inhibitors (celecoxib, rofecoxib, etoricoxib, meloxicam), low dose aspirin (acetylsalicylic acid ≤ 100 mg per day, carbasalate calcium ≤ 100 mg per day), and statins (simvastatin, lovastatin, atorvastatin, fluvastatin, pravastatin) from the time of BE diagnosis to the most recent endoscopy or to the endoscopy that resulted in the diagnosis of HGD or EAC. For each patient, the total duration of filled prescriptions for PPIs, NSAIDs, aspirin, and statins was calculated by adding the duration of individual prescriptions and subtracting overlap in dates. Patients were classified as users of PPIs, NSAIDs, aspirin or

statins, when the pharmacy had provided medication for at least 1 month, to ensure that patients with single-use were not identified as users.

Ethics
The study protocol was approved by the Medical Ethics Committee of the Erasmus University Medical Center, and by the local Medical Ethics Committees of all participating hospitals. Before the first endoscopy, written informed consent was obtained from all patients. Patients also gave written informed consent to obtain their pharmacy record.

Statistical analysis
The incidence rate of neoplastic progression was calculated by dividing the number of patients with HGD or EAC by the total person-years of follow-up in the study. The effect of NSAID, aspirin and statin use on the risk of neoplastic progression was estimated in Cox proportional-hazards models. Follow-up time was defined as the time from inclusion in the study to the most recent surveillance endoscopy, or to the endoscopy that resulted in a diagnosis of HGD or EAC. Incident cases of HGD or EAC were defined as the development of HGD or EAC at least 9 months after the index endoscopy. Cox-regression models were used to calculate hazard ratios (HR) and 95% confidence intervals (CI). The exposure of interest was use of NSAIDs, aspirin and statins after BE diagnosis. The 5-year cumulative incidence of neoplastic progression was estimated using Kaplan Meier curves. In multivariable Cox proportional-hazards models, we calculated hazard ratios and 95% confidence intervals, adjusted for age, gender, BE length, baseline histology and use of other medications. Several cumulative durations of medication use were evaluated, to investigate a possible duration-response relationship. To assess how users of NSAIDs and statins differed from non-users, we evaluated the patient characteristics for each exposure of interest. Mann-Whitney-Wilcoxon tests were used for continuous variables and Chi-squared tests for categorical variables. In addition, we constructed Kaplan-Meier curves of the cumulative incidence of neoplastic progression stratified by medication use, and we compared these curves using the log-rank test for equality. Two sided P-values <0.05 were considered to be statistically significant. Data were analysed using SPSS Statistics (version 17.0, Chicago, Illinois, USA).

Results

Patient characteristics

A total of 570 BE patients were included in this study. The median age at inclusion was 60 years and 412 (72%) patients were of male gender. Patients were followed for a median duration of 4.5 years. After inclusion in the study, 26 patients developed HGD, and another 12 patients developed EAC during a follow-up period of 2738 patient years. The incidence rate of HGD and EAC together was 1.4 per 100 patient-years and the incidence rate of EAC alone was 0.4 per 100 patient-years. The risk of neoplastic progression significantly increased with age. Other significant risk factors for developing HGD or EAC during follow-up were BE length and low grade dysplasia (Table 1).

Table1. Patient characteristics and the risk of neoplastic progression

		Cohort n = 570	Progression n = 38	HR (95%CI)
Follow-up	Median, years	4.5 (4.0-5.9)	3.3 (2.0-5.3)	
	Total, person-years	2738	133	
Age	Median, years	60 (53-68)	66 (56-73)	1.04 (1.01-1.08)
Gender	Male	412 (72%)	31 (82%)	1.53 (0.67-3.47)
BMI	≤ 25 kg/m^2	164 (29%)	14 (37%)	Reference
	> 25-30 kg/m^2	286 (50%)	16 (42%)	0.62 (0.30-1.27)
	> 30 kg/m^2	108 (19%)	8 (21%)	0.82 (0.34-1.96)
Smoking	Never	190 (33%)	9 (24%)	Reference
	Former	260 (46%)	21 (55%)	1.63 (0.75-3.57)
	Current	111 (20%)	8 (21%)	1.52 (0.58-3.93)
Alcohol use	Never	74 (13%)	3 (8%)	Reference
	Former	49 (9%)	5 (13%)	2.48 (0.59-10.37)
	Current	437 (77%)	30 (79%)	1.62 (0.49-5.31)
Symptoms	Reflux	170 (30%)	15 (40%)	1.33 (0.70-2.57)
	Regurgitation	139 (24%)	12 (32%)	1.38 (0.69-2.75)
	Dysphagia	70 (12%)	8 (21%)	1.59 (0.73-3.48)
BE diagnosis	At inclusion	82 (14%)	8 (21%)	1.34 (0.61-2.93)
BE length	> 4 cm	201 (35%)	21 (55%)	2.14 (1.13-4.05)
Histology	LGD	79 (14%)	17 (45%)	5.11 (2.69-9.70)

HR, hazard ratio; CI, confidence interval; BMI, bod y mass index; BE, Barrett's esophagus; LGD, low-grade dysplasia

Medication use

After BE diagnosis, 562 (99%) patients were prescribed PPIs for median duration of 9.0 years, 318 (56%) patients used NSAIDs for a median duration of 2 months, either prescribed or as over-the-counter medication, 161 (28%) patients used aspirin for a median duration of 5.4 years, and 209 (37%) patients used statins for a median duration of 5.3 years. Of the patients using NSAIDs, 87 (15%) patients used COX-2-inhibitors, and 289 (51%) non-selective NSAIDs. Of the 532 patients without neoplastic progression, 303 (57%) patients used NSAIDs, 153 (29%) used aspirin, and 200 (38%) used statins. Of the 38 patients who developed HGD or EAC during follow-up, 15 (40%) patients used NSAIDs, 8 (21%) used aspirin, and 9 (24%) used statins (Table 2).

Table 2. Medication use and the risk of neoplastic progression

	Cohort n = 570	Progression n = 38	HR (95% CI)	HR (95% CI)[a]
NSAID				
Any use ≥ 1 month	318 (56%)	15 (40%)	0.51 (0.27-0.99)	0.47 (0.24-0.93)
Median duration	0.2 (0.1-0.3)	0.2 (0.1-0.3)		
Non selective NSAID				
Any use ≥ 1 month	289 (51%)	13 (34%)	0.50 (0.26-0.97)	0.43 (0.22-0.88)
Median duration	0.2 (0.1-0.3)	0.2 (0.1-0.3)		
COX-2 inhibitor				
Any use ≥ 1 month	87 (15%)	4 (11%)	0.67 (0.24-1.90)	1.07 (0.37-3.13)
Median duration	0.3 (0.1-0.6)	0.1 (0.1-0.3)		
Low dose aspirin				
Any use ≥ 1 month	161 (28%)	8 (21%)	0.67 (0.31-1.46)	0.66 (0.27-1.65)
Median duration	5.4 (1.4-9.3)	6.1 (1.4-12.8)		
Statin				
Any use ≥ 1 month	209 (37%)	9 (24%)	0.52 (0.25-1.09)	0.46 (0.21-0.99)
Median duration	5.3 (1.9-8.3)	6.8 (2.2-11.2)		

HR, hazard ratio; CI, confidence interval; NSAID, nonsteroidal anti-inflammatory drug
[a] Adjusted for age, gender, BE length, baseline histology and use of other medications

Effect of NSAID use

NSAID use was associated with a reduced risk of neoplastic progression (HR 0.51; 0.27-0.99) and remained associated with a reduced risk of developing HGD or EAC (HR 0.47; 0.24-0.93) after adjusting for age, gender, BE length, baseline histology, and use of other medications. The effect was the same for

men and woman, and for patients younger and older than 60 years. When considering use of COX-2-inhibitors and non-selective NSAIDs separately, only use of non-selective NSAIDs was associated with a significantly reduced risk of neoplastic progression (HR 0.43; 0.22-0.88). Figure 1 shows the cumulative incidence of neoplastic progression during follow-up, stratified by NSAID use. Patients, who did not use NSAIDs, had a higher risk of developing HGD or EAC than patients using NSAIDs (P=.041).

To investigate a possible duration-response relationship, we evaluated different cumulative durations of NSAID use. NSAID use for more than 2 months was associated with a trend towards a lower risk of neoplastic progression than NSAID use for 2 months or less (Table 3). To assess how users of NSAIDs differed from non-users, we evaluated the patient characteristics of both groups. NSAID users were slightly younger and had a higher body mass index (BMI) than patients who did not use NSAIDs. There were no differences in gender, year of BE diagnosis, BE length, histology and duration of follow-up (Table 4).

Table 3. Duration of medication use and the risk of neoplastic progression

		Cohort n = 570	Progression n = 38	HR (95% CI)	HR (95% CI)[a]
NSAID	None	252 (44%)	23 (61%)	Reference	Reference
	≤ 2 months	165 (29%)	10 (26%)	0.66 (0.32-1.39)	0.58 (0.27-1.25)
	> 2 months	153 (27%)	5 (13%)	0.36 (0.14-0.94)	0.32 (0.12-0.86)
Statin	None	361 (63%)	29 (76%)	Reference	Reference
	≤ 5 years	102 (18%)	4 (11%)	0.52 (0.18-1.48)	0.51 (0.18-1.47)
	> 5 years	107 (19%)	5 (13%)	0.52 (0.20-1.34)	0.49 (0.18-1.29)

HR, hazard ratio; CI, confidence interval; NSAID, nonsteroidal anti-inflammatory drug
[a] Adjusted for age, gender, BE length, baseline histology and use of other medications

Effect of low dose aspirin use

Use of low dose aspirin did not change the risk of neoplastic progression (HR 0.67; 0.31-1.46). Although the effect of aspirin use was not significant, the hazard ratio pointed in the direction of a protective effect.

Effect of statin use

Statin use was associated with a trend towards a reduced risk of neoplastic progression (HR 0.52; 0.25-1.09). Figure 2 shows the cumulative incidence of

HGD and EAC during follow-up, stratified by statin use. A trend was seen towards a higher risk of neoplastic progression in patients who did not use statins (P=.079). In a multivariable model statin use was associated with a significantly reduced risk of neoplastic progression (HR 0.46; 0.21-0.99) after adjusting for age, gender, BE length, baseline histology, and use of other medications. The effect of statins was only seen in men and in patients older than 60 years. To investigate a possible duration-response relationship, we evaluated different cumulative durations of statin use. No duration-response relationship was found (Table 3).

However, most patients used statins for several years. To assess how users of statins differed from non-users, we evaluated the patient characteristics of both groups. Statin users were older than patients not using statins. However, there were no differences in gender, BMI, year of BE diagnosis, BE length, baseline histology and duration of follow-up (Table 4).

Table 4. Characteristics of patients using NSAIDs or statins

		Cohort n = 570	NSAID use n = 318	P-value	Statin use n = 209	P-value
Follow-up	Median, years	4.5 (4.0-5.9)	4.4 (4.0-5,9)	.605	4.7 (4.1-5.9)	.372
Age	Median, years	60 (53-68)	59 (52-67)	.009	63 (57-70)	<.001
Gender	Male	412 (72%)	221 (70%)	.095	155 (74%)	.445
BMI	≤ 25 kg/m^2	164 (29%)	82 (26%)	.024	56 (27%)	.697
	> 25-30 kg/m^2	286 (50%)	164 (52%)		111 (53%)	
	> 30 kg/m^2	108 (19%)	72 (23%)		42 (20%)	
Smoking	Current	111 (20%)	72 (23%)	.082	46 (22%)	.085
Alcohol use	Current	437 (77%)	247 (78%)	.601	161 (77%)	.956
Symptoms	Reflux	170 (30%)	97 (31%)	.691	55 (26%)	.164
	Regurgitation	139 (24%)	83 (26%)	.284	46 (22%)	.315
	Dysphagia	70 (12%)	39 (12%)	.989	21 (10%)	.217
Diagnosis	At inclusion	152 (26%)	83 (26%)	.903	55 (26%)	.965
BE length	> 4 cm	201 (35%)	104 (33%)	.151	75 (36%)	.813
Histology	LGD	79 (14%)	42 (13%)	.613	30 (14%)	.795

NSAID, nonsteroidal anti-inflammatory drug; BMI, body mass index; BE, Barrett's esophagus; LGD, low-grade dysplasia

Figure 1. Cumulative incidence of neoplastic progression, stratified by use of nonsteroidal anti-inflammatory drugs (NSAIDs)
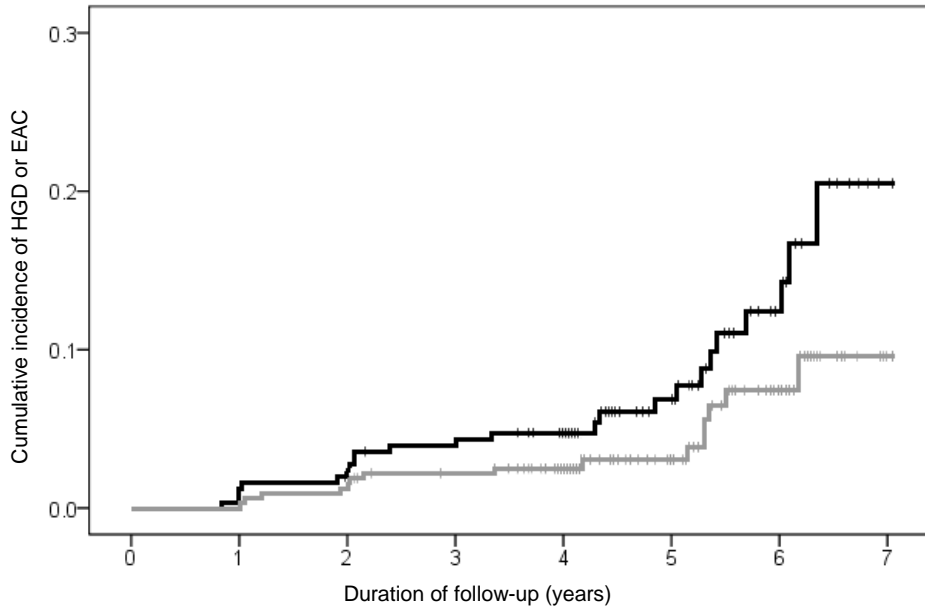━ No NSAID use, ━ NSAID use, Log rank P=.041

Duration of follow-up (years)

Figure 2. Cumulative incidence of neoplastic progression, stratified by use of statins. ━ No statin use, ━ Statin use, Log rank P=.079
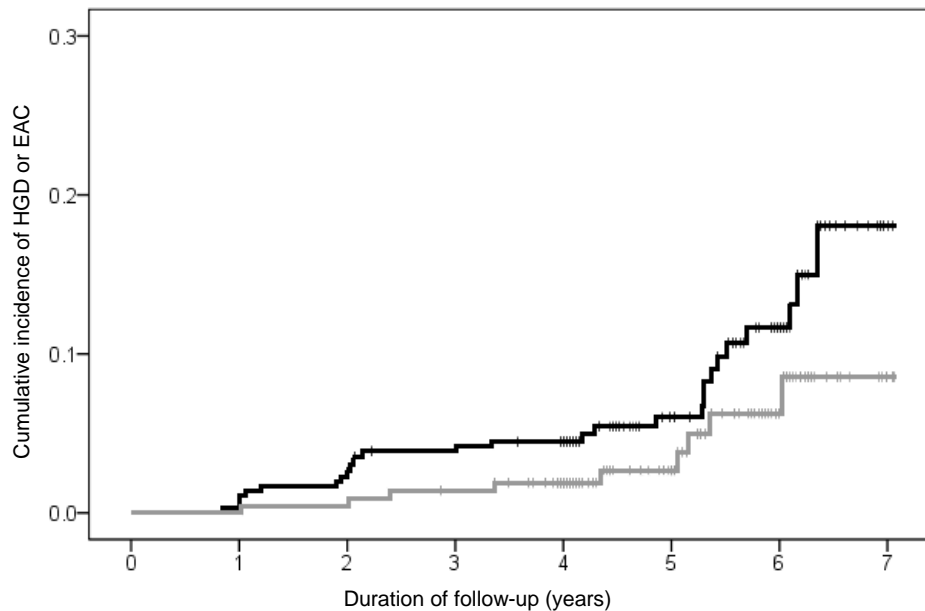

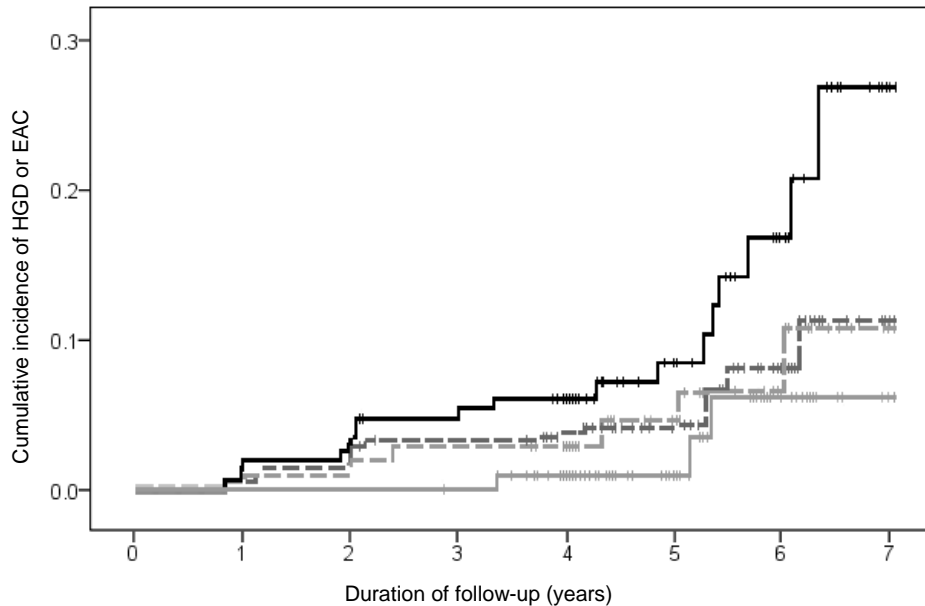Duration of follow-up (years)

Figure 3. Cumulative incidence of neoplastic progression, stratified by use of nonsteroidal anti-inflammatory drugs (NSAIDs) and statins

— None, - - NSAID or statin, — NSAID and statin, Log rank P=.036

Effect of NSAID and statin use

After BE diagnosis, 150 (26%) patients used neither NSAIDs nor statins, 211 (37%) patients used only NSAIDs, 102 (18%) used only statins and 107 (19%) used both NSAIDs and statins (Table 5). Use of NSAIDs or statins was associated with a trend towards a lower risk of neoplastic progression (HR 0.48; 0.23-1.01 for NSAIDs and HR 0.48; 0.19-1.21 for statins). Use of both NSAIDs and statins was associated with an even lower risk of developing HGD or EAC (HR 0.24; 0.07-0.82). In a multivariable model, use of both NSAIDs and statins remained associated with an additive protective effect (HR 0.22; 0.06-0.85). Figure 3 shows the cumulative incidence of neoplastic progression during follow-up, stratified by both NSAID and statin use. The cumulative incidence of HGD or EAC was lower in patients using an NSAID or statin, than in patients using neither (log rank P=.048 for NSAIDs and log rank P=.113 for statins). In patients using both NSAIDs and statins the cumulative incidence of neoplastic progression was even lower (log rank P=.014).

Table 5. Use of both NSAIDs and statins and the risk of neoplastic progression

	Cohort n = 570	Progression n = 38	HR (95% CI)	HR (95% CI)[a]
NSAID and statin				
None	150 (26%)	17 (45%)	Reference	Reference
NSAID only	211 (37%)	12 (31%)	0.48 (0.23-1.01)	0.46 (0.22-0.99)
Statin only	102 (18%)	6 (16%)	0.48 (0.19-1.21)	0.51 (0.18-1.42)
NSAID and statin	107 (19%)	3 (8%)	0.24 (0.07-0.82)	0.22 (0.06-0.85)

Discussion

In this large prospective cohort study, NSAID and statin use were associated with 50% reduction in the risk of neoplastic progression in BE patients. Use of both NSAIDs and statins had an additive protective effect and was associated with approximately 75% reduction in the risk of developing HGD or EAC. These associations were independent of age, gender, BE length, baseline histology and use of other medication.

The reduced risk of neoplastic progression with NSAID use appeared to be related to the duration of medication use. However, patients used NSAIDs for a relatively short period with a median duration of 2 months raising concerns of uncontrolled confounding. Use of low dose aspirin was associated with a smaller, non-significant reduction in the risk of neoplastic progression than use of NSAIDs, indicating a dose-response relationship. The presence of duration-response and dose-response relationship, supports a causal association between NSAID use and the risk of neoplastic progression in BE. Although chemoprevention with NSAIDs seems more effective than chemoprevention with low dose aspirin, use of NSAIDs will also lead to more serious side effects. The results of our study are consistent with previous published studies investigating the effect of NSAID use on neoplastic progression in BE. A case-control study with 114 EAC patients and 382 BE patients observed that NSAID use was more prevalent in BE patients than in patients with EAC (38% versus 26%, P=.02).[23] Furthermore, in a prospective cohort study with 350 BE patients, current users of NSAIDs were at lower risk for developing EAC than never users of NSAIDs (HR 0.20; 0.10-0.41).[15] Neither of these studies investigated concomitant PPI or statin use. In a more recent observational study with 344 BE patients, NSAID use was associated with a non-significant trend toward a lower

incidence of HGD or EAC (HR 0.56; 0.27-1.18).[24] The same study group also performed a case-control study with 116 EAC patients and 696 matched BE patients, which confirmed that NSAID use was significantly associated with a reduced risk of EAC (incidence density ratio 0.64; 0.42-0.97).[13]

Previous studies have also investigated the working mechanism of NSAIDs in chemoprevention. NSAIDs inhibit the cyclooxygenase enzymes COX-1 and COX-2. COX-1 is constitutively expressed in human tissue, whereas COX-2 expression is induced in response to cytokines, growth factors and mitogens. Inhibition of COX-2 restores apoptosis, inhibits cell growth, decreases cell proliferation and inhibits angiogenesis in human tissue.[25] In Barrett patients, inhibition of cyclooxygenase enzymes by NSAIDs may therefore lead to a decreased risk of neoplastic progression.

Statin use was also associated with a reduced risk of neoplastic progression in our study. Only 2 previous studies have investigated the effect of statin use in BE patients. In a first observational study, statin use did not affect the risk of dysplasia or EAC (HR 0.73; 0.30-1.78), but this study was underpowered with only 87 patients using statins.[24] The results of our study were consistent with the results of a further case-control study, in which statin use reduced the risk of EAC (incidence density ratio 0.55; 0.36-0.86).[13] The patients included in this study were veterans receiving care in the Veterans' Affairs health care system. These patients were therefore more likely to be of male and of old age than the overall BE population. This may limit the generalizability of these results.

The working mechanism of statins in chemoprevention has also been investigated. Statins competitively inhibit 3-hydroxy-3-methylglutaryl coenzyme A reductase, the rate-limiting enzyme in the biosynthesis of cholesterol. Although this is the most appreciated biological action, there are several other import roles of statins. Farnesyl and geranylgeranyl are formed in the cholesterol biosynthesis pathway and are essential for the activation of intracellular proteins though prenylation. Several important proteins involved in intracellular signalling such as Ras, Rho and Rac are dependent on prenylation. Ras is the activator of the ERK mitogen-activated pathway and possibly the activator of the Akt pathway as well. Both cascades provide cell proliferation and cell survival signals in human cells. As a result statins may inhibit proliferation and induce apoptosis in BE epithelium leading to a reduced risk of neoplastic progression.[14, 26-27]

In the current study, use of both NSAIDs and statins provided a stronger risk reduction than use of NSAIDs or statins alone. This is de first study

investigating the effect of both NSAID and statin use on the risk of neoplastic progression in BE. A previous study in patients with colorectal cancer also demonstrated that chemoprevention with the combination of low-dose aspirin and statins provided a stronger risk reduction than either of the single drugs.[16] Several in-vitro studies have investigated the effect of chemoprevention with a combination of NSAIDs and statins. These studies have demonstrated synergistic effects of NSAIDs and statins in inhibition of cell growth and induction of apoptosis in cancer cells. How NSAIDs and statins work in a synergistic fashion is largely unresolved.[16-17]

This study has several strengths including the large sample size and long follow-up time. All patients presenting with BE at the endoscopy unit of 3 academic and 12 regional hospitals were included in this study. As a result our cohort should be representative for the BE population in the Netherlands. The incidence of EAC during follow-up was 0.4 per 100 person-years, which is equal to the incidence reported in most studies and which supports that our population is representative. There were strict criteria for BE diagnosis and for inclusion in the study. In addition, there was a stringent follow-up scheme, a standardized endoscopy protocol and a standardized biopsy protocol. All biopsies were reviewed by at least 2 pathologists to obtain a consensus diagnosis. During follow-up, clinical information was collected prospectively and recorded in a central computerized database. Information on medication use was collected prospectively and patients also filled out a questionnaire on their use of over-the-counter medication. All information on medication use was cross-checked using pharmacy records. Because PPI use is one of the mainstays in the treatment of BE patients it may act as a possible confounder. Therefore we also collected detailed information on PPI use.

Our study also has some limitations. Since this is an observational study we cannot exclude uncontrolled confounding. However, we have collected much information on potential confounding factors and we corrected for these factors in the final analysis. Unfortunately, we did not have information on the indication of NSAID and statin use. As a result we were not able to adjust for this possible confounder. Users of NSAIDs and statins may have differed in other ways from other BE patients than in the use of these drugs. To assess how users of NSAIDs and statins differed from non-users, we evaluated the patient characteristics for each exposure of interest. NSAIDs users were slightly younger and statin users were slightly older than other BE patients, but otherwise there were no major differences. Despite the large sample size of this

cohort, the number of events was small in some strata of exposure, limiting the interpretation. This was especially the case when looking at the effect of treatment duration and the interaction between NSAID and statin use. In addition, patients were informed about any changes seen during endoscopy or histological assessment, which may have influenced compliance or lifestyle.

In conclusion, this large prospective cohort study shows that use of NSAIDs and statins is associated with a significantly reduced risk of neoplastic progression in patients with BE. Use of both NSAIDs and statins appears to have an additive protective effect. As a result chemoprevention with NSAIDs and statins may have a role in the treatment of patients with BE.

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Chapter 5

Aberrant p53 protein expression is associated with an increased risk of neoplastic progression in patients with Barrett's esophagus

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Abstract

Introduction: The value of surveillance for patients with Barrett's esophagus is under discussion given the overall low incidence of neoplastic progression and lack of discriminative tests for risk stratification. Histological diagnosis of low-grade dysplasia is the only accepted predictor for neoplastic progression to date, but has a low predictive value. The aim of this study was therefore to evaluate the value of p53 immunohistochemistry for predicting neoplastic progression in patients with Barrett's esophagus.

Methods: We conducted a case-control study within a prospective cohort of 720 patients with Barrett's esophagus. Patients who developed high-grade dysplasia or esophageal adenocarcinoma were classified as cases and patients without neoplastic progression were classified as controls. P53 protein expression was determined by immunohistochemistry in more than 12.000 biopsies from 635 patients and was scored independently by 2 expert pathologists who were blinded for long-term outcome.

Results: During follow-up, 49 (8%) patients developed high-grade dysplasia or esophageal adenocarcinoma. P53 overexpression was associated with an increased risk of neoplastic progression in patients with Barrett's esophagus after adjusting for age, gender, Barrett's esophagus length and esophagitis (RR[a] 5.6; 95% CI 3.1-10.3), but the risk was even higher with loss of p53 expression (RR[a] 14.0; 95% CI 5.3-37.2). The positive predictive value for neoplastic progression increased from 15% with histological diagnosis of low-grade dysplasia to 33% with low-grade dysplasia and concurrent aberrant p53 expression.

Conclusion: Aberrant P53 protein expression is associated with an increased risk of neoplastic progression in patients with Barrett's esophagus and appears to be a more powerful predictor for neoplastic progression than histological diagnosis of low-grade dysplasia.

Introduction

Over the past decades, the incidence of esophageal adenocarcinoma (EAC) has been rising at a rate exceeding that of any other cancer.[1] In many cases the development of EAC is related to Barrett's esophagus (BE), a premalignant condition in which the normal squamous epithelium of the distal esophagus is replaced by columnar epithelium containing goblet cells.[2] BE patients have an increased risk of developing EAC with an estimated incidence of 0.5% per year.[3, 4] The development of EAC is thought to be a gradual process, in which metaplastic epithelium evolves to low-grade dysplasia (LGD), high-grade dysplasia (HGD), and eventually EAC under the influence of esophageal acid exposure.[5, 6] Early identification of a premalignant stage provides the opportunity to prevent progression to EAC and endoscopic surveillance is therefore recommended for BE patients.[7] However, the value of BE surveillance is under discussion given the overall low incidence of neoplastic progression, the large screening base which is estimated at 1-2% of the general population, and lack of discriminative tests for risk stratification.[8, 9]

Histological diagnosis of LGD is currently the only accepted predictor for neoplastic progression and international guidelines recommend more intensive surveillance in BE patients with LGD (yearly instead of every 3 years).[7, 10] However, histological diagnosis of LGD is subject to sample error and considerable interobserver variation, mainly because features of dysplasia may overlap with features of non-neoplastic regenerative changes.[11] Although the predictive value of LGD increases with consensus of multiple pathologists, still one third of BE patients is diagnosed with LGD during surveillance, while the 10-year cumulative incidence of neoplastic progression is only around 15% in this subgroup.[12, 13] Use of biomarkers in addition to histological assessment may improve risk stratification for these patients, whereby more stringent surveillance is applied to individuals at high risk for neoplastic progression, while surveillance intervals are prolonged in those at low risk. P53 appears to be one of the most promising biomarkers and previous studies have shown that p53 overexpression is associated with an increased risk of neoplastic progression.[12, 14-18] P53 overexpression can be caused by TP53 mutations which stabilize the inactivated protein. On the other hand truncating TP53 mutations or epigenetic silencing may result in protein inactivation and subsequently loss of p53 expression. Although little is known about loss of p53 expression, the first results indicate that it is also associated with an increased

risk of neoplastic progression.[19] The aim of the present study was therefore to evaluate the value of p53 immunohistochemistry for predicting neoplastic progression in patients with BE.

Methods

Study design
We conducted a case-control study within a large prospective cohort of BE patients. In this cohort 720 patients were included with known or newly diagnosed BE of at least 2 cm, confirmed by the presence of intestinal metaplasia and without a history of HGD or EAC. Patients were included between November 2003 and December 2004 in 3 University Medical Centers and 12 regional hospitals throughout the Netherlands and had endoscopic surveillance according to the guidelines of the American College of Gastroenterology.[7] Patients without dysplasia underwent upper endoscopy with biopsy sampling every 3 years and patients with LGD every year. All endoscopic procedures were performed by experienced gastroenterologists, according to a standardized protocol. Endoscopic landmarks such as the diaphragm impression, gastroesophageal junction and squamocolumnar junction were noted, the presence of esophagitis was graded according to the Los Angeles Classification, and abnormalities were reported including nodules, ulcers and erosions.[20] At each endoscopy targeted biopsies were taken from mucosal abnormalities and quadrant biopsies were taken every 2 cm from the most distal to the most proximal part of the BE epithelium, according to the Seattle protocol.[21]

Histology
Biopsy specimens were fixed with buffered formalin and embedded in paraffin, according to standard procedures. From each biopsy set 4 µm thick sections were cut and stained with haematoxylin-eosin to assess the presence of BE and to define the grade of dysplasia. After examining all biopsies, the highest degree of abnormality was reported for each endoscopy. Slides were first graded by a local pathologist and then by an expert pathologist for second opinion. When both pathologists disagreed on the grade of dysplasia, the slides were reviewed by a second expert pathologist. Pathologists were blinded to the diagnosis of each other and a final diagnosis was made only if at least 2 pathologists agreed on the grade of dysplasia. If there was still disagreement, a

panel of expert pathologists reviewed the slides as well and a final diagnosis was made based on consensus agreement.

Patient selection

We collected paraffin material suitable for immunohistochemistry from all patients in our BE cohort. Paraffin material was not available in 85 patients, leaving 635 patients to be included in this analysis. Patients who developed HGD or EAC during follow-up were identified as cases and patients without neoplastic progression were identified as controls (Figure 1).

Immunohistochemistry was performed on paraffin material of all surveillance endoscopies of patients who developed any form of dysplasia. In patients without any dysplasia, immunohistochemistry was performed on biopsies of a random surveillance endoscopy.

Figure 1. Flowchart of patients included in the study. Patients with neoplastic progression were classified as cases and patients without progression as controls.
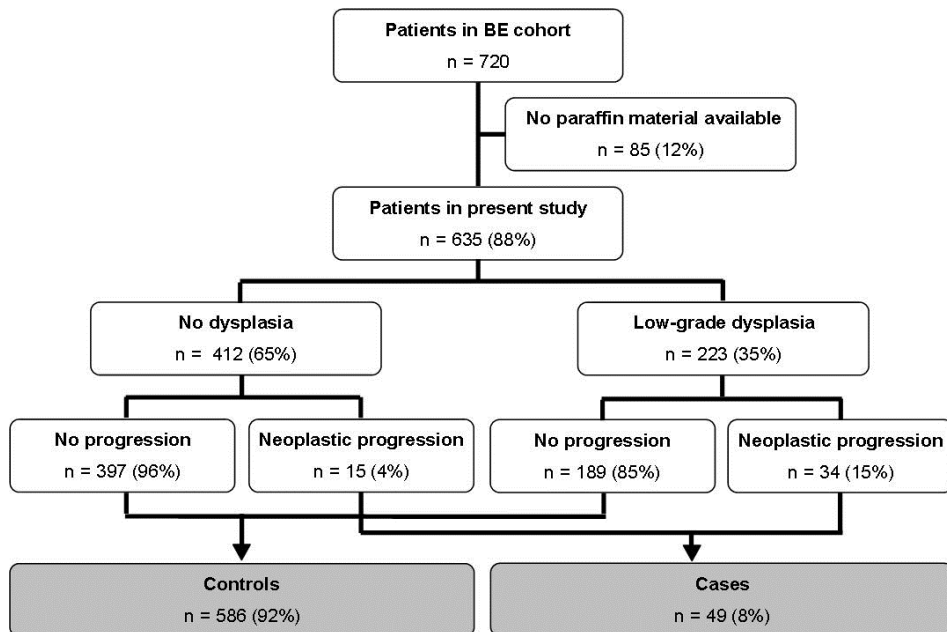
Immunohistochemistry

Immunohistochemistry was performed as a single batch at the pathology department of the Erasmus University Medical Center (Rotterdam, the Netherlands) using an automatic immunohistochemical staining machine (Ventana Medical Systems, Tucson, Arizona, USA). A sample of tumor tissue was used as positive control for each section. Sections were deparaffinized prior to the staining procedure and heat-induced epitope retrieval was performed at 97ºC for 15 minutes. Endogenous peroxidase activity was blocked by incubating the slides for 15 minutes in a solution of 0.3% hydrogen peroxide in phosphate-buffered saline. Monoclonal mouse anti-human p53-protein was used as the primary antibody for immunohistochemistry with a dilution of 1:25 (Clone DO-7, Dako, Glostrup, Denmark). The slides were incubated for 30 minutes with the primary antibody. Then amplification and visualization was performed by using the Dako REAL EnVision system (peroxidase/DAB, Rabbit/Mouse, Dako, Glostrup, Denmark). Finally, slides were counterstained with haematoxylin.

Immunohistochemical stained slides were examined with haematoxylin-eosin stained slides to determine p53 expression in areas with dysplasia. P53 expression was scored independently by 2 expert pathologists who were blinded for long-term outcome on a 3 point scale (normal expression, overexpression, or complete loss of expression). Only intense nuclear staining for p53 was considered as overexpression (Figure 2). Aberrant expression was defined as either p53 overexpression or complete loss of p53 expression. P53 protein expression was considered as aberrant when at least one gland showed overexpression or complete loss of expression. After examining all biopsies, the highest degree of abnormality was reported for each endoscopy. When there was disagreement between the pathologists, the slides were evaluated by both pathologists simultaneously to reach a consensus diagnosis.

Ethics

The study protocol was approved by the Institutional Review Boards of the Erasmus University Medical Center, as well as those of all participating hospitals. Before the first endoscopy, written informed consent was obtained from all patients.
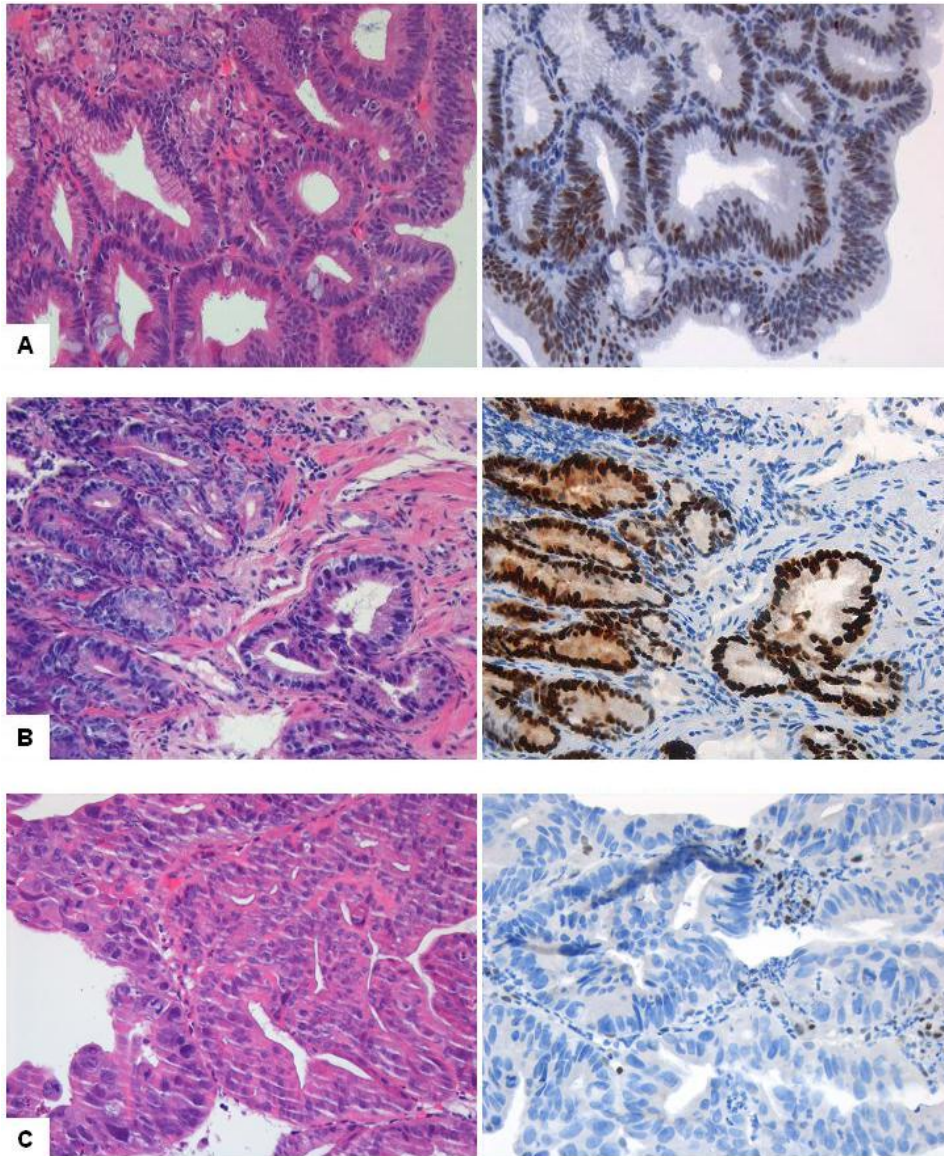
Figure 2. Haematoxylin-eosin staining and p53 immunohistochemistry of

A. Barrett's esophagus with low-grade dysplasia and normal p53 expression
B. Barrett's esophagus with low-grade dysplasia and p53 overexpression
C. Esophageal adenocarcinoma with loss of p53 expression

Statistical analysis

Characteristics of cases and controls were compared using Mann-Whitney U tests for continuous variables and Chi-squared tests for categorical variables. To compare p53 expression in biopsies with different grades of dysplasia Mann-Whitney U tests and Kruskal-Wallis tests were used, thereby ignoring that multiple biopsies could be from the same patient. The value of p53 immunohistochemistry for predicting neoplastic progression was estimated in loglinear regression models. Since immunohistochemistry was not performed on all biopsy series, data were split up by endoscopy. Neoplastic progression was defined as the development of HGD or EAC after inclusion in the study and follow-up time was defined as the time between each endoscopy and the next surveillance endoscopy. Loglinear regression models were used to calculate relative risks (RR) and 95% confidence intervals (CI) with the logarithm of follow-up time as offset variable. In multivariable models, relative risks were calculated adjusted for age, gender, BE length and esophagitis. With 49 cases and 586 controls 80% power was provided to detect a relative risk of at least 2.5 at a significance level of 5%. Interobserver agreement for p53 expression was determined using Cohen kappa (κ) statistics. Two sided P-values <0.05 were considered to be statistically significant. Data were analysed using SPSS Statistics (version 20.0, Chicago, Illinois, USA).

Results

Patient characteristics

Six hundred thirty-five BE patients (73% male, median age 60 years (interquartile range (IQR) 53-69)) were included in this study and followed during surveillance for a median duration of 6.6 years (IQR 5.1-7.3) and with a median of 4 follow-up endoscopies (IQR 4-5). Thirty-five (6%) patients developed HGD and 14 (2%) patients developed EAC during surveillance, resulting in 49 (8%) patients with neoplastic progression which were identified as cases. The remaining 586 (92%) patients without neoplastic progression were identified as controls (Figure 1). The incidence rate of HGD and EAC together was 1.4 per 100 patient-years (95% CI 1.0-1.8) and the incidence rate of EAC alone was 0.4 per 100 patient-years (95% CI 0.2-0.6). Histology and p53 expression were assessed in biopsy series of 1481 endoscopies. The highest degree of abnormality was reported for each endoscopy after examining all biopsies. In total, more than 12.000 biopsies were reviewed. Cases had a

smaller number of follow-up endoscopies and longer BE length than controls, but otherwise there were no significant differences (Table 1).

Histology

Normal BE without dysplasia was seen in 1085 (73%) biopsy series, LGD in 347 (23%), HGD in 35 (3%) and EAC in 14 (1%). Presence of LGD was more common in biopsy series of cases (44%) than in biopsy series of controls (22%) and was associated with an increased risk of neoplastic progression (relative risk (RR) 4.2; 95% CI 2.4-7.3). This association remained after adjusting for age, gender, BE length and esophagitis (RR[a] 4.0; 95% CI 2.3-7.0) (Table 2). In total 223 (35%) patients were diagnosed with LGD during follow-up with an incidence rate of 3.6 per 100 patient-years (95% CI 3.0-4.4). Of these patients 34 (15%) eventually developed HGD or EAC with an incidence rate of 4.2 per 100 patient-years (95% CI 2.8-5.9). The percentage of patients with LGD gradually increased from 5 years before neoplastic progression. The sensitivity of LGD for predicting neoplastic progression was 44% with a specificity of 78%.

Table 1. Characteristics of cases and controls

		Controls n = 586	Cases n = 49	P-value
Follow-up	Median, years	6.8 (5.7-7.4)	3.0 (2.0-5.3)	<0.001
Endoscopies	Median number	4 (4-5)	3 (2-4)	<0.001
Biopsies	Median per endoscopy Total number	7 (4-10) 10.346	8 (6-12) 1781	0.042
Age	Median, years	60 (53-69)	65 (55-70)	0.171
Gender	Male	426 (73%)	40 (82%)	0.174
Alcohol use	Current	453 (79%)	38 (78%)	0.969
Smoking	Current	122 (21%)	12 (25%)	0.464
NSAID use	≥ 1 month	185 (32%)	14 (29%)	0.664
Reflux	Current	176 (31%)	19 (39%)	0.230
BE diagnosis	At inclusion	143 (25%)	13 (27%)	0.512
BE length	Median, cm	4 (3-6)	5 (4-8)	0.004
Esophagitis	Current	110 (19%)	14 (29%)	0.096

NSAID, nonsteroidal anti-inflammatory drugs; BE, Barrett's esophagus

Table 2. Histology and p53 immunohistochemistry in cases and controls

	Controls n = 1300*	Cases n = 132*	RR (95% CI)	RR[a] (95% CI)
Histology				
No dysplasia	1011 (78%)	74 (56%)	Reference	Reference
Low-grade dysplasia	289 (22%)	58 (44%)	4.2 (2.4-7.3)	4.0 (2.3-7.0)
P53 expression				
Normal p53 expression	1115 (86%)	67 (51%)	Reference	Reference
Aberrant p53 expression	185 (14%)	65 (49%)	6.2 (3.6-10.9)	6.4 (3.6-11.3)
P53 overexpression	169 (13%)	58 (44%)	5.5 (3.1-10.0)	5.6 (3.1-10.3)
Loss of p53 expression	16 (1%)	7 (5%)	13.4 (5.1-35.3)	14.0 (5.3-37.2)
Histology and p53 expression				
ND & normal p53 expression	918 (71%)	50 (38%)	Reference	Reference
LGD & normal p53 expression	197 (15%)	17 (13%)	2.4 (0.9-6.0)	2.2 (0.8-5.5)
ND & aberrant p53 expression	93 (7%)	24 (18%)	4.5 (2.0-10.0)	4.3 (1.9-9.8)
LGD & aberrant p53 expression	92 (7%)	41 (31%)	11.2 (5.7-22.0)	12.2 (6.1-24.5)

ND, no dysplasia; LGD, low-grade dysplasia; RR, relative risk

[a] Adjusted for age, gender, BE length and esophagitis

* The highest degree of abnormality was reported for each endoscopy after examining all biopsies

P53 immunohistochemistry

Normal p53 expression was seen in 1188 (80%) biopsy series, p53 overexpression in 262 (18%) and loss of p53 expression in 31 (2%). Aberrant p53 expression was more common with higher grades of dysplasia and was seen in 11% of biopsy series without dysplasia, 38% of biopsy series with LGD, 83% of biopsy series with HGD and all biopsy series with EAC (p<0.001) (Figure 3). Loss of p53 expression was especially seen in biopsy series with HGD (6%) and EAC (43%). Aberrant p53 expression was more common in biopsy series of cases (49%) than in biopsy series of controls (14%) and was associated with an increased risk of neoplastic progression (RR 6.2; 95%CI 3.6-10.9). This association remained after adjusting for age, gender, BE length and esophagitis (RR[a] 6.4; 95%CI 3.6-11.3) and was seen in both biopsy series without dysplasia and biopsy series with LGD. Not only p53 overexpression, but also loss of p53 expression was associated with an increased risk of neoplastic progression (RR[a] 5.6; 95% CI 3.1-10.3 and RR[a] 14.0; 95% CI 5.3-37.2) (Table 2). In total 118 (19%) patients were diagnosed with aberrant p53 expression during follow-up. Of these patients 31 (26%) eventually developed HGD or EAC with an incidence rate of 7.4 per 100 patient-years (95% CI 5.0-10.5). During

follow-up, aberrant p53 expression was confirmed in 37% of biopsy series without dysplasia, 78% of biopsy series with LGD and all biopsy series with HGD or EAC. In approximately 45% of patients, aberrant p53 expression was already seen up to 5 years before neoplastic progression and this percentage remained stable over time (Figure 4). The sensitivity of aberrant p53 expression for predicting neoplastic progression was 49% with a specificity of 86%. Interobserver agreement for p53 expression was good (κ = 0.79; 95%CI 0.75-0.83). Both expert pathologists agreed on p53 expression in 1379 (93%) biopsy series (Table 3). When p53 expression was scored on a 2-point scale (normal or aberrant expression) interobserver agreement was similar.

Figure 3. Percentage of biopsy series with aberrant p53 expression, stratified by grade of dysplasia. ■ p53 overexpression ■ loss of p53 expression
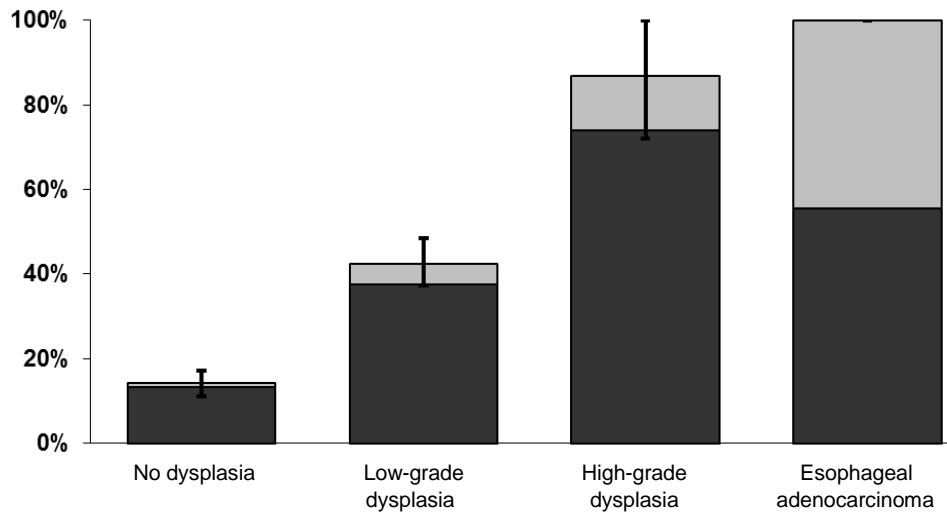

**Table 3.** Interobserver agreement for p53 expression in expert pathologists

P53 expression	Normal expression	Overexpression	Loss of expression	κ
Normal expression	1126 (76%)	56 (4%)	7 (0%)	0.793
Overexpression	24 (2%)	234 (16%)	-	
Loss of expression	14 (1%)	-	19 (1%)	

The highest degree of abnormality was reported for each endoscopy after examining all biopsies

Figure 4. Percentage of patients with (A) low-grade dysplasia (LGD) and (B) aberrant p53 expression before neoplastic progression. ▬ cases ··· controls
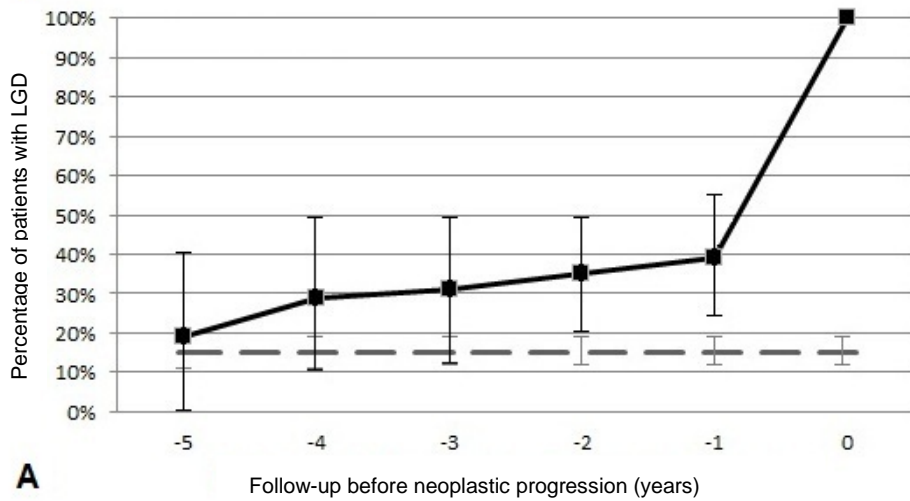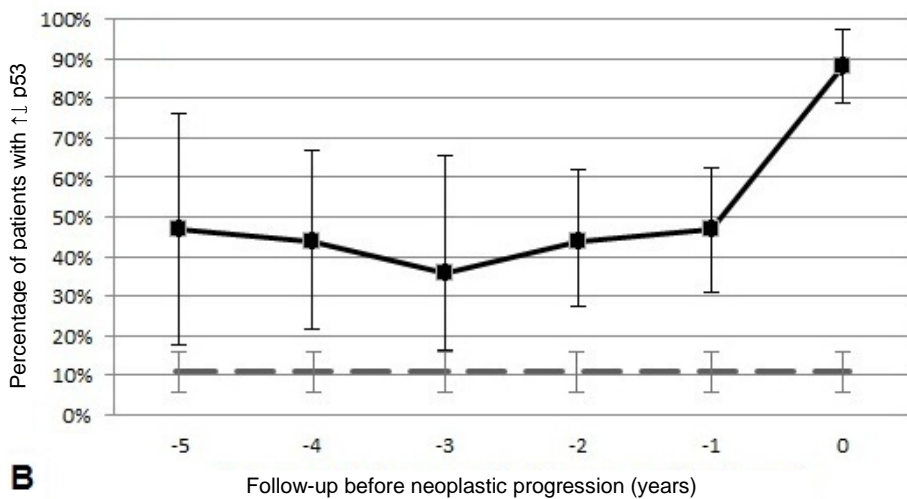
Histology and p53 immunohistochemistry

Normal BE with normal p53 expression was seen in 968 (68%) biopsy series, LGD with normal p53 expression in 214 (15%), normal BE with aberrant p53 expression in 117 (8%) and LGD with aberrant p53 expression in 133 (9%). Aberrant p53 expression was more common in biopsy series of cases than in biopsy series controls, not only in normal BE (18% versus 7%), but also in BE with LGD (31% versus 7%). Aberrant p53 expression in normal BE was

associated with an increased risk of neoplastic progression (RR[a] 4.3; 95% CI 1.9-9.8), but the risk was even higher with concurrent LGD (RR[a] 12.2; 95% CI 6.1-24.5) (Table 2). During follow-up 73 (11%) patients were diagnosed with LGD and concurrent aberrant p53 expression. Of these patients 24 (33%) eventually developed HGD or EAC with an incidence rate of 11.2 per 100 patient-years (95% CI 7.1-16.8).

Discussion

In this case control study, we evaluated the value of p53 immunohistochemistry for predicting neoplastic progression in patients with BE. P53 overexpression was associated with an increased risk of neoplastic progression, but the risk was even higher with complete loss of p53 expression. Although aberrant p53 expression appeared to be a more powerful predictor than histological diagnosis of LGD, the risk of neoplastic progression was the highest in patients with LGD and concurrent aberrant p53 expression.

During surveillance, up to 35% of patients were diagnosed with LGD, while only 15% of these patients eventually developed HGD or EAC. The predictive value of LGD was thus low, despite using a consensus diagnosis for dysplasia. The incidence rate of LGD was 3.6% per year in our study, which is similar to rates observed previously.[22, 23] Although patients with LGD were at increased risk of neoplastic progression, the absolute risk of developing HGD or EAC was low with an incidence rate of 4.2% per year. Results of previous studies are highly variable, but show an average incidence rate of 1-2% per year (range 0.6% to 13%), which is only slightly lower than the incidence rate observed in our study.[24-30] Aberrant p53 expression was observed more frequently with higher grades of dysplasia. The percentage of biopsy series with aberrant p53 expression increased from 11% in samples without dysplasia to even 100% in samples with EAC. These findings correspond to results reported in previous studies.[31-34] Aberrant p53 expression was identified more frequently in cases than in controls and was associated with an increased risk of neoplastic progression. Not only p53 overexpression was associated with an increased risk of developing HGD or EAC, but the risk was even higher with complete loss of p53 expression. The positive predictive value for neoplastic progression increased from 15% with histological diagnosis of LGD to 33% with LGD and concurrent aberrant p53 expression. To our knowledge this is the first large case-control study evaluating the value of both p53 overexpression and loss of

p53 expression for predicting neoplastic progression in BE. Previous studies have shown that p53 overexpression is associated with an increased risk of neoplastic progression in non-dysplastic BE as well as BE with LGD. The results of these studies are in line with the results of our study.[12, 16, 35]

In the present study we have also shown good interobserver agreement for the assessment of p53 expression, which indicates that p53 is not only a theoretical but also a clinically suitable marker for predicting progression in BE. Although routine p53 immunohistochemistry is associated with higher costs than histological assessment alone, application of this marker may lead to the identification of a much smaller high-risk group needing intensive surveillance. At this moment all patients with LGD receive intensive surveillance, which can be up to one third of all BE patients. In the present study we have shown that only 11% of patients is diagnosed with both LGD and aberrant p53 expression and that the risk of neoplastic progression is much higher in this subgroup. Surveillance of such a small high-risk group may result in lower costs, less burden on endoscopy units and higher quality of life for BE patients.

Previous studies have shown that p53 is an early molecular marker of genetic instability and may precede the development of dysplasia. In addition, studies have shown that aberrant p53 expression can be detected in non-dysplastic epithelium of patients with dysplasia.[34] This may explain why aberrant p53 expression was also associated with an increased risk of neoplastic progression in biopsy series without dysplasia. Although aberrant p53 expression was more common in cases it was also seen in controls. The development of EAC in BE is thought to be a gradual process and it is unknown how much time this process of progression takes.[5] It is therefore possible that patients with aberrant p53 expression but without neoplastic progression will develop HGD or EAC in the future. On the other hand, not all patients with neoplastic progression showed aberrant p53 expression. It may be that these patients actually have mutations in p53, but that these mutations not lead to accumulation or complete loss of p53 in the cell nucleus.

This study has several strengths including the large cohort of BE patients and long follow-up time. Patients were prospectively followed according to a stringent follow-up scheme and during follow-up clinical and pathological data were collected. In addition, a standardized endoscopy and biopsy protocol were used. All slides were reviewed by at least 2 pathologists to obtain a final diagnosis based on consensus. In contrast to previous studies, the slides were not only evaluated for p53 overexpression, but also for loss of p53 expression.[36]

Our study also has some limitations. Although patients were only classified as controls when they did not develop HGD or EAC during follow-up, we cannot exclude that these patients will develop HGD or EAC in the future. If so, this may have led to an underestimation of the value of p53 for predicting progression. Secondly, a disadvantage of p53 immunohistochemistry is that the antibody directed to p53 not only stains protein derived from the mutant TP53 but also wild-type TP53. Nevertheless, p53 expression is considered indicative for the presence of mutant TP53, because the latter has a longer half-life than wild-type p53 and is not degraded in the normal way. This results in accumulation of nuclear p53, which is detectable by immunohistochemistry.[37]

In conclusion, this case control study shows that aberrant p53 expression is associated with an increased risk of neoplastic progression in patients with BE. Aberrant p53 expression appears to be a more powerful predictor for neoplastic progression than histological diagnosis of LGD. P53 immunohistochemistry may be useful as a discriminative test to improve risk stratification and hence the cost-effectiveness of BE surveillance programs.

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Chapter 6

Value of alpha-methylacyl-CoA racemase for predicting neoplastic progression in patients with Barrett's esophagus

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Abstract

Introduction: The value of surveillance for patients with Barrett's esophagus is under discussion given the overall low incidence of neoplastic progression and lack of discriminative tests for risk stratification. Histological diagnosis of low-grade dysplasia is the only accepted predictor for neoplastic progression to date, but has a low predictive value. The aim of this study was to evaluate the value of Alpha-MethylAcyl-CoA Racemase (AMACR) immunohistochemistry for predicting neoplastic progression in patients with Barrett's esophagus.

Methods: We conducted a case-control study within a prospective cohort of 720 patients with Barrett's esophagus. Patients who developed high-grade dysplasia or esophageal adenocarcinoma were classified as cases and patients without neoplastic progression were classified as controls. AMACR expression was determined by immunohistochemistry in 12.127 biopsies from 635 patients and was scored independently by 2 expert pathologists on a 3 point scale. Relative risks (RR[a]) adjusted for age, gender, Barrett's esophagus length and esophagitis were calculated in log-linear models.

Results: During a median follow-up of 6.6 years, 49 patients (8%) developed high-grade dysplasia or esophageal adenocarcinoma. Although mild AMACR expression was associated with a trend towards an increased risk of neoplastic progression (RR[a] 1.6; 95%CI 0.9-3.1), the risk of neoplastic progression was especially elevated with strong AMACR expression (RR[a] 4.8; 95%CI 1.9-12.6). The positive predictive value of strong AMACR expression was slightly higher than that of low-grade dysplasia (22% versus 15%). The negative predictive value was slightly lower than that of low-grade dysplasia (91 versus 93%).

Conclusions: Strong AMACR expression is associated with an increased risk of neoplastic progression in patients with Barrett's esophagus. However, AMACR expression appears to be a less powerful predictor for neoplastic progression than low-grade dysplasia.

Introduction

Barrett's esophagus (BE) is a premalignant condition, in which the normal squamous epithelium of the distal esophagus is replaced by columnar epithelium containing goblet cells.[1] BE patients have an increased risk of developing esophageal adenocarcinoma (EAC) with an estimated incidence of 0.1-0.5% per year.[2, 3] The development of EAC is thought to be a gradual process, in which metaplastic epithelium evolves to low-grade dysplasia (LGD), high-grade dysplasia (HGD), and eventually EAC under the influence of chronic esophageal acid exposure.[4] Early identification of a premalignant stage followed by endoscopic treatment provides the opportunity to prevent progression to EAC and endoscopic surveillance is therefore recommended for BE patients. However, the value of BE surveillance is under discussion given the overall low incidence of neoplastic progression, the large screening base which is estimated at 1-2% of the general population, and the lack of discriminative tests for risk stratification.[5, 6] Histological diagnosis of low-grade dysplasia (LGD) is the only accepted predictor for neoplastic progression to date but has a low predictive value due to sample error and considerable interobserver variation.[7, 8] Application of biomarkers in addition to standard histological assessment may contribute to the identification of patients at high risk for neoplastic progression and may thereby improve risk stratification. Alpha-Methylacyl-CoA Racemase (AMACR) is a cytoplasmic enzyme that plays an essential role in the β-oxidation of branched-chain fatty acids by catalyzing the conversion of (2R)-methyl-branched-chain fatty acyl-coenzyme A esters to their (S)-stereoisomers. AMACR is normally expressed in hepatocytes, renal tubular cells, gallbladder epithelium and bronchial epithelium and is an established biomarker for prostate cancer. It is expressed in both colon adenomas and adenocarcinomas but not in normal colon epithelium, which suggest that it may play a role in the development of gastrointestinal malignancies.[9, 10] Several studies showed that AMACR may also be expressed in BE epithelium, with an increasing prevalence along the metaplasia-dysplasia-adenocarcinoma sequence.[9-14] The aim of this study was therefore to investigate the value of AMACR immunohistochemistry for predicting neoplastic progression in a large cohort of patients with BE.

Methods

Study design

We conducted a case-control study within a large prospective cohort of BE patients. In this cohort 720 patients were included with known or newly diagnosed BE of at least 2 cm, confirmed by the presence of intestinal metaplasia and without a history of HGD or EAC. Patients were included between November 2003 and December 2004 in 3 University Medical Centers and 12 regional hospitals throughout the Netherlands and had endoscopic surveillance according to the guidelines of the American College of Gastroenterology.[15] Patients without dysplasia underwent upper endoscopy with biopsy sampling every 3 years and patients with LGD every year. All endoscopic procedures were performed by experienced gastroenterologists, according to a standardized protocol. Endoscopic landmarks such as the diaphragm impression, gastroesophageal junction and squamocolumnar junction were noted, the presence of esophagitis was graded according to the Los Angeles Classification, and abnormalities were reported including nodules, ulcers and erosions.[16, 17] At each endoscopy targeted biopsies were taken from mucosal abnormalities and quadrant biopsies were taken every 2 cm from the most distal to the most proximal part of the BE epithelium, according to the Seattle protocol.[18]
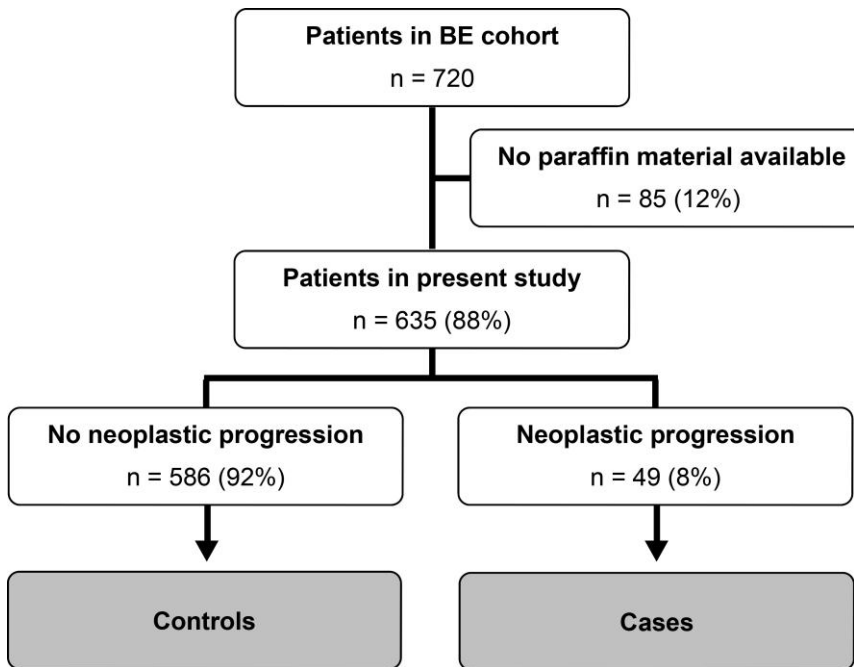
Histology

Biopsy specimens were fixed with buffered formalin and embedded in paraffin, according to standard procedures. From each biopsy set 4 µm thick sections were cut and stained with haematoxylin-eosin to assess the presence of BE and to define the grade of dysplasia. After examining all biopsies, the highest degree of abnormality was reported for each endoscopy. Slides were first graded by a local pathologist and then by an expert pathologist for second opinion. When the local pathologist and expert pathologist disagreed on the grade of dysplasia, the slides were reviewed by a second expert pathologist. Pathologists were blinded to the diagnosis of each other and a final diagnosis was made only if at least 2 pathologists agreed on the grade of dysplasia. If there was still disagreement, a panel of expert pathologists reviewed the slides as well and a final diagnosis was made based on consensus agreement.

Patient selection

We collected paraffin material suitable for immunohistochemistry from all patients in our BE cohort. Paraffin material was not available in 85 patients, leaving 635 individual patients to be included in this analysis. Patients who developed HGD or EAC during follow-up were classified as cases and patients without neoplastic progression were classified as controls (Figure 1). Immunohistochemistry was performed on paraffin material of all surveillance endoscopies of patients who developed LGD, HGD or EAC. In patients without dysplasia, immunohistochemistry was performed on biopsies of a random surveillance endoscopy.

Figure 1. Flowchart of patients included in the study. Patients with neoplastic progression were classified as cases and patients without progression as controls.

Immunohistochemistry

Immunohistochemistry was performed as a single batch at the pathology department of the Erasmus University Medical Center (Rotterdam, the Netherlands) using an automatic immunohistochemical staining machine

(Ventana Medical Systems, Tucson, Arizona, USA). A sample of prostate cancer was used as positive control for each section. Sections were deparaffinized prior to the staining procedure and heat-induced epitope retrieval was performed at 97ºC for 15 minutes. Endogenous peroxidase activity was blocked by incubating the slides for 15 minutes in a solution of 0.3% hydrogen peroxide in phosphate-buffered saline. Monoclonal rabbit anti-human AMACR polypeptide was used as the primary antibody with a dilution of 1:200 (Clone 13H4, Thermo scientific, Cheshire, UK). The slides were incubated for 30 minutes with the primary antibody followed by amplification and visualization using the Dako REAL EnVision system (peroxidase/DAB, Rabbit/Mouse, Dako, Glostrup, Denmark) and counterstaining with haematoxylin-eosin.

Immunohistochemical stained slides were examined in tandem with haematoxylin-eosin stained slides to determine AMACR expression in areas with dysplasia. Cytoplasmic AMACR expression was scored on a 3 point scale (no expression, mild expression or strong expression) by 2 independent expert pathologists who were blinded for long-term outcome (Figure 2). After examining all biopsies, the highest degree of abnormality was reported for each endoscopy. When there was disagreement between the 2 pathologists, the slides were evaluated by both pathologists simultaneously to reach a consensus diagnosis.

Ethics

The study protocol was approved by the Institutional Review Boards of the Erasmus University Medical Centre, as well as those of all participating hospitals. Before the first endoscopy, written informed consent was obtained from all patients.

Statistical analysis

Characteristics of cases and controls were compared using Mann-Whitney U tests for continuous variables and Chi-squared tests for categorical variables. To compare AMACR expression in biopsies with different grades of dysplasia Mann-Whitney U tests and Kruskal-Wallis tests were used, thereby ignoring that multiple biopsies could be from one patient. The value of AMACR immunohistochemistry for predicting neoplastic progression was estimated in loglinear regression models. Since immunohistochemistry was not performed on all biopsy series, data were split up by endoscopy.
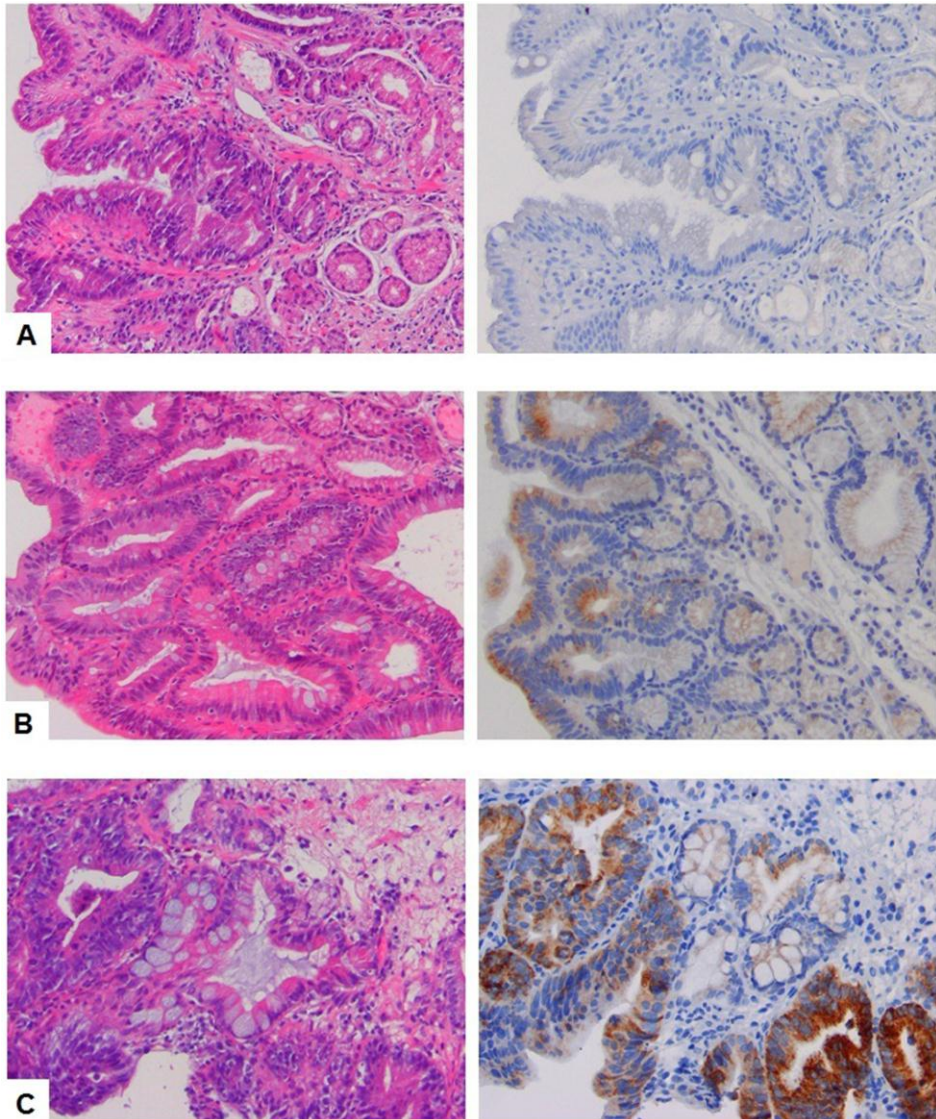
Figure 2. Haematoxylin-eosin staining and AMACR immunohistochemistry of

A. Barrett's esophagus without dysplasia and no AMACR expression
B. Barrett's esophagus with low-grade dysplasia and mild AMACR expression
C. Barrett's esophagus with high-grade dysplasia and strong AMACR expression

Neoplastic progression was defined as the development of HGD or EAC after inclusion in the study and follow-up time was defined as the time between each endoscopy and the next surveillance endoscopy. Loglinear regression models were used to calculate relative risks (RR) and 95% confidence intervals (CI) with the logarithm of follow-up time as offset variable. In multivariable models, relative risks were adjusted for age, gender, BE length, and presence of esophagitis. The 49 cases and 586 controls provided 80% power to detect a relative risk of at least 2.5 at a significance level of 5%. Interobserver agreement for AMACR expression was determined using Cohen kappa (κ) statistics. Two sided P-values <0.05 were considered to be statistically significant. Data were analysed using SPSS Statistics (version 20.0, Chicago, Illinois, USA).

Results

Patient characteristics
Six hundred thirty-five BE patients (73% male, median age 60 years (IQR 53-69)) were included in this study and followed during surveillance for a median duration of 6.6 years (IQR 5.1-7.3) and with a median of 4 follow-up endoscopies (IQR 4-5). In total 223 (35%) patients were diagnosed with LGD during follow-up with an incidence rate of 3.6 per 100 patient-years (95% CI 3.0-4.4). Thirty-five (6%) patients developed HGD and 14 (2%) patients developed EAC after a median follow-up period of 3.0 (2.0-5.3) years. These 49 (8%) patients with neoplastic progression were classified as cases and the remaining 586 (92%) patients without neoplastic progression were classified as controls (Figure 1). The incidence of HGD and EAC together was 1.4 per 100 patient-years (95% CI 1.0-1.8) and the incidence of EAC alone was 0.4 per 100 patient-years (95% CI 0.2-0.6). AMACR expression was assessed in biopsy series of 1481 endoscopies. This included 1085 (73%) biopsy series without dysplasia, 347 (23%) with LGD, 35 (3%) with HGD and 14 (1%) with EAC. The highest degree of abnormality was reported for each endoscopy after examining all biopsies. In total 12.127 biopsies were reviewed. Cases had a smaller number of follow-up endoscopies, longer BE length and were more likely to be diagnosed with LGD than controls, but otherwise there were no significant differences (Table 1).

Table 1. Characteristics of cases and controls

		Controls n = 586	Cases n = 49	P-value
Follow-up	Median, years	6.8 (5.7-7.4)	3.0 (2.0-5.3)	<0.001
Endoscopies	Median number	4 (4-5)	3 (2-4)	<0.001
Biopsies	Median per endoscopy Total number	7 (4-10) 10.346	8 (6-12) 1781	0.042
Age	Median, years	60 (53-69)	65 (55-70)	0.171
Gender	Male	426 (73%)	40 (82%)	0.174
Alcohol use	Current	453 (79%)	38 (78%)	0.969
Smoking	Current	122 (21%)	12 (25%)	0.464
NSAID use	≥ 1 month	185 (32%)	14 (29%)	0.664
Reflux	Current	176 (31%)	19 (39%)	0.230
BE diagnosis	At inclusion	143 (25%)	13 (27%)	0.512
BE length	Median, cm	4 (3-6)	5 (4-8)	0.004
Histology	LGD	90 (15%)	22 (45%)	<0.001
Esophagitis	Current	110 (19%)	14 (29%)	0.096

NSAID, nonsteroidal anti-inflammatory drugs; BE, Barrett's esophagus; LGD, low-grade dysplasia

AMACR expression

No AMACR expression was seen in 696 (47%) biopsy series, mild AMACR expression in 707 (48%) and strong AMACR expression in 78 (5%). AMACR expression was more common in biopsy series with a higher grade of dysplasia and was seen in 526 (49%) biopsy series without dysplasia, 218 (63%) with LGD, 32 (91%) with HGD and 10 (71%) with EAC (P<0.001) (Figure 3). Mild AMACR expression was seen in 46% of biopsy series without dysplasia, 53% with LGD, 64% with HGD and 57% with EAC. Strong AMACR expression was seen in 3% of biopsy series without dysplasia, 10% with LGD, 27% with HGD and 14% with EAC. Mild AMACR expression was seen in the base of glands as well as in surface epithelium without an evident pattern. Strong AMACR expression was especially seen in the base of glands. In about 1 in 5 patients, strong AMACR expression was also seen in surface epithelium. AMACR expression was more common in biopsy series of cases (67%) than in biopsy series of controls (50%) and was associated with an increased risk of developing HGD or EAC during follow-up (RR 2.0; 95% CI 1.1-3.6). This

association remained after adjusting for age, gender, BE length and esophagitis (adjusted RR 1.9; 95%CI 1.0-3.4). Although mild AMACR expression was associated with a trend towards an increased risk of neoplastic progression (adjusted RR 1.6; 95%CI 0.9-3.1), the risk was especially elevated with strong AMACR expression (adjusted RR 4.8; 95%CI 1.9-12.6) (Table 2). After adjusting for histological diagnosis, only strong AMACR expression remained associated with a significantly increased risk of neoplastic progression (adjusted RR 1.4; 95%CI 0.8-2.6 and 3.3; 95%CI 1.3-8.4 respectively).

In total 344 (54%) patients were diagnosed with AMACR expression during follow-up. Of these patients 39 (11%) eventually developed HGD or EAC with an incidence of 2.9 per 100 patient-years (95% CI 2.0-3.9). The sensitivity of AMACR expression for predicting neoplastic progression was 67% with a specificity of 50%. The positive predictive value for neoplastic progression was 11% with a negative predictive value of 97%. Forty-one (6%) patients were diagnosed with strong AMACR expression during follow-up. Of these patients 9 (22%) eventually developed HGD or EAC with an incidence rate of 7.1 per 100 patient-years (95% CI 3.3-13.5). The sensitivity of strong AMACR expression for predicting neoplastic progression was 10% with a specificity of 96%. The positive predictive value for neoplastic progression was 22% with a negative predictive value of 93%.

Figure 3. Percentage of biopsy series with AMACR expression, stratified by grade of dysplasia. ▪ mild expression ▪ strong expression
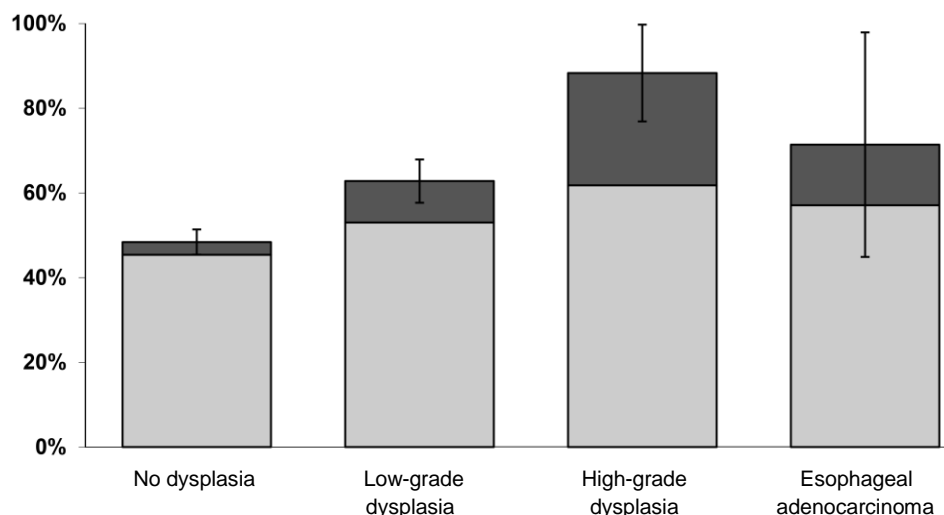
Table 2. AMACR expression in biopsy series of cases and controls

AMACR expression	Controls n = 1300*	Cases n = 132*	RR (95% CI)	RR[a] (95% CI)
No expression	645 (50%)	43 (33%)	Reference	Reference
Mild expression	601 (46%)	76 (57%)	1.7 (0.9-3.2)	1.6 (0.9-3.1)
Strong expression	54 (4%)	13 (10%)	5.1 (2.0-13.1)	4.8 (1.9-12.6)

RR, relative risk; CI, confidence interval

[a] Adjusted for age, gender, BE length and esophagitis.

* The highest degree of abnormality was reported for each endoscopy after examining all biopsies

Histology and AMACR expression

Normal BE with no or mild AMACR expression was seen in 1052 (74%) biopsy series, normal BE with strong AMACR expression in 33 (2%), LGD with no or mild AMACR expression in 313 (22%), and LGD with strong AMACR expression in 34 (2%). LGD with no or mild AMACR expression was associated with an increased risk of neoplastic progression (RR[a] 3.4; 95% CI 1.9-6.3), but the risk was higher with LGD and concurrent strong AMACR expression (RR[a] 11.0; 95% CI 4.1-30.0) (Table 3). During follow-up 23 (4%) patients were diagnosed with LGD and concurrent strong AMACR expression. Of these patients 7 (30%) eventually developed HGD or EAC with an incidence rate of 12.2 per 100 patient-years (95% CI 4.9-25.1).

Table 3. Histology and AMACR expression in cases and controls

Histology and AMACR expression	Controls n = 1300*	Cases n = 132*	RR (95% CI)	RR[a] (95% CI)
ND and no/mild AMACR expression	982 (76%)	70 (53%)	Reference	Reference
ND and strong AMACR expression	29 (2%)	4 (3%)	1.6 (0.2-11.6)	1.5 (0.2-10.3)
LGD and no/mild AMACR expression	264 (20%)	49 (37%)	3.6 (2.0-6.6)	3.4 (1.9-6.3)
LGD and strong AMACR expression	25 (2%)	9 (7%)	10.7 (4.1-28.0)	11.0 (4.1-30.0)

ND, no dysplasia; LGD, low-grade dysplasia; RR, relative risk; CI, confidence interval

[a] Adjusted for age, gender, BE length and esophagitis.

* The highest degree of abnormality was reported for each endoscopy after examining all biopsies

Interobserver agreement

Interobserver agreement for histological diagnosis was fair (κ = 0.34; 95%CI 0.29-0.39). The local pathologists and expert pathologists agreed on histological

diagnosis in 1028 (69%) samples. It was especially hard to distinguish LGD. Local pathologists diagnosed dysplasia in 27% of all samples. Of these samples 46% was downgraded by expert pathologists and 0.8% was upgraded.

Interobserver agreement for AMACR expression was moderate (κ = 0.44; 95%CI 0.41-0.48). Both expert pathologists agreed on AMACR expression in 997 (67%) samples. It was especially hard to distinguish mild AMACR expression. When AMACR expression was scored on a 2 point scale (no or mild expression versus strong expression) interobserver agreement was substantial (κ = 0.78; 95%CI 0.71-0.84). Both expert pathologists agreed on AMACR expression in 1444 (98%) samples (Table 4).

Table 4. Interobserver agreement for AMACR expression in expert pathologists

AMACR expression	No expression	Mild expression	Strong expression	κ
No expression	493 (33%)	426 (29%)	2 (0%)	0.444
Mild expression	21 (2%)	435 (29%)	35 (2%)	
Strong expression	-	-	69 (5%)	

AMACR expression	No or mild expression		Strong expression	κ
No or mild expression	1375 (93%)		37 (2%)	0.776
Strong expression	-		69 (5%)	

The highest degree of abnormality was reported for each endoscopy after examining all biopsies

Discussion

In this case control study, the value of AMACR immunohistochemistry was evaluated for predicting neoplastic progression in patients with BE. Although mild AMACR expression was associated with a trend toward an increased risk of neoplastic progression, the risk of developing HGD or EAC was especially elevated with strong AMACR expression. This association was independent of age, gender, BE length, esophagitis and histology.

AMACR is an enzyme that plays an essential role in the β-oxidation of branched-chain fatty acids and is normally expressed in peroxisomes and mitochondria of hepatocytes, renal tubular cells, gallbladder epithelium and bronchial epithelium. AMACR is especially expressed in malignancies that have been associated with a high fat diet such as prostate and colon cancer. As AMACR is known to be involved in the metabolism of lipids, this protein may

lead to alterations in the balance of cellular oxidants that may contribute to the development of cancer. AMACR has been most extensively studied in the prostate gland, where the overall sensitivity and specificity for the detection of carcinoma ranges from 80 to 100%. In the colon, AMACR is expressed in up to 75% of adenomas as well as 75% of well-differentiated adenocarcinomas.[9, 10]

In the present study AMACR expression was evaluated in a large cohort of BE patients. The percentage of patients with AMACR expression increased along the metaplasia-dysplasia-adenocarcinoma sequence. AMACR was detected in 49% of samples without dysplasia, 63% with LGD, 91% with HGD and 77% with EAC. Previous studies reported AMACR expression in 60 to 96% of samples with HGD and 67 to 96% of samples with EAC, which corresponds to the results of our study.[9-14] These previous studies showed much more variation in AMACR expression in LGD, ranging from 11 to even 93% of the investigated samples. This wide range may be explained by the observer heterogeneity of samples with LGD, the small sample size of these studies and variable interpretation of the immunohistochemistry results. This is supported by the present study with mild AMACR expression detected in 63% of samples with LGD, which is around the average of previous studies. Previous studies have also shown inconsistent results for BE without dysplasia. Most studies reported no or little AMACR overexpression in BE without dysplasia, but one study reported AMACR overexpression in up to 83% of samples. We found AMACR overexpression in about half of samples without dysplasia. Although AMACR expression increased along the metaplasia-dysplasia-adenocarcinoma sequence and thus may help to detect dysplasia, AMACR overexpression was not sufficient to make a diagnosis of dysplasia.

To our knowledge this is the first study investigating the value of AMACR immunohistochemistry for predicting neoplastic progression in patients with BE. Although strong AMACR expression was associated with an increased risk of neoplastic progression, the predictive value was only slightly higher than that of LGD (22% versus 15%). The negative predictive value of strong AMACR expression was slightly lower than that of LGD (91 versus 93%). Thus AMACR has not enough power as a single biomarker for neoplastic progression.

In addition to AMACR, many other biomarkers have been proposed for risk stratification in BE. However, only few biomarkers have been shown to be predictive for progression to EAC. P53 immunohistochemistry appears to be one of the most promising biomarkers. Previous studies have shown that aberrant p53 expression is associated with an increased risk of neoplastic

progression in BE. However, high rates of false negatives and false positives hamper the use of p53 immunohistochemistry in clinical practice. Other biomarkers predictive for neoplastic progression are cyclin D1, p53 loss of heterozygosity, as well as aneuploidy and tetraploidy.[9, 10, 19]

This study has several strengths including the large cohort of BE patients and long follow-up time. Patients were prospectively followed according to a stringent follow-up scheme and during follow-up clinical and pathological data were collected. In addition, a standardized endoscopy and biopsy protocol were used. All slides were reviewed by at least 2 pathologists to obtain a final diagnosis based on consensus. One of the limitations of this study is that although patients were only classified as controls when they did not develop HGD or EAC during follow-up, we cannot exclude that these patients will develop HGD or EAC in the future. If so, we may have underestimated the value of AMACR for predicting neoplastic progression. In addition, AMACR immunohistochemistry was performed on all samples of patients with dysplasia, but only on one random sample of patients without dysplasia. In the analysis we accounted for this variation by splitting up data per endoscopy. As a sensitivity analysis we repeated the analysis using one random of all patients, which yielded similar results.

In conclusion, this case control study shows that strong AMACR expression is associated with an increased risk of neoplastic progression in patients with BE. However, AMACR expression appears to be a less powerful predictor for neoplastic progression than LGD.

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Chapter 7

Surveillance in patients with long-segment Barrett's esophagus: a cost-effectiveness analysis

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Abstract

Introduction: Surveillance is recommended for Barrett's esophagus (BE) to detect early esophageal adenocarcinoma (EAC). The aim of this study was to evaluate the cost-effectiveness of surveillance.

Methods: We included 714 patients with long-segment BE in a multicenter prospective cohort study and used a multi-state-Markov model to calculate progression rates from no dysplasia (ND) to low-grade dysplasia (LGD), high-grade dysplasia (HGD) and EAC. Progression rates were incorporated in a decision-analytic model, including costs and quality of life data. We evaluated different surveillance intervals for ND and LGD, endoscopic mucosal resection (EMR), radiofrequency ablation (RFA) and esophagectomy for HGD or early EAC and esophagectomy for advanced EAC. Incremental cost-effectiveness ratios (ICER) were calculated in costs per quality-adjusted life year (QALY).

Results: The annual progression rate was 2% for ND to LGD, 4% for LGD to HGD or early EAC, and 25% for HGD or early EAC to advanced EAC. Surveillance every 5 or 4 years with RFA for HGD or early EAC and esophagectomy for advanced EAC had ICERs of €5.283 and €62.619 per QALY for ND. Surveillance every 5 to 1 year had ICERs of €4.922, €30.067, €32.531, €41.499, and €75.601 per QALY for LGD. EMR prior to RFA was slightly more expensive, but important for tumor staging.

Conclusion: Based on a Dutch healthcare perspective and assuming a willingness-to-pay threshold of €35.000 per QALY, surveillance with EMR and RFA for HGD or early EAC, and esophagectomy for advanced EAC is cost-effective every 5-years for ND and every 3-years for LGD.

Introduction

Barrett's esophagus (BE) is a premalignant condition in which patients have an increased risk of developing esophageal adenocarcinoma (EAC) with an estimated incidence of 0.1 to 0.5% per year.[1-4] The development of EAC in BE is a gradual process, in which metaplastic epithelium with no dysplasia (ND) evolves to low-grade dysplasia (LGD), high-grade dysplasia (HGD) and eventually EAC under the influence of chronic esophageal acid exposure.[5] Once a patient has developed EAC the prognosis is poor with a 5-year survival of less than 20%.[6, 7] Endoscopic surveillance is therefore recommended for BE to detect EAC at an early stage, when curative treatment is still feasible.[8, 9] Histological diagnosis of dysplasia is the golden standard for predicting neoplastic progression in BE and is therefore used for defining surveillance intervals. Current guidelines recommend surveillance every 3 to 5 years in patients with ND, every 6 to 12 months in patients with LGD and every 3 months in patients with HGD (in absence of endoscopic therapy). Most patients with BE belong to the group with ND and have an overall low risk of neoplastic progression. The majority of patients with non-dysplastic BE will never develop HGD or EAC and die of causes not related to BE, which makes surveillance controversial in this patient group.[10] In patients with LGD the risk of neoplastic progression is increased, which makes surveillance more effective. However, histological diagnosis of LGD is subject to considerable intra- en interobserver variation which limits its predictive value.[11, 12]

Over the past years there has been a major shift in the treatment of BE patients with the introduction of endoscopic treatment modalities such as endoscopic mucosal resection (EMR) and radiofrequency ablation (RFA). EMR is used to remove visible mucosal irregularities and has a role in tumor staging, while RFA is used to eradicate residual intestinal metaplasia. Although use of RFA alone is still controversial, some studies suggest that this might be just as effective.[13, 14] Nowadays endoscopic treatment with EMR and RFA is the preferred strategy for HGD and early EAC.[8, 9] Recently was suggested that RFA might also be suitable for patients without neoplastic progression, especially for those with confirmed LGD. However, it is difficult to make a reliable diagnosis of LGD and the risk of progression may vary greatly among these patients. Therefore no strict recommendations are made for patients with LGD.[9] Esophagectomy is still the mainstay for curative treatment of advanced EAC, but is complemented with neoadjuvant chemoradiotherapy.[15] Chemotherapy, esophageal stenting and

brachytherapy have been added to the palliative treatment of EAC.[16] One of the key questions in the discussion about BE surveillance is whether surveillance and (endoscopic) treatment is cost-effective. The cost-effectiveness of BE surveillance has been investigated in previous studies, where transition rates to HGD and EAC were mostly based on pooled literature data.[17-26] For a more accurate representation of the natural history of BE and its progression to EAC, true transition and misclassification rates can be calculated in a multi-state Markov (MSM) model using prospectively collected follow-up data from a large cohort of BE patients.[27] The aim of this study was to evaluate the cost-effectiveness of different surveillance intervals and treatment strategies for patients without dysplasia and LGD in long-segment BE, within a large multicenter prospective cohort study.

Methods

Study Design

We conducted a large multicenter prospective cohort study in 3 university medical centers and 12 regional hospitals throughout the Netherlands. Between November 2003 and December 2004, 714 consecutive patients were included presenting with known or newly diagnosed BE of at least 2 cm, without a history of HGD or EAC. The diagnosis was confirmed by the presence of intestinal metaplasia. Patients were followed according to the guidelines of the American College of Gastroenterology.[9] During follow-up incident cases of HGD and EAC were identified. Patients who developed HGD or EAC were considered to have reached an endpoint and received appropriate treatment. At each follow-up endoscopy targeted biopsies were taken from mucosal abnormalities and four-quadrant biopsies were taken every 2 cm from the most distal to the most proximal part of the BE epithelium. Biopsies were first graded by a local pathologist and then by an expert pathologist for second opinion. After examining all biopsies, the highest degree of abnormality was reported for each endoscopy. When the local and expert pathologist disagreed on the grade of dysplasia, slides were reviewed by a second expert pathologist. Pathologists were blinded to the diagnosis of each other and a final diagnosis was made only if at least 2 pathologists agreed on the grade of dysplasia. HGD and EAC limited to the mucosa (T1a) were considered as one category (HGD or early EAC), since both are treated similarly. Carcinomas invading the submucosa

(T1b), muscularis propria (T2), adventitia (T3) or adjacent structures (T4) were considered as another category (advanced EAC).

*Incidence, misclassification and transition rates*

The incidence rates of LGD, HGD and EAC were calculated by dividing the number of incident cases by the total number of follow-up years. Since neoplastic progression is thought to be a gradual process, patients who developed HGD or EAC were supposed to have passed the stage of LGD. When LGD was not observed, the time till the development of LGD was estimated to be half of the follow-up time in patients who developed HGD or early EAC and one third of the follow-up time in patients who developed advanced EAC. Patients who developed advanced EAC were supposed to have passed the stage of HGD. When HGD was not observed, the time till the development of HGD was estimated to be two third of the follow-up time in patients with ND and half of the follow-up time in patients with LGD. Since histological diagnosis is subject to misclassification due to sampling error and interobserver variation, the histological diagnosis observed at each endoscopy may not represent the true histological diagnosis (or "true state"). The observed state is dependent on the true state as well as the misclassification rates (Figure 1). In a MSM model misclassification rates can be estimated based on observed follow-up data.[27] The assumption was made that advanced EAC was not observed in patients with true ND and that ND or LGD was not observed in patients with true advanced EAC. The misclassification rates were used to convert observed transition rates into true transition rates. Since patients who developed HGD or EAC were excluded from further follow-up, we were not able to observe the transition rate from HGD or early EAC to advanced EAC. Therefore we added one patient with HGD to our follow-up data who developed advanced EAC after 4 years of follow-up, based on observations in another Dutch BE cohort.[28] Although regression of dysplasia was observed in some patients, we assumed that true regression of dysplasia was not possible and that the observed regression was due to sampling error and observer variability.

Surveillance strategies

We evaluated the cost-effectiveness of 16 different surveillance strategies. The first strategy consisted of upper endoscopy only in case of symptoms such as dysphagia or pyrosis and esophagectomy with neoadjuvant chemoradiotherapy in patients with EAC (no surveillance). The other 15 strategies consisted of

surveillance with different intervals (1 to 5 years) for patients with ND or LGD and endoscopic or surgical intervention for patients with HGD or EAC. Treatment strategies for HGD or early EAC consisted of RFA alone, EMR followed by RFA, or esophagectomy with neoadjuvant chemoradiotherapy. We assumed that complications occurred in 2% after EMR, 7% after RFA and 23% after esophagectomy and considered costs associated with additional treatment.[29-31] After endoscopic treatment with EMR or RFA we assumed that patients returned to ND and surveillance was resumed. We assumed that 5 to 10% of patients had early recurrence for which they received endoscopic treatment. After endoscopic treatment, patients remained at risk for neoplastic progression. Treatment of advanced EAC consisted of esophagectomy with neoadjuvant chemoradiotherapy. Palliative treatment of EAC consisted of chemotherapy, esophageal stenting or brachytherapy and terminal care.

Figure 1. Multi-state Markov model
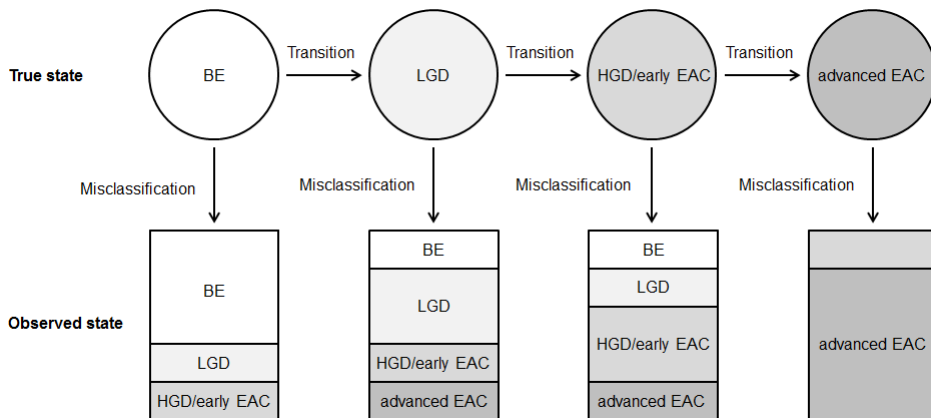
BE, Barrett's esophagus; LGD, low-grade dysplasia; HGD, high-grade dysplasia; EAC, esophageal adenocarcinoma

Costs and quality of life

The cost-effectiveness analysis was performed from a health care perspective. Direct medical true costs of endoscopic and surgical procedures, neoadjuvant and palliative treatment, and inpatient and outpatient care were obtained using the 2012 reimbursement rates per diagnosis and intervention as provided by the Dutch healthcare authority (NZa).[32] Direct medical costs include costs of

medical procedures, equipment, overhead, personnel and honoraria of medical specialists. Hospitals get these costs reimbursed by the health insurance. Data on quality of life (utilities) associated with different health states were derived from the published literature and were used to convert absolute life-years of survival into quality-adjusted-life-years (QALYs).[33-35] Costs and utilities were discounted at an annual rate of 5%, which allows to compare our results to those of previous studies (Table 1).

Table 1. Variables included in cost-effectiveness analysis

Variables		Base value	Reference
Transition rates (per year)			
ND to LGD		0.023	Own data
LGD to HGD or early EAC		0.043	Own data
HGD or early EAC to advanced EAC		0.250	[27]
Misclassification rates			
True state	*Observed state*		
ND	LGD	0.086	Own data
ND	HGD or early EAC	0.004	Own data
LGD	ND	0.247	Own data
LGD	HGD or early EAC	0.123	Own data
LGD	Advanced EAC	0.008	Own data
HGD or early EAC	LGD	0.016	Own data
HGD or early EAC	Advanced EAC	0.287	Own data
Advanced EAC	HGD or early EAC	0.036	Own data
Probabilities			
Probability of surgery		0.600	Cancer register
Probability of curative treatment		0.500	Cancer register
Probability of dying from surgery		0.018	[44]
Probability of complications from surgery		0.229	[45]
Probability of complications from endoscopy		0.001	[46]
Probability of complications from EMR		0.022	[47]
Probability of complications from RFA		0.065	[38]

Variables	Base value	Reference
Costs		
Cost of endoscopy	€ 629	NZa
Cost of endoscopy with complication	€ 1677	NZa
Cost of EMR	€ 1925	Expert opinion
Cost of EMR with complication	€ 3425	Expert opinion
Cost of RFA	€ 6210	Expert opinion
Cost of RFA with complication	€ 8710	Expert opinion
Cost of staging adenocarcinoma	€ 2499	NZa
Cost of esophagectomy	€ 17.887	NZa
Cost of esophagectomy with complication	€ 38.930	NZa
Cost of postoperative follow-up, per year	€ 948	NZa
Cost of neoadjuvant chemoradiation	€ 8792	NZa
Cost of palliative chemotherapy	€ 3867	NZa
Cost of palliative stenting	€ 1215	NZa
Cost of brachytherapy	€ 3004	NZa
Cost of terminal care, per year	€ 32565	[18]
Quality of life		
Quality of life after HGD diagnosis	0.84	[29, 31]
Quality of life after EAC diagnosis	0.66	[29, 31]
Quality of life after endoscopic treatment (short term)	0.93	[29, 31]
Quality of life after esophagectomy (short term)	0.86	[30]
Quality of life after esophagectomy (long term)	0.90	[30]
Duration of short term morbidity		
After endoscopic treatment	3 days	[38]
After esophagectomy	4 weeks	[30]
Discount rate	0.05	[18]

ND, no dysplasia; LGD, low-grade dysplasia; HGD, high-grade dysplasia; EAC, esophageal adenocarcinoma; EMR, endoscopic mucosal resection; RFA, radiofrequency ablation; NZa, Dutch healthcare authority

Ethics

The study protocol was approved by the Institutional Review Boards of the Erasmus University Medical Center, as well as those of all participating hospitals. Before the first endoscopy, written informed consent was obtained.

Cost-effectiveness analysis

For the analysis we used a modification of a previously published decision-analytic Markov model, which was constructed in Windows Decision Maker (Beta test version 2010).[22] In this computer model a BE cohort was simulated with as base case a 55-years old male BE patient with ND or LGD. The natural history of the BE cohort was modelled to examine the costs of no surveillance and its effects on quality of life. Subsequently, the effect of multiple surveillance strategies was evaluated with various surveillance intervals for patients with ND or LGD and endoscopic or surgical interventions for patients with HGD or EAC. Simulation of the BE cohort started with baseline endoscopy and was continued with cycles of 3 months until death. True progression rates from ND to LGD, HGD, and advanced EAC were estimated in a MSM model based on the progression and misclassification rates observed in our cohort. Death from other causes than EAC was possible in any state and was modelled as a time-dependent variable with the risk increasing with age.

Statistical analysis

Primary outcome of the study was the incremental cost-effectiveness ratio (ICER) of each surveillance strategy. The ICER is defined as the difference in cost between 2 surveillance strategies, divided by the change in QALY's. Whether a surveillance strategy is cost-effective depends on the willingness-to-pay threshold, which is highly variable among countries. In the Netherlands a willingness-to-pay threshold is used of € 20.000 to € 80.000, depending on the severity of the condition.[36] In the United States of America and the United Kingdom a willingness-to-pay threshold of € 35.000 is used.[37, 38] In one-way sensitivity analyses we evaluated the effect of halving or doubling all individual input variables, while keeping the other input variables unchanged. In addition we performed analyses using a discount rate of 3% and using transition rates of 200%, 50% and 25% of the calculated values.

Results

Patient characteristics

Seven hundred fourteen patients (73% male, median age 61 years) with a median Barrett length of 4 centimeters were included and followed during surveillance with a median duration of 6 years and a total of 3992 person-years of follow-up. Most patients (74%) were already known with BE before inclusion in the study for a median duration of 5 years (Table 2).

Table 2. Characteristics of patients with Barrett's esophagus

		Cohort n = 714
Follow-up	Median, years (IQR)	6.1 (4.4-7.0)
	Total, person-years	3992
Age	Median, years (IQR)	61 (53-69)
Gender	Male	520 (73%)
BE diagnosis	< Inclusion	529 (74%)
	≥ Inclusion	185 (26%)
BE length	Median, cm (IQR)	4 (2-6)
Baseline esophagitis	Yes	72 (10%)
Baseline histology	No dysplasia	606 (85%)
	Low-grade dysplasia	108 (15%)

IQR, interquartile range; BE, Barrett's esophagus

Incidence and transition rates

At baseline, 606 (85%) patients had ND and 108 (15%) LGD. In patients with ND the observed incidence of LGD was 6% per year. In patients with LGD the observed annual incidence was 13% for progression to HGD or early EAC and 57% for regression to ND. During follow-up 46 (6%) patients developed HGD or early EAC and 4 (1%) patients developed advanced EAC with an annual incidence of 1.2% (95% CI 0.9-1.6) for HGD or early EAC and 0.1% (95% CI 0.0-0.3) for advanced EAC, which was stable over time and similar for patients with incident and prevalent BE.(Table 3). After neoplastic progression, 33 patients were treated with EMR. In 75% of cases the histological diagnosis was confirmed in the EMR specimen, in 20% the histological diagnosis was downgraded and in 5% upgraded after evaluation of the EMR specimen.

Table 3. Observed annual incidence rates in patients with Barrett's esophagus

Transition	Cases			Follow-up in years	Incidence rate with 95% CI
	Observed	Interpolated	Total		
ND to LGD	180	27	207	3640	5.7% (4.9-6.5)
LGD to HGD/early EAC	18	28	46	350	13% (9.6-18)
LGD to ND	198	-	198	350	57% (49-65)
ND/LGD to HGD/early EAC	42	4	46	3990	1.2% (0.9-1.6)
ND/LGD to advanced EAC	4	-	4	3992	0.1% (0.0-0.3)

CI, confidence interval; ND, no dysplasia; LGD, low-grade dysplasia; HGD, high-grade dysplasia; EAC, esophageal adenocarcinoma

The true annual transition rate was estimated to be 2.3% for ND to LGD, 4.3% for LGD to HGD or early EAC, and 25% for HGD or early EAC to advanced EAC. The true incidence rate of HGD or EAC was estimated to be 0.1% per year in ND and 4.9% per year in LGD.

Surveillance in patients with no dysplasia

In patients with ND, the costs of no surveillance were € 5.695 for 12.62 discounted QALYs. Surveillance every 5 years with RFA for HGD or early EAC and esophagectomy for advanced EAC resulted in an increase in life expectancy by 0.25 QALYs and an increase in costs by €1.324, representing an ICER of €5.283 per QALY. Surveillance every 4 years resulted in an additional increase in life expectancy by 0.02 QALYs and an additional increase in costs by €802, representing an ICER of €62.619 per QALY gained. Strategies with surveillance intervals shorter than 4 years provided substantial higher costs with similar or less QALYs gained (Table 4). Strategies using EMR prior to RFA had similar effects on QALYs compared to strategies using RFA alone, but were slightly more expensive. Strategies using esophagectomy were much more expensive with less QALYs. However, use of RFA alone is still controversial and EMR contributed significantly to tumor staging, which may justify the slightly higher costs. In summary, when assuming a willingness-to-pay threshold of €35.000 per QALY, surveillance every 5 years with EMR followed by RFA or RFA alone for HGD or early EAC and esophagectomy with neoadjuvant chemoradiotherapy for advanced EAC is a cost-effective strategy for long-segment BE with ND. When assuming a willingness-to-pay threshold of €80.000 per QALY, surveillance every 4 years is cost-effective (Figure 2).

Table 4. Cost-effectiveness of surveillance in patients without dysplasia

Strategy	No dysplasia		
	Costs	QALYs	ICER
No surveillance	€ 5.695	12.62	
Surveillance every 5 years with RFA	€ 7.019	12.87	€ 5.283
Surveillance every 5 years with EMR followed by RFA	€ 7.247	12.87	x
Surveillance every 5 years with esophagectomy	€ 13.965	12.64	x
Surveillance every 4 years with RFA	€ 7.821	12.89	€ 62.619
Surveillance every 4 years with EMR followed by RFA	€ 8.086	12.89	x
Surveillance every 4 years with esophagectomy	€ 15.229	12.63	x
Surveillance every 3 years with RFA	€ 9.005	12.90	€ 105.755
Surveillance every 3 years with EMR followed by RFA	€ 9.277	12.90	x
Surveillance every 3 years with esophagectomy	€ 16.890	12.61	x
Surveillance every 2 years with RFA	€ 10.984	12.90	€ 324.420
Surveillance every 2 years with EMR followed by RFA	€ 11.286	12.90	x
Surveillance every 2 years with esophagectomy	€ 19.325	12.59	x
Surveillance every year with RFA	€ 15.074	12.89	x
Surveillance every year with EMR followed by RFA	€ 15.421	12.89	x
Surveillance every year with esophagectomy	€ 23.686	12.54	x

QALYs, quality-adjusted-life-years; ICER, incremental cost-effectiveness ratio ;RFA, radiofrequency ablation; EMR, endoscopic mucosal resection; x, strategy dominated by alternative

Surveillance in patients with low-grade dysplasia

In patients with LGD, the costs of no surveillance were € 21.806 for 10.95 discounted QALYs. Surveillance every 5 years with RFA for HGD or early EAC and esophagectomy for advanced EAC resulted in an increase in life expectancy by 0.96 QALYs and an increase in costs by €4.756, representing an ICER of €4.922 per QALY. Surveillance every 1 to 4 years resulted in an additional increase in life expectancy, but at increasing costs (Table 5). EMR followed by RFA for patients with HGD or early EAC had similar effects on QALYs compared to strategies using RFA alone, but costs were slightly higher. Esophagectomy was much more expensive with less QALYs gained.

Table 5. Cost-effectiveness of surveillance in patients with low-grade dysplasia.

Strategy	Low-grade dysplasia		
	Costs	QALYs	ICER
No surveillance	€ 21.806	10.95	
Surveillance every 5 years with RFA	€ 26.562	11.91	€ 4.922
Surveillance every 5 years with EMR followed by RFA	€ 28.245	11.91	x
Surveillance every 5 years with esophagectomy	€ 50.909	11.33	x
Surveillance every 4 years with RFA	€ 28.964	11.99	€ 30.067
Surveillance every 4 years with EMR followed by RFA	€ 30.856	11.99	x
Surveillance every 4 years with esophagectomy	€ 51.835	11.34	x
Surveillance every 3 years with RFA	€ 32.071	12.09	€ 32.531
Surveillance every 3 years with EMR followed by RFA	€ 34.238	12.09	x
Surveillance every 3 years with esophagectomy	€ 52.851	11.34	x
Surveillance every 2 years with RFA	€ 36.242	12.19	€ 41.499
Surveillance every 2 years with EMR followed by RFA	€ 38.779	12.19	x
Surveillance every 2 years with esophagectomy	€ 53.960	11.34	x
Surveillance every year with RFA	€ 42.086	12.27	€ 75.601
Surveillance every year with EMR followed by RFA	€ 45.133	12.27	x
Surveillance every year with esophagectomy	€ 55.159	11.34	x

QALYs, quality-adjusted-life-years; ICER, incremental cost-effectiveness ratio ;RFA, radiofrequency ablation; EMR, endoscopic mucosal resection; x, strategy dominated by alternative

When assuming a willingness-to-pay threshold of €35.000 per QALY, surveillance every 3 years with EMR followed by RFA or RFA alone for HGD or early EAC and esophagectomy with neoadjuvant chemoradiotherapy for advanced EAC is a cost-effective strategy for long-segment BE with LGD. When assuming a willingness-to-pay threshold of €80.000 per QALY, surveillance every year is cost-effective.

The most critical variables in the cost-effectiveness analysis were the true progression rates. When progression rates were doubled, surveillance every 2 years was cost-effective for BE with ND and every year for LGD with ICERs of €27.073 and €17.973 per QALY (Table 6). When progression rates were halved, surveillance every 5 years was cost-effective for both ND and LGD with ICERs of €29.802 and €7.631 per QALY. When progression rates were only 25% of the calculated values, surveillance was only cost-effective for LGD, with intervals of 5 years and an ICER of 11.753 per QALY. Changes in costs and quality of life data had less impact on the cost-effectiveness of surveillance. When using a discount rate of 3% instead of 5%, results were similar.

Table 6. Cost-effectiveness in case of higher or lower transition rates.

No dysplasia

Surveillance	Transition rates 200%			Transition rates 50%		
	Costs	QALYs	ICER	Costs	QALYs	ICER
None	€ 9.886	11.89		€ 3.501	12.87	
Every 5 years with RFA	€ 9.731	12.54	x	€ 5.864	12.95	€ 29.802
Every 4 years with RFA	€ 10.510	12.60	€ 12.560	€ 6.667	12.95	x
Every 3 years with RFA	€ 11.624	12.67	€ 16.152	€ 7.868	12.95	x
Every 2 years with RFA	€ 13.473	12.74	€ 27.073	€ 9.883	12.94	x
Every year with RFA	€ 17.403	12.78	€ 87.727	€ 10.411	12.93	x

Low-grade dysplasia

Surveillance	Transition rates 200%			Transition rates 50%		
	Costs	QALYs	ICER	Costs	QALYs	ICER
None	€ 24.747	9.44		€ 19.772	11.84	
Every 5 years with RFA	€ 29.778	10.76	€ 3.817	€ 24.548	12.46	€ 7.631
Every 4 years with RFA	€ 32.095	10.90	€ 16.398	€ 27.034	12.48	€ 135.848
Every 3 years with RFA	€ 35.053	11.11	€ 14.100	€ 30.249	12.50	€ 206.087
Every 2 years with RFA	€ 39.024	11.39	€ 14.080	€ 34.540	12.50	€ 670.480
Every year with RFA	€ 44.671	11.70	€ 17.973	€ 40.499	12.49	x

Figure 2. Costs and quality-adjusted life years (QALYs) associated with different surveillance strategies in patients with no dysplasia (A) or low-grade dysplasia (B). ● No surveillance, ▲ Surveillance with radiofrequency ablation for high-grade dysplasia (HGD) or early esophageal adenocarcinoma (EAC) and esophagectomy for advanced EAC, ◆ Surveillance with esophagectomy for HGD or EAC
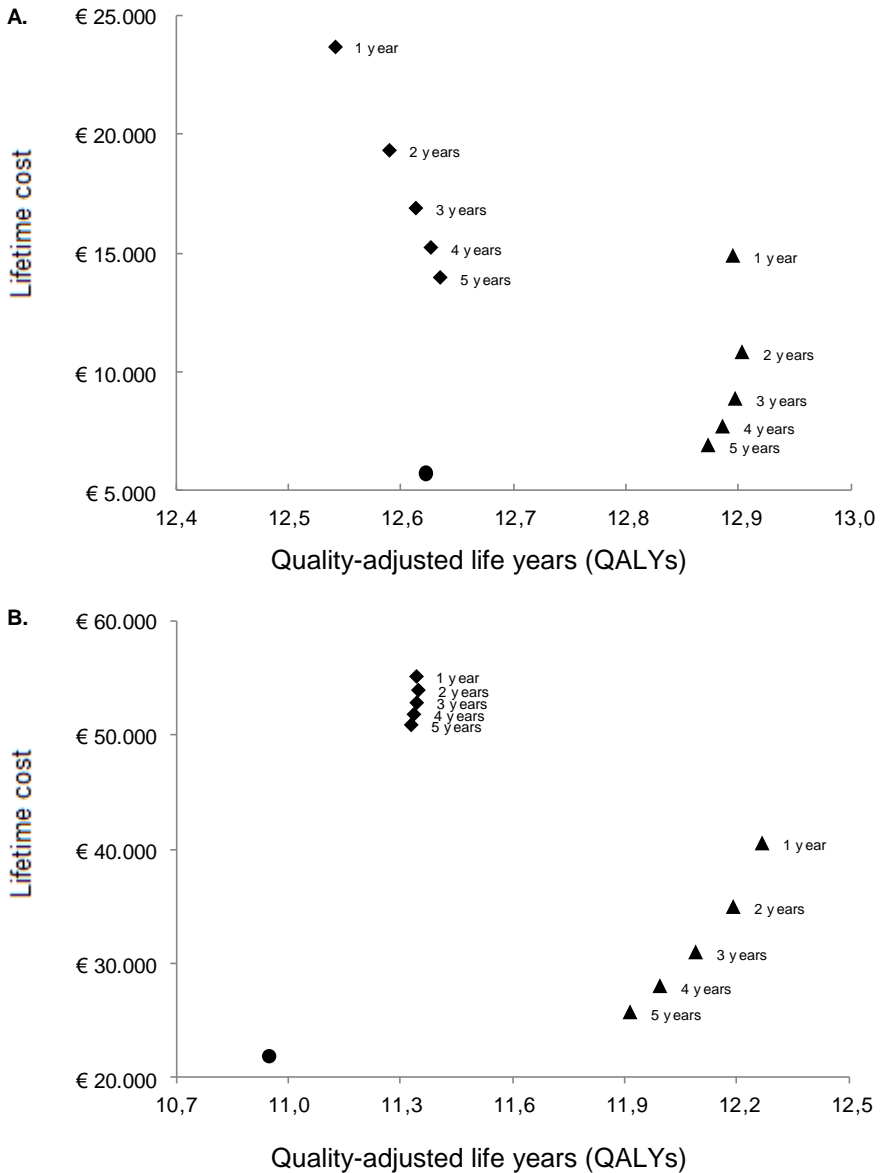
Discussion

In this large prospective study, we evaluated the cost-effectiveness of different surveillance intervals and treatment strategies in patients with long-segment BE. Assuming a willingness-to-pay threshold of € 35.000 per QALY, endoscopic surveillance is cost-effective with intervals of 5 years, EMR followed by RFA for HGD or early EAC, and esophagectomy with neoadjuvant chemoradiotherapy for advanced EAC in patients with non-dysplastic BE. Surveillance every 3 years is cost-effective for patients with LGD. For patients with ND, the results of our study correspond to recommendations made in current guidelines.[8, 9] For patients with LGD however, surveillance is recommended with intervals of 6 to 12 months, while according to our study intervals should be at least 3 years in order to be cost-effective. When histology is used as the only predictor for neoplastic progression, surveillance intervals should be prolonged to 3 years in patients with LGD to be cost-effective. However, with prolongation of the surveillance intervals, the risk of interval carcinomas increases. Identification of additional risk factors may improve risk-stratification and thereby the cost-effectiveness of surveillance with short intervals.

Previous studies investigating the cost-effectiveness of BE surveillance have shown highly variable results, mainly due to different assumptions about progression rates and quality of life associated with different health states. Surveillance was reported to be cost-effective in 4 studies with surveillance intervals ranging from 2 to 5 years.[20, 21, 23, 24] However, in 4 other studies surveillance was not cost-effective with sometimes even higher costs and less quality of life than without surveillance.[17, 19, 22, 26]

Over the past years there has been a major shift in the treatment BE patients with the introduction of endoscopic treatment strategies. We therefore included EMR and RFA in this cost-effectiveness analysis.[8, 9] An advantage of EMR is that it not only removes mucosal abnormalities suspect for dysplasia, but also allows for evaluation of tissue invasion.[39, 40] RFA is used in addition to EMR for complete eradication of BE, but may also be used as a single treatment modality.[30, 41] Previous studies have shown that RFA is effective in eradicating HGD, early EAC and complete segments of BE with low complication rates.[30, 41-43] The current study shows that RFA is also cost-effective, which corresponds to the results of previous studies.[17-26] Some recent studies suggested that RFA might also be cost-effective in patients with confirmed LGD.[43, 44] However, it is hard to make a reliable diagnosis of LGD which limits its feasibility. Therefore

we did not include RFA as a treatment strategy for LGD. Use of EMR in addition to RFA was associated with similar effects on quality of life, but was slightly more expensive. As a result, strategies using EMR followed by RFA were dominated by strategies using RFA alone. In 2 recent retrospective studies was shown that use of EMR before RFA had no additional benefit, which suggests that RFA alone might be a suitable treatment for patients with HGD or early EAC.[13, 14] However, use of RFA alone is still controversial and although use of additional EMR might be slightly more expensive, it allows for evaluation of tissue invasion and is therefore useful for tumor staging. The current study shows that in 25% of patients histological diagnosis was changed after evaluation of the EMR specimens and in some patients another treatment strategy was preferred based on these results. We therefore believe there is an additional role for EMR prior to RFA, which corresponds to recommendations in current guidelines.[8, 9]

The cost-effectiveness of a surveillance strategy not only depends on the costs and effects on quality of life, but also on the willingness-to-pay threshold.[22] We considered a willingness-to-pay threshold between €20.000 to €80.000 per QALY with special emphasis on the threshold of €35.000 per QALY, which is used in the United Kingdom and the United States of America.[36-38] The most critical variables in the cost-effectiveness analysis were the true progression rates. We used advanced statistical techniques to estimate these rates from prospectively collected follow-up data. The incidence of EAC was estimated at 0.1% per year which corresponds to the results of recent population-based studies, which confirms that our model is a good reflection of the natural history of neoplastic progression in BE.[2] For patients with LGD, the incidence rate of EAC was estimated at 4.9% per year. Previous studies have shown highly variable results for LGD with incidence rates of 0-26% and 1.7% in a recent meta-analysis.[45] The estimated progression rate in the current study was higher than in the meta-analysis which can be explained by the fact that we only included patients with long-segment BE, that LGD diagnosis was made only when at least 2 pathologists agreed on the diagnosis and that patients were under strict surveillance. When progression rates were halved, surveillance every 5 years was cost-effective for both ND and LGD. When progression rates were 25% of the calculated values, surveillance was only cost-effective for LGD. Changes in other variables such as costs and quality of life data had less impact on outcome.

One of the strengths of this study is that the transition rates were estimated based on follow-up data from our own large prospective BE cohort instead of using pooled literature data. Transition rates based on pooled literature data are likely to overestimate the true incidence rate of neoplastic progression due to publication and selection bias. Transition rates based on large epidemiological studies are likely to underestimate the true incidence rate of neoplastic progression since these patients are not necessarily under strict surveillance, which is of major importance to detect HGD or early EAC. With the use of our own follow-up data, we obtained a more accurate representation of the natural history of BE and its progression to EAC. In addition, patients with EAC were stratified according to TNM stage. As a result endoscopic intervention could be applied to patients with HGD as well as patients with early EAC. Furthermore, we incorporated new treatment strategies such as EMR and RFA for HGD or early EAC, neoadjuvant chemoradiotherapy for patients who underwent esophagectomy, and chemotherapy, esophageal stenting and brachytherapy for palliative treatment.

Our study also has some limitations. Although progression rates were estimated based on prospective follow-up data, the number of patients who developed HGD or EAC was relatively low which limits the accuracy of the estimate. When longer follow-up becomes available, a more reliable estimate can be made. Secondly, we were not able to observe the transition from HGD or early EAC to advanced EAC since these patients were excluded from further follow-up and received appropriate treatment. Instead we used data from another Dutch BE cohort. Thirdly, we only included patients with BE of at least 2 centimeters and therefore our results cannot be applied universally to all BE patients. Since long-segment BE is associated with a higher risk of neoplastic progression we believe that our cohort is representative for the clinically relevant population with patients with long-segment BE, which are the patients who are most likely to benefit from surveillance. Finally, we did not include any other risk factors than histology. To date histological diagnosis of dysplasia is the only accepted predictor for neoplastic progression and therefore used for defining surveillance intervals. Other potential risk factors are insufficiently validated in large studies and are therefore not yet ready for use. However, when new risk factors become available they can be used to identify patients at high risk for neoplastic progression. By targeting surveillance to those at high risk the cost-effectiveness of surveillance can be improved. In previous studies we have already shown promising results of chemoprevention with proton pump

inhibitors, nonsteroidal anti-inflammatory drugs and statins and use of biomarkers such as p53.[46-48] When new risk factors become available our model needs to be updated for a more personalized surveillance strategy.

In conclusion this study shows that surveillance every 5 years with EMR followed by RFA for HGD or early EAC and esophagectomy with neoadjuvant chemoradiotherapy for advanced EAC is a cost-effective strategy in patients with long-segment BE without dysplasia, assuming a willingness-to-pay threshold of € 35.000 per QALY. In patients with LGD surveillance every 3 years with EMR followed by RFA for HGD or early EAC and esophagectomy with neoadjuvant chemoradiotherapy for advanced EAC is cost-effective. In the future new risk factors or biomarkers may identify patients at high risk for neoplastic progression and thereby improve the cost-effectiveness of BE surveillance.

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Chapter 8

Impact of surveillance for long segment Barrett's esophagus on tumor stage and survival of patients with neoplastic progression: results of a large multicenter prospective cohort study

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Abstract

Introduction: The value of surveillance for Barrett's esophagus (BE) is under discussion given the overall low incidence of neoplastic progression and lack of evidence that it prevents advanced esophageal adenocarcinoma (EAC). The aim of this study was to evaluate the impact of BE surveillance on tumor stage and survival of patients with neoplastic progression.

Methods: 783 patients with BE of at least 2 centimeter were included in a multicenter prospective cohort study and followed during surveillance according to the ACG guidelines. Incident cases of high-grade dysplasia (HGD) and EAC were identified during follow-up. EAC staging was performed according to the 7th UICC-AJCC classification. Survival data were collected and cross-checked using death and municipal registries. Data from patients with EAC in the general population were obtained from the Dutch cancer registry. We compared survival of BE patients with neoplastic progression during surveillance to those of patients without neoplastic progression and patients with different stages of EAC in the general population.

Results: 53 BE patients developed HGD or EAC during surveillance. Thirty-five patients (66%) were classified as stage 0, 14 (26%) as stage 1, and 4 (8%) as stage 2. EAC was diagnosed at an earlier stage during BE surveillance than in the general population (P<0.001). The survival of patients with neoplastic progression during BE surveillance was not significantly worse than those of patients without neoplastic progression and similar to the survival of patients with stage 0 or 1 EAC in the general population.

Conclusion: BE surveillance enables the detection of EAC at an early stage with good survival rates.

Introduction

Barrett's esophagus (BE) is a premalignant condition in which patients have an increased risk of developing esophageal adenocarcinoma (EAC) with an estimated incidence of 0.1 to 0.5% per year.[1-4] The development of EAC in BE is thought to be a gradual process, in which metaplastic epithelium without dysplasia evolves to low-grade dysplasia (LGD), high-grade dysplasia (HGD) and eventually EAC under the influence of chronic esophageal acid exposure.[5,6] Once a patient has developed EAC the prognosis is poor with a 5-year survival of less than 20%.[7,8] Endoscopic surveillance is therefore recommended for BE to detect EAC at an early stage, when curative treatment is still feasible.[9,10] Current guidelines recommend surveillance every 3 to 5 years in patients with non-dysplastic BE, surveillance every 6 to 12 months in patients with LGD and (endoscopic) treatment in patients with established HGD or EAC. Over the past years there has been a major shift in the treatment of patients with neoplastic progression in BE with the introduction of endoscopic mucosal resection (EMR) and ablation techniques such as radiofrequency ablation (RFA), photodynamic therapy (PDT) and argon plasma coagulation (APC).[11] Endoscopic treatment is effective, less burdensome, associated with low morbidity and mortality rates, and may improve survival of patients with neoplastic progression.[12] Although esophagectomy is still the mainstay for advanced EAC, esophagectomy is nowadays complemented by neoadjuvant chemoradiotherapy.[13] Chemotherapy, esophageal stenting and brachytherapy have been added to the palliative treatment of EAC.[14]

Recently, the value of BE surveillance has been under discussion given the overall low incidence of neoplastic progression and lack of evidence that surveillance reduces the risk of advanced EAC and improves survival.[15-17] These key questions has been evaluated in case-control studies, population-based studies and small prospective cohort studies with conflicting results.[11, 18-30] Although most studies suggest that surveillance enables the detection of early EAC with good survival rates, some other studies reported no effect on mortality.[17] Furthermore in most studies BE patients were included independent of BE length. However the risk of neoplastic progression is much lower in patients with short-segment BE (SSBE) than in patients with long-segment BE.[31, 32] The aim of the present study was to evaluate the impact of surveillance of patients with long-segment BE according to current guidelines, on tumor stage and survival of patients with EAC.

Methods

Study design

We conducted a large multicenter prospective cohort study in 3 academic and 12 regional hospitals throughout the Netherlands. Between November 2003 and December 2004, 783 patients were included with known or newly diagnosed BE of at least 2 centimeters (long-segment BE). The endoscopic diagnosis was confirmed by the presence of intestinal metaplasia and patients with HGD or EAC in the past or at the index endoscopy were excluded. Endoscopic surveillance was performed according to guidelines of the American College of Gastroenterology.[10] Patients without dysplasia underwent upper endoscopy with biopsy sampling every 3 years and patients with LGD every year. Endoscopic procedures were performed by experienced gastroenterologists, according to a standardized protocol. At each endoscopy targeted biopsies were taken from mucosal abnormalities and quadrant biopsies were taken every 2 centimeters from the most distal to the most proximal part of the BE epithelium, according to the Seattle protocol.[33]

Histology

Biopsy specimens were fixed with buffered formalin and embedded in paraffin, according to standard procedures. From each biopsy set 4 µm thick sections were cut and stained with haematoxylin-eosin to assess the presence of BE and define the grade of dysplasia. After examining all biopsies, the highest degree of abnormality was reported for each endoscopy. Slides were first graded by a local pathologist and then by an expert pathologist for second opinion. When the local and expert pathologists disagreed on the grade of dysplasia, the slides were reviewed by a second expert pathologist. Pathologists were blinded to the diagnosis of each other and a final diagnosis was made only if at least 2 pathologists agreed on the grade of dysplasia. If there was disagreement, a panel of expert pathologists reviewed the slides and a final diagnosis was made based on consensus agreement.

Neoplastic progression

Neoplastic progression was defined as the development of HGD or EAC after inclusion in the study. The diagnosis was made only if at least 2 pathologists, including an expert pathologist, agreed on the presence of HGD or EAC. Patients with neoplastic progression were treated according to the guidelines of

the American College of Gastroenterology. Patients with HGD received intensive endoscopic surveillance (every 3 months) or were treated as early EAC with EMR, ablation techniques such as PDT, APC and RFA, or a combination of both depending on local expertise. Patients with advanced EAC received esophagectomy with or without neoadjuvant chemoradiotherapy.[10] EAC staging was performed according to the 7th UICC-AJCC classification. The stage of the primary tumor was based on histological assessment of biopsies, EMR specimens or resection specimens, whichever was available. The highest tumor stage was reported for each patient. After endoscopic or surgical treatment surveillance was resumed. During follow-up occurrence of complications, recurrence and metastasis was recorded.

Survival

Survival data were collected from all BE patients included in the study. Since surveillance intervals were up to 3 years and some patients dropped out of surveillance, survival of patients was cross-checked using death registries and municipal administrations. When a patient was deceased, the cause of death was obtained from the attending gastroenterologist or general practitioner. Survival data from patients with EAC in the Netherlands, independent of the cause of death and stratified by age, gender, stage, and year of diagnosis, were obtained from the Dutch cancer registry.[7] Data on the cause of death in the general Dutch population, stratified by age, gender and year of death, were obtained from the Dutch central statistical office.[34]

Ethics

The study protocol was approved by the Institutional Review Boards of the Erasmus University Medical Center, as well as those of all participating hospitals. Before the first endoscopy, written informed consent was obtained from all patients.

Statistical analysis

The incidence rate of neoplastic progression was calculated by dividing the number of patients with HGD or EAC by the total person-years of follow-up in the study. Chi-squared tests were used to compare EAC stage at diagnosis in BE patients undergoing surveillance and in patients with EAC in the general Dutch population. The survival of BE patients with and without neoplastic progression during surveillance was compared in Cox proportional-hazards

models adjusted for age and gender, whereby neoplastic progression was modelled as a time-dependent variable. Follow-up time was defined as the time from inclusion in the study to death or 1 January 2014, whichever came first. When no information was available from death or municipal registries, follow-up time was defined as the time from inclusion in the study to the last surveillance endoscopy. Cox-regression models were used to calculate hazard ratios (HR) and 95% confidence intervals (CI). In addition, survival of patients with different EAC stages in the general population was evaluated and compared to survival of BE patients with neoplastic progression during surveillance. The 5-year cumulative survival was estimated using survival tables and Kaplan-Meier curves. In addition, we evaluated cause of death in BE patients and in individuals with similar age and gender in the general population. Two sided P-values <0.05 were considered to be statistically significant. Data were analysed using SPSS Statistics (version 20.0, Chicago, Illinois, USA).

Results

Patient characteristics
Seven hundred eighty-three patients (73% male, median age 61 years) were included in the study and followed during surveillance with a median duration of 7 years (interquartile range (IQR) 4-8 years) and a total of 4556 person-years of follow-up (Table 1). The majority of patients (72%) was known with BE before inclusion in the study. At baseline, patients had a median BE length of 4 cm (IQR 2-6 cm), 78 (10%) patients were diagnosed with esophagitis and 117 (15%) with LGD.

Table 1. Characteristics of patients with Barrett's esophagus and patients with esophageal adenocarcinoma in the general population

|	Cohort	HGD or EAC	EAC general population
	n = 783	n = 53	n = 8855
Age, median years (IQR)	61 (53-70)	65 (55-70)	68 (60-77)
Male gender, number	573 (73%)	44 (83%)	7164 (81%)
Follow-up, median years (IQR)	7 (4-8)	5 (2-7)	1 (0-2)

BE, Barrett's esophagus; HGD, high-grade dysplasia; EAC. Esophageal adenocarcinoma

Neoplastic progression

After a median follow-up of 3 years 53 patients (83% male, median age 66 years) developed HGD or EAC with an incidence rate of 1.2 per 100 person-years (IQR 0.9-1.5), which was stable over time (Figure 1). The incidence rate was 0.3 per 100 person-years (IQR 0.2-0.6) for EAC (all stages) and 0.1 per 100 person-years (IQR 0.02-0.2) for advanced EAC (at least stage 2). Thirty-five patients (66%) developed HGD, 12 (22%) T1a EAC, 2 (4%) T1b EAC, 2 (4%) T2 EAC, and 2 (4%) T3 EAC. In 2 patients with T2 EAC, metastases were found in regional lymph nodes (N1). In none of the other patients lymph node metastases were found (N0). At the time of diagnosis, there was no evidence of distant metastases in any of the patients (M0). Thirty-five patients (66%) were classified as stage 0 disease, 14 (26%) as stage 1, and 4 (8%) as stage 2. EAC stage at diagnosis did not significantly change over time. Three patients (75%) with stage 2 EAC were previously diagnosed with LGD, for which they received annual surveillance. The remaining patient was never diagnosed with dysplasia and received surveillance every 3 years. Two patients (50%) with LGD at inclusion were diagnosed with stage 2 EAC one year later.

Figure 1. Incidence of neoplastic progression during BE surveillance.
━ HGD and EAC, ⋯ HGD, ━ EAC (all stages), ⋯ advanced EAC (≥ stage 2)
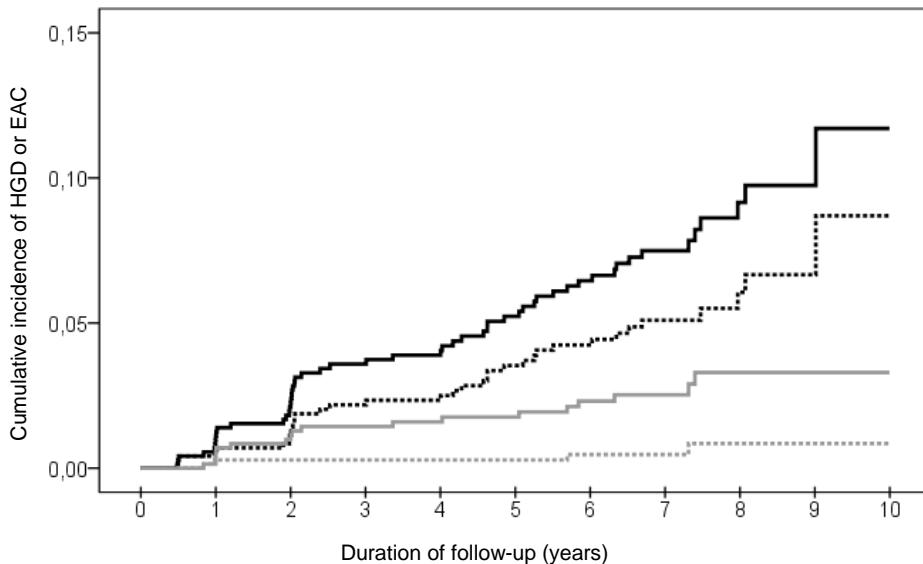
In the Netherlands, 8855 patients (81% male, median age 68 years) were diagnosed with EAC between 2004 and 2012 according to data of the Dutch cancer registry.[7] One percent of patients was classified as stage 0 disease, 14% as stage 1, 16% as stage 2, 23% as stage 3, and 46% as stage 4. EAC was diagnosed in a significantly earlier stage during BE surveillance than in the general Dutch population (P<0.001) (Figure 2).

Figure 2. Stage of esophageal adenocarcinoma at the time of diagnosis in BE patients undergoing surveillance (■) and in the general Dutch population (■)
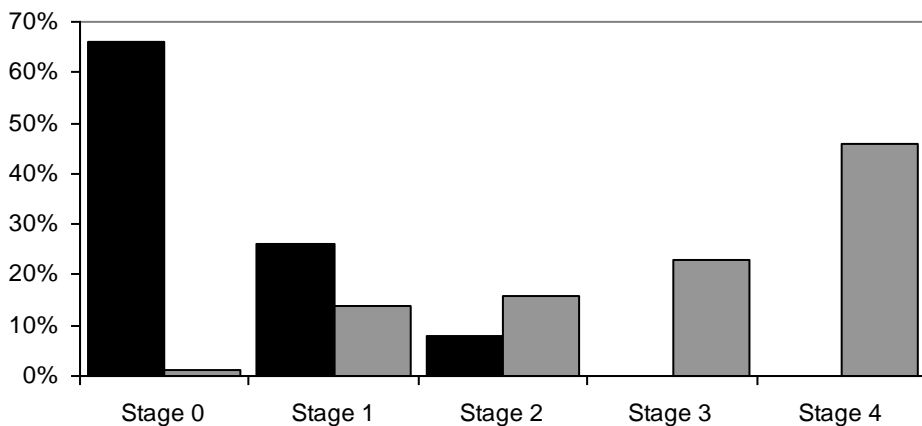
Treatment

During surveillance 10 patients were diagnosed with focal HGD without mucosal abnormalities for which they received intensive surveillance. Although the initial diagnosis of HGD was confirmed by expert pathologists, in none of these patients HGD was confirmed during further follow-up. Therefore it was chosen to refrain from endoscopic treatment and follow a policy of watchful waiting. The remaining 25 patients with HGD received endoscopic treatment. Two patients were treated with PDT, 11 with EMR, 7 with EMR followed by PDT, and 5 with EMR followed by RFA. One patient developed a stenosis after EMR for which dilatation was performed and 1 patient had a perforation for which a stent was placed. Five patients had recurrence of HGD or early EAC during follow-up for which they were treated successfully with EMR and RFA. Of the 12 patients with T1a EAC 2 were treated with EMR, 7 with EMR followed by PDT and 2 with EMR followed by RFA. One patient died prior to treatment, 1 patient developed a stenosis for which dilatation was performed and 2 patients had recurrence for

which they were treated successfully with EMR and RFA. The remaining 6 patients with T1b, T2 or T3 EAC were treated with transhiatal esophagectomy, which in 2 patients was complemented by neoadjuvant chemoradiotherapy. Two patients developed postoperative anastomotic leakage. One patient died due to postoperative complications and two patients due to advanced EAC after a median follow-up of 2 years (Figure 3).

Figure 3. Treatment of patients with neoplastic progression during surveillance.
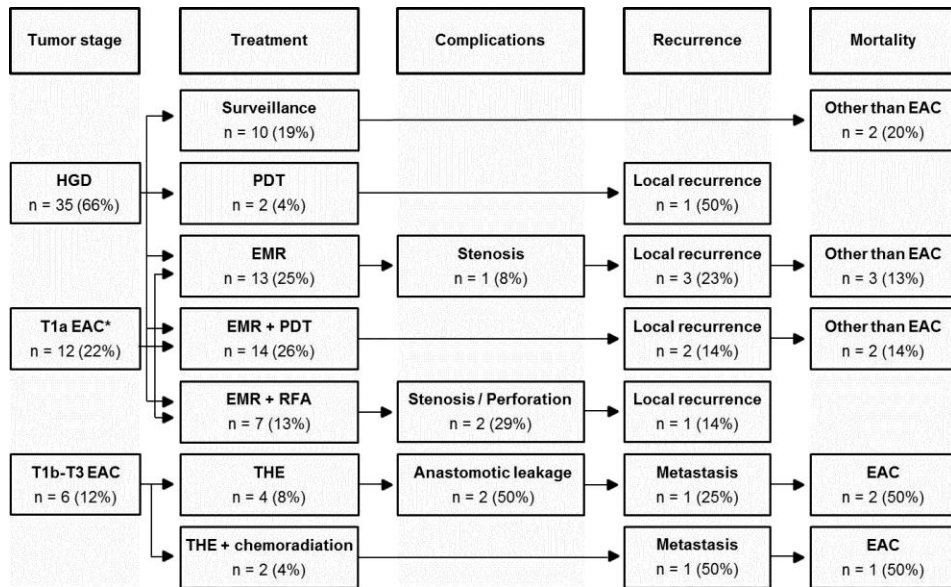
PDT, photodynamic therapy; EMR, endoscopic mucosal resection; RFA, radiofrequency ablation; THE, transhiatal esophagectomy; * 1 patient died prior to treatment

Survival

Of all 53 patients with neoplastic progression during surveillance, 12 patients (23%) (83% male, median age 73 years) died after a median follow-up of 2 years (IQR 1-4 years). The all cause 5-year survival of patients with neoplastic progression during surveillance was 74% (95% CI 60-87%). The 5-year survival was 80% for patients with stage 0 disease (n=35), 68% for stage 1 (n=14), and 33% for stage 2 (n=4). Of the remaining 730 BE patients in the cohort, 100 patients (14%) (76% male, median age 78 years) died after a median follow-up of 7 years (IQR 3-8 years). The all cause 5-years survival of BE patients without neoplastic progression was 94% (95% CI 92-96%). Of the 8855 patients with

EAC in the general Dutch population, 6352 patients (72%) (81% male, median age 71 years) died after a median follow-up of 7 months (IQR 3-15 months). The all cause 5-year survival of patients with EAC in the Netherlands was 17% (95% CI 16-18%). The 5-year survival was 62% for patients with stage 0, 65% for stage 1, 30% for stage 2, 14% for stage 3, and 3% for stage 4 (Figure 4).
The survival of BE patients with neoplastic progression during surveillance was only slightly (and not statistically significant) worse than those of BE patients without neoplastic progression during surveillance (HR 1.8, 95% CI 0.9-3.3), and similar to the survival of patients with stage 0 or stage 1 EAC in the general Dutch population (HR 0.8, 95% CI 0.3-1.8 and HR 0.7, 95% CI 0.4-1.2 respectively). Results were similar when excluding BE patients with HGD.

Figure 4. Cumulative survival of Barrett's esophagus (BE) patients with neoplastic progression during surveillance and patients with different stages of esophageal adenocarcinoma (EAC) in the general population.

━ BE with neoplastic progression during surveillance, ⋯ EAC stage 0,
━ EAC stage 1, ⋯ EAC stage 2, ━ EAC stage 3, ⋯ EAC stage 4
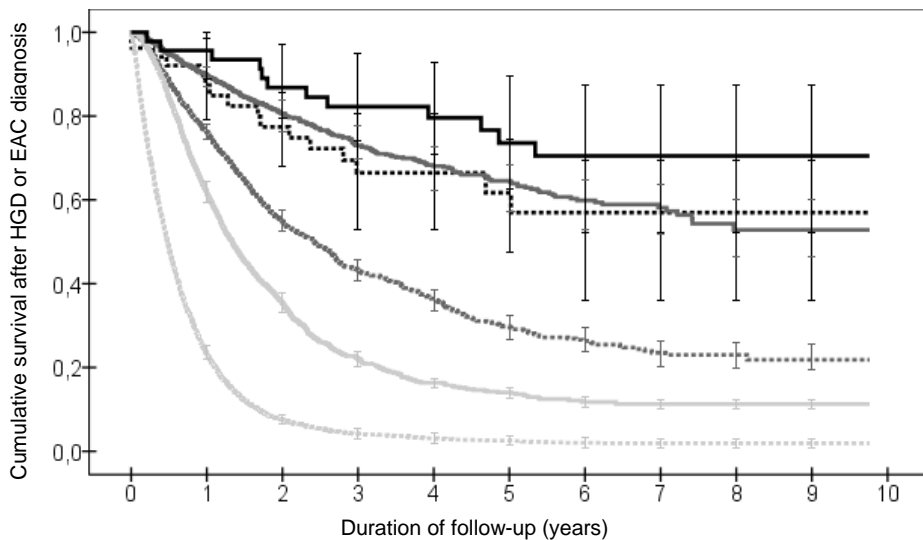
Cause of death

Of the 783 BE patients included in the cohort, 112 patients (14%) died after a median follow-up of 6 years. The majority of patients died due to malignancies (36%) or cardiovascular diseases (29%). Four percent of patients died due to EAC after a median follow-up of 2 years. Of all 53 BE patients with neoplastic progression during surveillance, 12 patients (23%) died after a median follow-up of 2 years. Two patients (17%) died due to cardiovascular diseases, 4 (33%) due to pulmonary diseases, and 6 (50%) due to malignancies, among which 3 (25%) due to EAC (Table 2). The cause of death for BE patients in our cohort was comparable to those of individuals with similar age and gender in the general Dutch population.

Table 1. Cause of death in patients with Barrett's esophagus and the general Dutch population

	Cohort n = 783	HGD or EAC n = 53	General Dutch population*
Deceased	**112 (14%)**	**12 (23%)**	
Cardiovascular diseases	32 (29%)	2 (17%)	29 %
Malignant neoplasms	40 (36%)	6 (50%)	36 %
Esophageal cancer	*4 (4%)*	*3 (25%)*	*2%*
Pulmonary diseases	7 (6%)	4 (33%)	10 %
(Un)intentional injuries	5 (4%)	-	4 %
Neuropsychiatric disorders	6 (5%)	-	3 %
Other	22 (20%)	-	18 %

* Individuals with similar age and gender in the same period

BE, Barrett's esophagus; HGD, high-grade dysplasia; EAC, esophageal adenocarcinoma

Discussion

In this large multicenter prospective cohort study, we evaluated the impact of BE surveillance according to current guidelines on tumor stage and survival of patients with EAC. The results of this study indicate that EAC is detected at an earlier stage during BE surveillance than in the general population, independent of age, gender and year of diagnosis, and that endoscopic treatment improves survival.

Surveillance is a process of periodic testing in patients at high risk for a certain disease. Key elements in the effectiveness of surveillance are whether disease is detected at an early and curable stage and whether survival is improved. In the present study we showed that EAC was detected at a significantly earlier stage during BE surveillance than in the general population. Of all patients with neoplastic progression during BE surveillance, 92% was diagnosed with early EAC (stage 0 or 1), compared to 15% in the general population. These results are in line with those of previous retrospective and small prospective studies, which reported early EAC in 60-95% of BE patients with neoplastic progression during surveillance and 10-40% of patients with EAC in the general population.[17, 19, 21, 23-25, 27, 30] In two previous studies surveillance failed to detect early EAC.[7, 10] One of the major shortcomings of those studies was that patients were not under strict surveillance, which is crucial for detection of HGD or early EAC.

In contrast to most previous studies, patients with early EAC in the present study received endoscopic treatment instead of esophagectomy, according to current guidelines. Since the majority of patients was diagnosed with early EAC, most patients were treated endoscopically and only 12% of patients needed esophagectomy. After endoscopic treatment 6 (17%) patients had complications and 7 (19%) had recurrence of HGD or EAC for which they received additional endoscopic treatment. None of the patients with early EAC died due to EAC or its treatment.

The overall 5-year survival was 74% in patients with EAC during BE surveillance and 17% in patients with EAC in the general population. Although it is difficult to compare survival of both groups due to different types of bias, including lead and length time bias, this large difference seems clinically relevant. The results correspond to those of previous retrospective and small prospective studies, which report an overall 5-year survival of 65-100% in patients with EAC during surveillance and 0-30% in patients with symptomatic EAC.[6, 9, 13, 15, 16, 19, 23, 34, 35] The majority of patients undergoing BE surveillance died due to cardiovascular diseases or malignancies and only 4% due to EAC, which was comparable to cause of death in individuals with similar age and gender in the general population. One in 4 patients with EAC during surveillance died due to EAC or its treatment. Unfortunately, no information was available on cause of death in patients with EAC in the general population. Since the cause of death in patients undergoing BE surveillance was comparable to those of individuals with similar age and gender in the general population, it is likely that excess mortality in patients with EAC in the general

population is caused by EAC itself or its treatment. This idea is supported by data from the Surveillance, Epidemiology, and End Results (SEER) database, which shows that approximately half of patients with EAC in the United States of America, dies due to EAC or its treatment.[36]

The present study shows that BE surveillance enables the detection of early EAC and improves survival, but the cost-effectiveness of BE surveillance is still controversial. Several recent studies among which one of our own study group, have shown that BE surveillance is cost-effective with intervals of five years for patients with non-dysplastic BE and three years for LGD.[37, 38] Although surveillance intervals were shorter in the current study (with surveillance every 3 years for non-dysplastic BE and every year for LGD), a minority of patients still developed advanced EAC. With prolongation of surveillance intervals, the risk of interval carcinomas will increase thereby limiting the protective effect. Identification of new risk factors is therefore needed to improve risk-stratification and thereby the cost-effectiveness of surveillance with short intervals.[35, 39, 40]

Our study has several strengths including the large sample size and long prospective follow-up. Consecutive BE patients were included presenting at the endoscopy unit of 3 academic and 12 regional hospitals throughout the Netherlands, resulting in a cohort that should be representative for the Dutch BE population. This is also supported by the annual incidence rate of EAC during follow-up of 0.3%, which corresponds to incidence rates reported in previous studies.[1, 3, 4] There were strict criteria for BE diagnosis and inclusion in the study, such as a BE length of at least 2 centimeters, presence of intestinal metaplasia in biopsies, and no presence or history of HGD or EAC. In addition, there was a stringent follow-up scheme and a standardized endoscopy and biopsy protocol. All biopsies were reviewed by at least 2 pathologists to obtain a diagnosis based on consensus. Surveillance and treatment of patients with neoplastic progression was performed according to current guidelines, which include endoscopic treatment modalities and neoadjuvant chemoradiotherapy for advanced EAC. Survival data were collected prospectively and were cross-checked using death registries and municipal administrations.

Our study also has limitations. Studies evaluating the effect of surveillance may be subject to lead and length time bias.[41] Lead time is the time between a preclinical stage detected during surveillance and the moment a disease becomes symptomatic. When improved survival is based on earlier detection during surveillance rather than postponement of death this is called lead time bias. Length time bias refers to the fact that surveillance enables the detection

of less aggressive disease with a mild course and thereby a better survival. Thus even in the absence of a true effect of surveillance it may improve survival due to lead and length time bias. Lead time bias is unlikely to affect the results of our study since improved survival was seen until 10 years after diagnosis, while the median survival of patients with symptomatic EAC was only 11 months. Although, a substantial proportion of patients was diagnosed with HGD instead of EAC, the results were similar when excluding patients with HGD, which makes length time bias unlikely as well. We compared the pathological stage and survival of patients with neoplastic progression during BE surveillance to those of patients with EAC in the general population based on data from the Dutch cancer registry. Since patients are included in this registry based on a clinical or pathological diagnosis of cancer, there is underreporting of HGD. However, since most patients were diagnosed with advanced EAC we assume this is not a major source for bias. Unfortunately, the Dutch cancer registry provides no information on previous participation in surveillance. It is therefore possible that some patients in the control group had previous surveillance, which may result in an underestimation of the surveillance effect. In addition the register provides no information on cause of death in patients with EAC. Finally, we only included patients with BE of at least 2 centimeters in the study and therefore our results cannot be applied universally to all BE patients. Since longer BE length is associated with a higher risk of neoplastic progression we believe that our cohort is representative for the patients who are most likely to benefit from surveillance.

In conclusion, regular endoscopic surveillance of BE patients enables the detection of EAC at an early and curable stage when endoscopic treatment is still feasible and leads to good survival. The results of this study therefore support current guidelines recommending endoscopic surveillance in long-segment BE patients.

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Chapter 9

General discussion

F. Kastelein

Introduction

Barrett's esophagus (BE) is a premalignant condition in which the squamous epithelium of the distal esophagus is replaced by columnar epithelium containing goblet cells as a complication of longstanding gastroesophageal reflux disease.[1-3] BE patients have an increased risk of developing esophageal adenocarcinoma (EAC) with an estimated incidence of 0.1-0.5% per year, which makes it the single most important risk factor for EAC.[4-7] The development of EAC in BE is thought to be a gradual process, in which metaplastic epithelium without dysplasia evolves to low-grade dysplasia (LGD), high-grade dysplasia (HGD) and eventually EAC under the influence of chronic esophageal acid exposure.[8] Once a patient has developed EAC the prognosis is poor with a 5 year survival of less than 20%.[9] Surveillance is therefore recommended for BE to detect EAC at an early stage when curative treatment is still feasible. Recommendations for BE surveillance in the Netherlands are based on guidelines of the American College of Gastroenterology, which recommend surveillance every 3-5 years for BE without dysplasia, surveillance every 6-12 months for BE with LGD, and (endoscopic) treatment for patients with established HGD or EAC.[10-13] However, the value of BE surveillance is under discussion given the overall low incidence of neoplastic progression, lack of discriminative tests for risk stratification and limited evidence that it prevents advanced EAC and improves survival. The aim of this thesis was to investigate whether chemoprevention and biomarkers can contribute to risk stratification in BE in order to optimize surveillance. In addition, we evaluated the effectiveness of surveillance according to current guidelines in terms of cost-effectiveness and survival. For this purpose, 783 patients with BE of at least 2 centimeters were included in a large multicenter prospective cohort study and followed during surveillance according to current guidelines.

Chemoprevention

Since esophageal acid exposure plays an import role in the initiation of BE and its progression toward EAC, acid suppression with proton pump inhibitors (PPIs) has become a mainstay in the treatment of BE.[3, 6, 14] Although chemoprevention with PPIs seems promising, it is not recommended in current guidelines in absence of long-term prospective trials.[10-13] Some observational studies investigated the effect of PPIs on the risk of neoplastic progression in

BE, but were unable to draw definite conclusions, as only small numbers of patients were included or essential clinical information was unavailable.[4, 15-18] For this purpose we collected data on the use of PPIs from 540 BE patients in a large multicenter prospective cohort study.[19] Information on medication use, including over-the-counter medication, was collected and was cross-checked using pharmacy records. Eighty-five percent of patients used a PPI at inclusion in the study and 99% during follow-up. In a time-dependent Cox-regression model, PPI use was associated with an approximately 75% reduced risk of neoplastic progression, independent of age, gender, BE length, esophagitis, histology, and use of other medications. The protective effect of PPIs increased with prolonged use and good adherence, and was similar for different PPIs, which supports a causal relationship. PPI use could not completely prevent neoplastic progression, probably because some patients continue to have pathological reflux despite PPI use.[20, 21] The results of our study are consistent with those of previous studies, which all reported an inverse relationship between PPI use and neoplastic progression in BE. [4, 15-18] Despite the large sample size, only a small minority of patients did not use PPIs and although this reflects clinical practice in Western countries, it limits the options for investigating the effect of PPIs. In contrast to previous studies we collected detailed information on medication use, which gave us the opportunity to perform time-dependent analyses. A randomized controlled trial would be the ideal way to investigate the effect of PPIs without the risk of confounding. However, since many BE patients suffer from reflux symptoms without the use of PPIs it is almost impossible to perform such a trial. The current study shows that use of PPIs is an effective strategy to prevent neoplastic progression in BE. In addition, PPIs are highly effective in relieving reflux symptoms and consequently patients are exceptionally compliant. PPI use is therefore justified and feasible in BE patients and should be recommended in guidelines.

On the other hand, studies have given support to chemoprevention with non-steroidal anti-inflammatory drugs (NSAIDs) and statins for the prevention of several cancers including EAC.[15, 16, 22-24] NSAIDs inhibit cyclooxygenase enzymes, resulting in decreased cell growth, proliferation and angiogenesis.[25] Statins not only inhibit the biosynthesis of cholesterol, but also decrease the activation of intracellular proteins through prenylation, which results in reduced cell proliferation and induced apoptosis.[23, 26, 27] Only limited studies have investigated the effect of NSAIDs and statins on the risk of neoplastic progression in BE, mostly with a small sample size and lack of clinical

information.[16, 23, 24] To our knowledge the current study is the first large prospective study investigating the combination of NSAID and statin use in BE. To investigate whether use of NSAIDs and statins reduces the risk of neoplastic progression in BE, we collected data on use of NSAIDs and statins from 570 BE patients in a large multicenter prospective cohort study. Information on medication use, including over-the-counter medication, was collected and was cross-checked using pharmacy records. Twenty-eight percent of patients used low dose aspirin, 56% NSAIDs, and 37% statins after BE diagnosis. Use of low dose aspirin did not significantly change the risk of HGD or EAC, but NSAID and statin use were both associated with an approximately 50% reduced risk of neoplastic progression, independent of age, gender, BE length, baseline histology and use of other medications. Use of both NSAIDs and statins was associated with an additive protective effect and an approximately 75% reduced risk of neoplastic progression. There appeared to be a duration- and dose-response relationship for NSAID use, which supports a causal connection. However, most patients used NSAIDs for a relatively short period (median 2 months) raising concerns of uncontrolled confounding. Although use of NSAIDs appears to have a more powerful chemopreventive effect than use of low dose aspirin, NSAIDs may also cause more serious side effects. The results of our study are consistent with those of previous studies investigating the effect of NSAIDs in BE, which all reported an inverse relationship between NSAID use and neoplastic progression in BE.[15, 16, 28, 29] Only 2 previous studies investigated the effect of statin use in BE, of which one was underpowered and one showed results similar to our study.[15, 16] Unfortunately, we had no information on the indication of NSAID and statin use and users of NSAIDs and statins may have differed from non-users in other ways than use of these drugs. Despite our efforts to consider this as much as possible, we cannot exclude uncontrolled confounding. The current study shows that use of NSAIDs and statins is an effective strategy to prevent neoplastic progression in BE. However, use of NSAIDs and statins may be accompanied with considerable side-effects which limits their use as active chemopreventive agents. Since NSAID and statin use are common among patients undergoing BE surveillance, information on NSAID and statin use may also be used for risk stratification.

Biomarkers

Histological diagnosis of dysplasia is currently the only accepted predictor for neoplastic progression in BE and is therefore used for defining surveillance intervals. However, histology is subject to sample error and considerable interobserver variation, which limits its predictive value. Use of biomarkers may contribute to the identification of patients at high risk for neoplastic progression and thereby improve risk stratification. Many biomarkers have been investigated of which immunohistochemical staining of p53 appears to be one of the most promising. TP53 is a tumor suppressor gene that plays an important role in regulation of the cell cycle and apoptosis. P53 overexpression can be caused by TP53 mutations which stabilize the inactivated protein and truncating TP53 mutations or epigenetic silencing may result in protein inactivation and loss of p53 expression. Previous studies have shown that p53 overexpression is associated with an increased risk of neoplastic progression in BE.[31-36] Although little is known about loss of p53 expression, the first results indicate that it may be predictive as well.[37] However, the value of p53 immunohistochemistry has not yet been validated in large prospective studies. Another potential biomarker for predicting neoplastic progression in BE is Alpha-Methylacyl-CoA Racemase (AMACR). AMACR is a cytoplasmic enzyme that plays an essential role in the β-oxidation of branched-chain fatty acids and is an established biomarker for prostate cancer. AMACR is expressed in adenomas and adenocarcinomas of the colon but not in normal colon epithelium, which suggests that it may play a role in the development of gastrointestinal malignancies.[38, 39] Although little is known about the value of AMACR in BE patients, the first small studies have shown promising results.[40-43] To investigate the value of p53 and AMACR immunohistochemistry for predicting neoplastic progression in BE we conducted a case-control study within a large multicenter prospective cohort study.[44, 45] Patients who developed HGD or EAC during follow-up were classified as cases and patients without neoplastic progression as controls. P53 and AMACR protein expression were determined by immunohistochemistry in more than 12.000 biopsies from 635 patients.

Aberrant p53 expression was more common with higher grades of dysplasia and was seen in 11% of biopsies without dysplasia, 38% with LGD, 83% with HGD and 100% with EAC. P53 overexpression was associated with an 5-fold increased risk of neoplastic progression and loss of p53 expression with an even 14-fold increased risk, independent of age, gender, BE length and

esophagitis. The sensitivity of aberrant p53 expression for predicting neoplastic progression was 49% with a specificity of 86%. The positive predictive value for neoplastic progression increased from 15% with LGD to 33% with LGD and concurrent aberrant p53 expression. To our knowledge this is the first large case-control study evaluating the value of both p53 overexpression and loss of expression for predicting neoplastic progression in BE. Previous studies have shown that p53 overexpression is associated with an increased risk of neoplastic progression, which corresponds to the results of the current study.[31, 34, 46] We have also shown good interobserver agreement for p53 expression, which indicates that p53 is not only a theoretical but also a clinically suitable biomarker for predicting neoplastic progression in BE. Although routine p53 immunohistochemistry is associated with higher costs than histology alone, application of this biomarker may lead to the identification of a much smaller high-risk group needing intensive surveillance. Surveillance of such a small risk group may eventually result in lower costs of surveillance, less burden on endoscopy units and higher quality of life for BE patients. The current study shows that aberrant p53 expression as determined by immunohistochemistry is a more powerful predictor for neoplastic progression than histological diagnosis of dysplasia and as a result implementation of p53 immunohistochemistry could improve risk stratification and hence the cost-effectiveness of BE surveillance.

AMACR expression was also more common in biopsies with a higher grade of dysplasia and was seen in 49% of biopsies without dysplasia, 63% with LGD, 91% with HGD and 71% with EAC. AMACR expression was associated with an 2-fold increased risk of neoplastic progression, independent of age, gender, BE length and *e*sophagitis. The risk was especially elevated with strong AMACR expression. The sensitivity for predicting progression was 10% with a specificity of 96%. Interobserver agreement was moderate. To our knowledge, this is the first study investigating AMACR immunohistochemistry for predicting neoplastic progression in BE. Although strong AMACR expression was associated with an increased risk of HGD or EAC, AMACR immunohistochemistry has not enough power to be used as a single biomarker.

Cost-effectiveness

Although BE surveillance seems reasonable and is incorporated in international guidelines, there is little scientific evidence that BE surveillance is beneficial. BE patients have a much higher risk of developing EAC compared to the general

population, but the absolute risk of neoplastic progression is low and which patients have the highest risk of neoplastic progression remains largely unknown. Over the past years there has been a major shift in the treatment of BE patients with the introduction of endoscopic treatment modalities such as endoscopic mucosal resection (EMR) and radiofrequency ablation (RFA) for patients with HGD or early EAC. Esophagectomy is still the mainstay for treatment of advanced EAC, but is nowadays complemented with neoadjuvant chemoradiotherapy.[47, 48] One of the key questions in the discussion about BE surveillance is whether it is cost-effective. Previous studies investigating the cost-effectiveness of BE surveillance have shown highly variable results, mainly due to different assumptions about progression rates and quality of life associated with different health states. In most studies, the incidence of EAC was estimated based on pooled literature data and esophagectomy was performed in case of HGD or EAC. To investigate the cost-effectiveness of BE surveillance according to current guidelines, we performed a cost-effectiveness analysis within a large multicenter prospective cohort with 714 BE patients. A multi-state-Markov model was used to calculate progression rates based on prospective follow-up data. These progression rates were incorporated in a decision-analytic model, which included costs and quality of life data. We evaluated different surveillance intervals for BE without dysplasia and LGD, EMR followed by RFA, RFA alone or esophagectomy for HGD or early EAC and esophagectomy with neoadjuvant chemoradiotherapy for advanced EAC. The incremental cost-effectiveness ratio (ICER) was calculated for each strategy in terms of costs per quality-adjusted life year (QALY) gained. Assuming a willingness-to-pay threshold of € 35.000 per QALY, endoscopic surveillance every 5 years is cost-effective for patients without dysplasia, with EMR followed by RFA in case of HGD or early EAC and esophagectomy for advanced EAC. Surveillance every 3 years is cost-effective for patients with LGD. Previous studies investigating the cost-effectiveness of BE surveillance have shown highly variable results. Surveillance was cost-effective in 4 studies with surveillance intervals ranging from 2 to 5 years.[49-52] However, in 4 other studies surveillance was not cost-effective with sometimes even higher costs and less quality of life than without surveillance.[53-56] For patients without dysplasia, the results of the current study correspond to recommendations made in current guidelines.[10, 57] For patients with LGD however, surveillance is recommended with intervals of 6-12 months in current guidelines, while according to our study intervals should be at least 3 years in order to be cost-effective. Identification of

additional risk factors besides histological diagnosis of dysplasia may improve risk-stratification and thereby the cost-effectiveness of surveillance with shorter intervals.

Survival

Other important issues in the discussion about BE surveillance are whether surveillance is able to prevent advanced EAC and improves survival. These key questions have been evaluated in previous case-control studies, population-based studies and small prospective cohort studies with conflicting results.[58-71] Although most studies suggest that surveillance enables the detection of early EAC with good survival rates, some other studies reported no effect on mortality.[72] For this purpose we evaluated the impact of BE surveillance according to current guidelines, on tumor stage and survival within a large multicenter prospective cohort of 783 BE patients. Patients were followed during surveillance according to current guidelines and during surveillance incident cases of HGD and EAC were identified. Survival data were collected and cross-checked using death and municipal registries and compared to data of patients with EAC in the general Dutch population. During surveillance 53 BE patients developed HGD or EAC, of which 92% was diagnosed with early EAC compared to 15% of patients with EAC in the general population. EAC was thus detected at an earlier stage during BE surveillance than in the general population, which was independent of age, gender and year of diagnosis. Since the majority of BE patients was diagnosed with early EAC, they were treated endoscopically and only 12% needed esophagectomy. The overall 5-year survival was 74% in patients with EAC during BE surveillance and 17% in patients with EAC in the general population, which seems clinically relevant. The survival of patients with EAC during BE surveillance was comparable to those of patients with stage 0 or 1 EAC in the general population. Most previous studies have shown that BE surveillance enables the detection of early EAC with good survival rates which is in line with the results of the current study. In 2 previous studies surveillance failed to detect early EAC. However, in these studies patients were not under strict surveillance, which is crucial for the detection of early EAC.[13, 73] Thus BE surveillance enables the detection of EAC at an early stage and leads to improved survival.

Conclusions

BE surveillance enables the detection of EAC at an early and curable stage when endoscopic treatment with EMR and RFA is still feasible, and which subsequently leads to improved survival. Although surveillance according to current guidelines is cost-effective for patients without dysplasia, annual surveillance for patients with LGD is not cost-effective in its current form. Therefore additional risk factors for neoplastic progression are needed to improve risk stratification and thereby the cost-effectiveness of BE surveillance. P53 immunohistochemistry is one of the most promising biomarkers for risk stratification in BE and appears to be a more powerful predictor for neoplastic progression than histological diagnosis of dysplasia. In contrast to most other biomarkers, P53 immunohistochemistry is widely available, affordable and associated with limited interobserver variation, which makes it also a clinically suitable biomarker. Another promising strategy to prevent neoplastic progression in BE is chemoprevention with NSAIDs, statins and PPIs. Use of NSAIDs and statins appears to be associated with a 50% reduced risk of neoplastic progression in BE, but may be accompanied with considerable side-effects which limits their use in chemoprevention. Use of PPI not only relieves reflux symptoms, but also reduces the risk of neoplastic progression in BE and should therefore be recommended in guidelines.

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Chapter 10

Summary

Samenvatting

F. Kastelein

Summary

Barrett's esophagus (BE) is a premalignant condition in which patients have an increased risk of developing esophageal adenocarcinoma (EAC) with an estimated incidence of 0.1 to 0.5% per year. The development of EAC in BE is thought to be a gradual process in which metaplastic columnar epithelium evolves to low-grade dysplasia (LGD), high-grade dysplasia (HGD) and eventually EAC under the influence of chronic esophageal acid exposure. Once a patient has developed EAC the prognosis is poor with a 5-year survival of less than 20%. Surveillance is therefore recommended for BE to detect EAC at an early stage when curative treatment is still feasible. Current guidelines recommend surveillance every 3 to 5 years in BE patients without dysplasia, surveillance every 6 to 12 months in patients with LGD, and (endoscopic) treatment in patients with established HGD or EAC. Histological diagnosis of dysplasia is the only accepted predictor for neoplastic progression in BE to date and is therefore used for defining surveillance intervals. However, histology is subject to sample error and considerable interobserver variation which limits its predictive value. Identification of additional predictors for neoplastic progression could improve risk stratification and hence the effectiveness of BE surveillance. The aim of this thesis was to evaluate whether chemoprevention and biomarkers can contribute to risk stratification in BE in order to optimize surveillance. In addition the effect of BE surveillance according to current guidelines was evaluated in terms of cost-effectiveness and survival.

BE usually develops in patients with gastroesophageal reflux disease and therefore esophageal acid exposure is thought to play an import role in the initiation of BE and its progression toward EAC. In **chapter 2** the existing literature is reviewed regarding the role of esophageal acid exposure in BE. Since many patients suffer from reflux symptoms, acid suppression with proton pump inhibitors (PPIs) has become a mainstay in the treatment of BE. In **chapter 3** we investigated whether PPI use also reduces the risk of neoplastic progression. For this purpose we included 540 BE patients in a large multicenter prospective cohort study. Information on medication use, including over-the-counter medication, was collected and was cross-checked using pharmacy records. PPI use during follow-up was associated with an approximately 75% reduced risk of neoplastic progression. PPI use is therefore justified in BE and should be recommended in guidelines.

In addition, studies have given support to chemoprevention with non-steroidal anti-inflammatory drugs (NSAIDs) and statins for the prevention of several cancers including EAC. In **chapter 4** we investigated whether use of NSAIDs and statins reduces the risk of neoplastic progression in BE. For this purpose, we collected data on use of NSAIDs and statins from 570 BE patients in a large multicenter prospective cohort study. Use of low dose aspirin did not significantly change the risk of neoplastic progression in BE, but NSAID and statin use were associated with an approximately 50% reduced risk. Use of both NSAIDs and statins was associated with an additive protective effect and an approximately 75% reduced risk of neoplastic progression. However, use of NSAIDs and statins may be accompanied with considerable side-effects which limits their use in chemoprevention. Since NSAID and statin use are common among BE patients, this information may be used for risk stratification.

Application of biomarkers in addition to histology may also contribute to the identification of patients at high risk for neoplastic progression. Many biomarkers have been investigated in BE of which immunohistochemical staining of p53 appears to be one of the most promising. In **chapter 5**, we investigated the value of p53 immunohistochemistry for predicting neoplastic progression in BE. For this purpose we conducted a case-control study within a large multicenter prospective cohort study. Patients who developed HGD or EAC during follow-up were classified as cases and patients without neoplastic progression were classified as controls. P53 expression was determined by immunohistochemistry in more than 12.000 biopsies from 635 patients. Overexpression of p53 was associated with an 5-fold increased risk of neoplastic progression in BE and loss of p53 expression with an even 14-fold increased risk. The sensitivity of aberrant p53 expression for predicting progression was 49% with a specificity of 86% and good interobserver agreement. Aberrant p53 expression appears to be a more powerful predictor for neoplastic progression in BE than histological diagnosis of dysplasia and as a result implementation of p53 immunohistochemistry could improve risk stratification and hence the cost-effectiveness of BE surveillance.

Another potential biomarker for predicting neoplastic progression in BE patients is Alpha-Methylacyl-CoA Racemase (AMACR), an established biomarker for prostate cancer. In **chapter 6**, we investigated the value of AMACR immunohistochemistry for predicting neoplastic progression in BE. AMACR expression was associated with an 2-fold increased risk of neoplastic progression in BE, especially in case of strong expression. The sensitivity of

strong AMACR expression for predicting progression was 10% with a specificity of 96% and moderate interobserver agreement. Although strong AMACR expression was associated with an increased risk of neoplastic progression, AMACR immunohistochemistry is not powerful enough to be used as a single biomarker.

Although BE surveillance seems reasonable and is incorporated in guidelines, there is little scientific evidence that BE surveillance is beneficial. One of the key questions in this discussion about BE surveillance is whether BE surveillance is cost-effective. In **chapter 7**, we investigated the cost-effectiveness of BE surveillance according to current guidelines. We performed a cost-effectiveness analysis within a large multicenter prospective cohort with 714 BE patients. A multi-state-Markov model was used to calculate progression rates based on prospective follow-up data. These progression rates were incorporated in a decision-analytic model, which included costs and quality of life data. We evaluated different surveillance intervals for BE patients without dysplasia and LGD, endoscopic mucosal resection (EMR) followed by radiofrequency ablation (RFA), RFA alone or esophagectomy for patients with HGD or early EAC and esophagectomy with neoadjuvant chemoradiotherapy for patients with advanced EAC. Assuming a willingness-to-pay threshold of €35.000 per quality-of-life year gained, endoscopic surveillance every 5 years is cost-effective for BE without dysplasia, with EMR followed by RFA for HGD or early EAC and esophagectomy with neoadjuvant chemoradiotherapy for advanced EAC. Surveillance every 3 years is cost-effective for LGD. Identification of additional risk factors besides histology may improve risk-stratification and hence the cost-effectiveness of surveillance with shorter intervals.

Another important issue in the discussion about BE surveillance is whether surveillance is able to prevent advanced EAC and improves survival. In **chapter 8**, we evaluated the impact of BE surveillance according to current guidelines, on tumor stage and survival of patients with EAC. For this purpose 783 BE patients were included in a large multicenter prospective cohort study and followed during surveillance according to current guidelines. During surveillance incident cases of HGD and EAC were identified. Survival data were collected and cross-checked using death and municipal registries and compared to data of patients with EAC in the general population. During BE surveillance 92% of patients with neoplastic progression was diagnosed with early EAC compared to 15% of patients with EAC in the general population. EAC was thus detected at an earlier stage during BE surveillance than in the general population. The

survival of BE patients with neoplastic progression during surveillance was not significantly worse than those of BE patients without neoplastic progression and comparable to those of patients with stage 0 or 1 EAC in the general population. Thus BE surveillance enables the detection of EAC at an early stage with good survival rates.

Samenvatting

Barrett slokdarm is een premaligne aandoening waarbij patiënten een verhoogd risico hebben op het ontwikkelen van een adenocarcinoom van de slokdarm met een geschatte incidentie van 0.1 tot 0.5% per jaar. De ontwikkeling van een adenocarcinoom in Barrett slokdarm is een geleidelijk proces waarbij metaplastisch cilinder epitheel veranderd in laaggradige dysplasie, hooggradige dysplasie en uiteindelijk adenocarcinoom onder invloed van chronische zuur expositie. Wanneer een patiënt eenmaal een adenocarcinoom heeft ontwikkeld is de prognose slecht met een 5-jaars overleving van minder dan 20%. Daarom wordt aan patiënten met een Barrett slokdarm surveillance geadviseerd om adenocarcinomen op te sporen in een vroegtijdig stadium wanneer curatieve behandeling nog mogelijk is. De huidige richtlijnen adviseren surveillance elke 3 tot 5 jaar voor patiënten zonder dysplasie, surveillance elke 6 tot 12 maanden voor patiënten met laaggradige dysplasie en (endoscopische) behandeling voor patiënten met hooggradige dysplasie of adenocarcinoom. Op dit moment is histologie de enige geaccepteerde voorspeller voor maligne ontaarding en histologie wordt daarom gebruikt voor bepaling van het surveillance interval. Histologie is echter onderhevig aan steekproeffouten en interobserver variatie wat de voorspellende waarde beperkt. Identificatie van andere voorspellers voor maligne ontaarding zou risicostratificatie van patiënten met een Barrett slokdarm kunnen verbeteren en daarmee ook de kosteneffectiviteit van surveillance. Het doel van dit proefschrift was te onderzoeken of gebruik van chemopreventie en biomarkers kan bijdragen aan risicostratificatie van patiënten met een Barrett slokdarm. Daarnaast evalueerden we het effect van surveillance volgens de huidige richtlijnen op kosteneffectiviteit en overleving.

Omdat Barrett slokdarm vaak wordt gevonden bij patiënten met gastro-oesofageale reflux ziekte, wordt gedacht dat zuur expositie een belangrijke rol speelt in zowel het ontstaan van Barrett slokdarm als de ontwikkeling van een adenocarcinoom. In **hoofdstuk 2** wordt de bestaande literatuur geëvalueerd met betrekking tot de rol van zuur expositie in het ontstaan van Barrett slokdarm en progressie naar adenocarcinoom. Omdat veel patiënten met een Barrett slokdarm last hebben van reflux klachten, speelt zuurremming met proton pomp remmers (PPIs) een belangrijke rol in de behandeling. In **hoofdstuk 3** hebben we onderzocht of gebruik van PPIs ook het risico op maligne ontaarding verminderd. We includeerden 540 Barrett patiënten in een grote multicenter prospectieve cohort studie en verzamelden informatie over medicijngebruik

inclusief zelfmedicatie. PPI gebruik tijdens surveillance was geassocieerd met een ongeveer 75% verlaagd risico op maligne ontaarding. PPI gebruik is daarom gerechtvaardigd voor patiënten met een Barrett slokdarm en moet worden geadviseerd in richtlijnen.

Daarnaast wordt gesuggereerd dat chemopreventie met NSAIDs en statines effectief zou kunnen zijn in de preventie van verschillende soorten kanker, waaronder ook adenocarcinomen van de slokdarm. In **hoofdstuk 4** hebben we onderzocht of gebruik van NSAIDs en statines ook het risico op maligne ontaarding verminderd bij patiënten met een Barrett slokdarm. Om dit te onderzoeken includeerden we 570 Barrett patiënten in een grote multicenter prospectieve cohort studie. Gebruik van lage dosering aspirine had geen invloed op het risico op maligne ontaarding, maar gebruik van NSAIDs en statines was geassocieerd met een ongeveer 50% verlaagd risico. Gebruik van zowel NSAIDs als statines was geassocieerd met een extra beschermend effect en een 75% verlaagd risico op maligne ontaarding. Omdat gebruik van een hoge dosering NSAIDs en statines echter gepaard kan gaan met aanzienlijke bijwerkingen is het gebruik in chemopreventie beperkt. Aangezien gebruik van NSAIDs en statines wel veel voorkomt onder Barrett patiënten, kan deze informatie ook worden gebruikt voor risicostratificatie.

Gebruik van biomarkers als aanvulling op histologie kan ook bijdragen aan de identificatie van Barrett patiënten met een hoog risico op maligne ontaarding. Er zijn veel verschillende biomarkers onderzocht in Barrett slokdarm, waarvan p53 immunohistochemie het meest veelbelovend lijkt te zijn. In **hoofdstuk 5** hebben we de waarde van p53 immunohistochemie onderzocht voor het voorspellen van maligne ontaarding in Barrett slokdarm. Hiervoor hebben we een case-control studie verricht binnen een grote multicenter prospectieve cohort studie. Patiënten die hooggradige dysplasie of adenocarcinoom ontwikkelden tijdens surveillance werd geclassificeerd als cases en de overige patiënten als controles. P53 expressie werd bepaald met immunohistochemie in meer dan 12.000 biopten van 635 patiënten. Overexpressie van p53 was geassocieerd met een 5 keer verhoogd risico op maligne ontaarding en verlies van p53 expressie met een 14 keer verhoogd risico. De sensitiviteit van afwijkende p53 expressie voor het voorspellen van maligne ontaarding was 49% met een specificiteit van 86% en goede interobserver overeenkomst. Afwijkende p53 expressie lijkt een krachtigere voorspeller te zijn voor maligne ontaarding in Barrett slokdarm dan histologische diagnose van dysplasie. Implementatie van

p53 immunohistochemie zou dus de risicostratificatie kunnen verbeteren en daarmee ook de kosteneffectiviteit van Barrett surveillance.

Een andere potentiele biomarker voor risicostratificatie in Barrett slokdarm is Alpha-Methylacyl-CoA Racemase (AMACR), een bekende biomarker voor prostaatkanker. In **hoofdstuk 6** hebben we de waarde van AMACR onderzocht voor het voorspellen van maligne ontaarding in Barrett slokdarm. AMACR expressie was geassocieerd met een 2 keer verhoogd risico op maligne ontaarding, vooral bij sterke expressie, met een sensitiviteit van 10%, een specificiteit van 96% en redelijke interobserver overeenkomt. Hoewel sterke AMACR expressie geassocieerd was met een verhoogd risico op maligne ontaarding in Barrett slokdarm lijkt AMACR immunohistochemie niet krachtig genoeg om gebruikt te worden als biomarker.

Hoewel surveillance van patiënten met een Barrett slokdarm logisch lijkt en wordt geadviseerd in richtlijnen is er weinig wetenschappelijk bewijs dat surveillance effectief is. In **hoofdstuk 7** werd de kosteneffectiviteit onderzocht van Barrett surveillance volgens de huidige richtlijnen. We hebben een kosteneffectiviteitsanalyse verricht binnen een grote multicenter prospectieve cohort studie met daarin 714 patiënten. Een multi-state-Markov model werd gebruikt om progressie kansen te berekenen op basis van follow-up gegevens, welke vervolgens werden opgenomen in een beslismodel met daarin gegevens over kosten en kwaliteit van leven. We evalueerden verschillende surveillance intervallen voor patiënten zonder dysplasie of met laaggradige dysplasie en endoscopische of chirurgische behandeling voor patiënten met hooggradige dysplasie of een adenocarcinoom. Surveillance elke 5 jaar, met endoscopische behandeling voor zowel hooggradige dysplasie als vroegcarcinomen en een slokdarmresectie met neoadjuvant chemoradiotherapie voor een gevorderd adenocarcinoom, lijkt kosteneffectief te zijn voor patiënten zonder dysplasie, uitgaande van een drempel van €35.000 per gewonnen levensjaar. Voor patiënten met laaggradige dysplasie lijkt surveillance elke 3 jaar kosteneffectief. Identificatie van aanvullende risicofactoren naast histologische diagnose van dysplasie kan de risicostratificatie verbeteren en daarmee de kosteneffectiviteit van surveillance met kortere intervallen.

In **hoofdstuk 8** hebben we onderzocht wat de invloed is van surveillance volgens de huidige richtlijnen op tumor stadium en overleving van patiënten met een adenocarcinoom van de slokdarm. Hiervoor hebben we 783 patiënten met een Barrett slokdarm geïncludeerd in een grote multicenter prospectieve cohort studie en gevolgd tijdens surveillance volgens de huidige richtlijnen. We

verzamelden overlevingsdata en vergeleken deze met overlevingsdata van patiënten met een adenocarcinoom van de slokdarm in de algemene bevolking. Tweeënnegentig procent van de Barrett patiënten met maligne ontaarding tijdens surveillance werd gediagnosticeerd in een vroeg stadium in vergelijking met 15% van de patiënten met een adenocarcinoom in de algemene bevolking. Adenocarcinomen van de slokdarm werden dus in een eerder stadium gevonden tijdens Barrett surveillance dan in de algemene bevolking. De overleving van Barrett patiënten met maligne ontaarding tijdens surveillance was niet significant slechter dan de overleving van Barrett patiënten zonder maligne ontaarding en gelijk aan de overleving van patiënten met stadium 0 of 1 adenocarcinoom in de algemene bevolking. Barrett surveillance maakt dus detectie van adenocarcinomen mogelijk in een vroeg stadium met daarbij goede overlevingskansen.

Chapter 11

Dankwoord

Curriculum Vitae

PhD portfolio

F. Kastelein

Dankwoord

Er zijn veel mensen die hebben bijgedragen aan de totstandkoming van dit proefschrift. Een aantal van hen wil ik graag in het bijzonder bedanken.

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Curriculum Vitae

Florine Kastelein werd geboren op 28 augustus 1985 in Voorburg. In 2003 behaalde zij cum laude haar gymnasium diploma aan College 't Loo in Voorburg. Vervolgens begon zij in datzelfde jaar aan de studie Geneeskunde aan de universiteit Leiden, waar zij in 2007 cum laude haar doctoraal behaalde. In 2009 doorliep zij haar oudste coschap op de afdeling Maag-, Darm- en Leverziekten van het Leids Universitair Medisch Centrum en haar keuze coschap op de afdeling Maag-, Darm- en Leverziekten van het Erasmus Medisch Centrum, waarna zij in augustus 2009 haar artsexamen behaalde. In oktober 2009 startte zij met promotieonderzoek op het gebied van Barrett slokdarm op de afdeling Maag-, Darm- en Leverziekten van het Erasmus Medisch Centrum onder begeleiding van prof.dr. M.J. Bruno en dr. M.C.W. Spaander. Op 1 januari 2013 startte zij met de opleiding Maag-, Darm en Leverziekten vanuit het Erasmus Medisch Centrum (opleider dr. R.A. de Man). De vooropleiding Interne Geneeskunde (opleider dr. E.F.M. Posthuma) en de eerste twee jaar van de opleiding Maag-, Darm- en Leverziekten (opleider dr. J.T. Brouwer) volgt zij in het Reinier de Graaf Gasthuis in Delft. Zij is getrouwd met Michel van Soolingen en woont in Pijnacker.

Publications

Publications related to this thesis:

F. Kastelein, M.C.W. Spaander, K. Biermann, B. Vucelic, E.J. Kuipers, and M.J. Bruno.
The role of acid suppression in the development and progression of dysplasia in patients with Barrett's esophagus.
Digestive diseases, 2011;29(5):499-506

F. Kastelein, M.C.W. Spaander, E.W. Steyerberg, K. Biermann, V.E. Valkhoff, E.J. Kuipers, and M.J. Bruno on behalf of the ProBar-study group.
Proton pump inhibitors reduce the risk of neoplastic progression in patients with Barrett's esophagus.
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F. Kastelein, M.C.W. Spaander, K. Biermann, E.W. Steyerberg, E.J. Kuipers, and M.J. Bruno on behalf of the ProBar-study group.
Nonsteroidal anti-inflammatory drugs and statins have chemopreventive effects in patients with Barrett's esophagus.
Gastroenterology, 2011 Dec;141(6): 2000-2008

F. Kastelein, K. Biermann, E.W. Steyerberg, J. Verheij, M. Kalisvaart, L.H.J. Looijenga, J.A. Stoop, L. Walter, E.J. Kuipers, M.C.W. Spaander, and M.J. Bruno[1] on behalf of the ProBar-study group.
Aberrant p53 protein expression is associated with an increased risk of neoplastic progression in patients with Barrett's esophagus.
Gut, 2013 Dec; 62(12):1676-1683

F. Kastelein, K. Biermann, E.W. Steyerberg, J. Verheij, M. Kalisvaart, L.H.J. Looijenga, J.A. Stoop, L. Walter, E.J. Kuipers, M.C.W. Spaander, and M.J. Bruno on behalf of the ProBar-study group.
Value of alpha-methylacyl-CoA racemase for predicting neoplastic progression in patients with Barrett's esophagus.
Histopathology, 2013 Nov;63(5):630-639

F. Kastelein, S. van Olphen, E.W. Steyerberg, M.C.W. Spaander, M. Sikkema, C.W.N. Looman, M.J.C. Eijkemans, J. Wong, D.T. Provenzale, E.J. Kuipers, P.D. Siersema, M.J. Bruno, and E.W. de Bekker-Grob on behalf of the ProBar-study group
Surveillance in patients with long-segment Barrett's esophagus: a cost-effectiveness analysis.
Gut, 2014

F. Kastelein, S. van Olphen, E.W. Steyerberg, M.C.W. Spaander, and M.J. Bruno on behalf of the ProBar-study group.
Impact of surveillance for long-segment Barrett's esophagus on tumor stage and survival of patients with neoplastic progression: results of a large multicenter prospective cohort study
Submitted for publication

Other publications:

S. van Olphen, K. Biermann, **F. Kastelein**, B.E. Hansen, J.A. Stoop, M.C.W. Spaander, M.J Bruno, and L.H.J. Looijenga on behalf of the ProBar-study group
SOX2 as a novel marker to predict neoplastic progression in Barrett's esophagus.
Submitted for publication

S. Zhang, X.Q. Zhang, X.W. Ding, R.K. Yang, S.L. Huang, **F. Kastelein**, M.J. Bruno, X.J. Yu, D. Zhou, and X.P. Zou.
Cyclooxygenase inhibitors use is associated with reduced risk of esophageal adenocarcinoma in patients with Barrett's esophagus: a meta-analysis.
British Journal of Cancer, 2014; Epub ahead of print

F. Kastelein, K. Biermann, L.H.J. Looijenga, and M.J. Bruno
P53 immunohistochemistry in patients with Barrett's esophagus
Treatment Strategies - Gastroenterology

F. Kastelein and E.J. Kuipers.
Surveillance voor Barrett slokdarm: de moeite waard?
Nederlands Tijdschrift voor Geneeskunde, 2012;156(49): A5497

M. Sikkema, C.W. Looman, E.W. Steyerberg, M. Kerkhof, **F. Kastelein**, H. van Dekken, A.J. van Vuuren, W.A. Bode, H. van der Valk, R.J. Ouwendijk, R. Giard, W. Lesterhuis, R. Heinhuis, E.C. Klinkenberg, G.A. Meijer, F. ter Borg, J.W. Arends, J.J. Kolkman, J. van Baarlen, R.A. de Vries, A.H. Mulder, A.J. van Tilburg, G.J. Offerhaus, F.J. ten Kate, J.G. Kusters, E.J. Kuipers, and P.D. Siersema.
Predictors for neoplastic progression in patients with Barrett's Esophagus: a prospective cohort study.
American Journal of Gastroenterology, 2011 Jul;106(7):1231-1238

PhD Portfolio

Oral presentations

2014   Impact of surveillance for Barrett's esophagus on survival of patients with neoplastic progression: results of a large multicenter prospective cohort study
United European Gastroenterology Week, Vienna, Austria

2014   Surveillance in patients with Barrett's esophagus: a cost-effectiveness analysis
Dutch Society of Gastroenterology, Veldhoven, the Netherlands

2013   Survival of patients with Barrett-related adenocarcinoma detected in a surveillance program: results of a large multicenter prospective cohort study
United European Gastroenterology Week, Berlin, Germany
Awarded with oral free paper prize

2013   P53 immunohistochemistry for predicting neoplastic progression in patients with Barrett's esophagus: results from a large multicentre prospective cohort
Dutch Society of Gastroenterology, Veldhoven, the Netherlands

2011   Proton pump inhibitors and the risk of neoplastic progression in Barrett's esophagus: results of a large prospective cohort study
United European Gastroenterology Week, Stockholm, Sweden

2011   Chemoprevention in Barrett's esophagus with NSAIDs and statins: results of a large multicenter prospective cohort study
United European Gastroenterology Week, Stockholm, Sweden
Awarded with oral free paper prize

2011   Chemoprevention in Barrett's esophagus with NSAIDs and statins: results of a large multicenter prospective cohort study
Digestive Diseases Week, Chicago, United States

2011   Chemoprevention in Barrett's esophagus with NSAIDs and statins: results of a large multicenter prospective cohort study
Dutch Society of Gastroenterology, Veldhoven, the Netherlands

2010   Prospective evaluation of the incidence of neoplastic progression in a large cohort of patients with Barrett's esophagus.
European Association for Gastroenterology bridging meeting, Berlin, Germany

Poster presentations

2014   Surveillance in patients with Barrett's esophagus: a cost-effectiveness analysis
        United European Gastroenterology Week, Vienna, Austria

2014   Surveillance in patients with Barrett's esophagus: a cost-effectiveness analysis
        Digestive Diseases Week, Chicago, United States

2013   Survival of patients with Barrett-related adenocarcinoma detected in a surveillance program: results of a large multicenter prospective cohort study
        Digestive Diseases Week, Orlando, United States

2013   AMACR immunohistochemistry for prediction of neoplastic progression in patients with Barrett's esophagus: results from a large multicenter prospective cohort
        Digestive Diseases Week, Orlando, United States

2013   P53 immunohistochemistry for prediction of neoplastic progression in patients with Barrett's esophagus: results from a large multicenter prospective cohort
        Digestive Diseases Week, Orlando, United States

2012   P53 and AMACR immunohistochemistry for predicting neoplastic progression in patients with Barrett's esophagus
        United European Gastroenterology Week, Amsterdam, the Netherlands

2012   P53 immunohistochemistry differentiates between low-grade dysplasia and regenerative changes in Barrett's esophagus
        United European Gastroenterology Week, Amsterdam, the Netherlands

2012   P53 immunohistochemistry differentiates between low-grade dysplasia and regenerative changes in Barrett's esophagus
        Digestive Diseases Week, San Diego, United States

2011   Proton pump inhibitors and the risk of neoplastic progression in Barrett's
        esophagus: results of a large prospective cohort study
        Digestive Diseases Week, Chicago, United States

Attended seminars and workshops

2012   Survival analysis for clinicians
        Netherlands Institute for Health Sciences, Rotterdam, the Netherlands

2012   Regression analysis for clinicians
        Netherlands Institute for Health Sciences, Rotterdam, the Netherlands

2011   Biomedical English writing and communication
        Erasmus University Medical Center, Rotterdam, the Netherlands

2011   Molecular diagnostics
        Molecular Medicine, Rotterdam, the Netherlands

2011   Biostatistics for clinicians
        Netherlands Institute for Health Sciences, Rotterdam, the Netherlands

2010   Short introduction course on statistics and survival analysis for MDs
        Molecular Medicine, Rotterdam, the Netherlands

2010   Principles of research in medicine
        Netherlands Institute for Health Sciences, Rotterdam, the Netherlands

Peer review activities

2013   Gut

2012   Gastroenterology

Membership

2010   Dutch Society of Gastroenterology