Incidence and Risk Factors of Neoplastic Progression in Barrett's Esophagus

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Incidence and Risk Factors of Neoplastic Progression in Barrett's Esophagus

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Chapter 1

General introduction and outline of the thesis

M. Sikkema

BARRETT'S ESOPHAGUS

Barrett's esophagus (BE) is a condition in which the normal stratified squamous epithelium lining the distal esophagus is replaced by columnar epithelium with specialized intestinal metaplasia (IM) containing goblet cells. The development of BE is a complication of chronic exposure to the gastric refluxate containing acid and bile.[1-3] BE is a premalignant condition which predisposes to the development of esophageal adenocarcinoma (EAC). The development of this malignancy is a gradual stepwise process from no dysplasia (ND) to low-grade dysplasia (LGD), high-grade dysplasia (HGD), and finally EAC (Figure 1).[1, 4]

During the past decades, the incidence of both BE[5] and EAC has been rising rapidly as demonstrated by a 7-fold increased incidence of EAC between 1973 and 2006.[6-8] In general, patients with BE have a 30- to 125-fold increased risk of developing EAC compared to the general population.[2, 9] However, the annual incidence of EAC in BE patients remains unclear, as it shows considerable variation among cohort studies, ranging from 0.2% to almost 3.5% per year.[10-12] The consensus is however that the incidence of EAC is approximately 0.5% per year.[13] EAC usually has a poor prognosis and a high mortality with a 5-year survival rate of less than 20%.[14] As a result of the malignant potential of BE, regular surveillance endoscopies are recommended in patients with BE. The intervals of surveillance depend on the grade of dysplasia present. The goal of surveillance is to detect neoplasia at an early stage, making early treatment with curative intention possible and reducing death from EAC.[15]

PREDICTORS OF NEOPLASTIC PROGRESSION AND RISK STRATIFICATION IN BARRETT'S ESOPHAGUS

Although the incidence of EAC has rapidly been increasing over the past decades, only a minority of BE patients will progress to EAC.[12, 13] As the absolute risk of neoplastic progression in BE is low, the majority of patients with BE will not benefit from a burdensome endoscopic surveillance program. Until now, it is unknown which subgroup of patients with BE will actually progress to HGD and EAC. Therefore, risk stratification could aid in identifying patients at the highest risk of developing neoplastic progression and requiring more frequent follow-up.

The major risk factor for BE and EAC development is gastroesophageal reflux disease (GERD).[17] Previous studies have shown that a hiatal hernia, a long BE segment and LGD are also associated with EAC development.[17-23] In addition, factors such as male gender, advanced age and Caucasian ethnicity are associated with GERD, BE and EAC

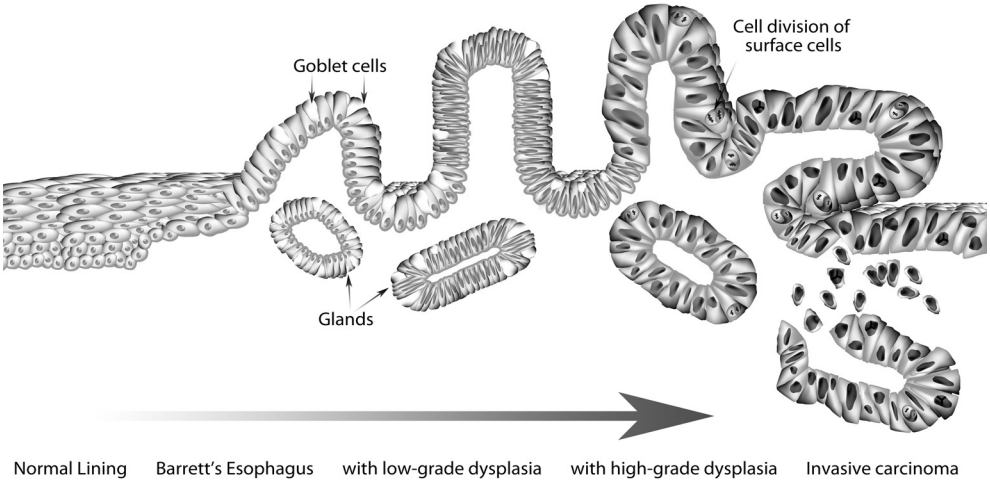

Figure 1. The morphologic development of Barrett's esophagus from normal, squamous epithelium to BE with intestinal metaplasia and goblet cells to low-grade dysplasia, high-grade dysplasia and finally to invasive esophageal adenocarcinoma.[16]

development. However, all these risk factors are not useful to discriminate between high- and low-risk patients because of their common prevalence in these patients and the often controversial results of different studies.[18, 22, 24]

Currently, histopathologic assessment of biopsies is the standard to determine the interval of endoscopic surveillance for a patient with BE. However, histological evaluation of biopsies obtained during endoscopy has its limitations. Due to sampling error and substantial interobserver variation, it misses tools to unambiguously identify the subset of patients who require more frequent surveillance.[25-27] As a result, research on biomarkers is still being performed to detect discriminative markers in addition to histology to identify patients at a high risk of progression.[28] Until now, no marker is ready for use in a clinical setting.[24]

So far, only the presence of dysplasia is used to determine the surveillance interval of patients with BE. Nonetheless, additional predictors of progression are needed to detect high-risk patients and to tailor surveillance programs.

SURVEILLANCE OF BARRETT'S ESOPHAGUS

Current recommendations for surveillance of patients with BE are based on guidelines from the American College of Gastroenterology.[15] In brief, patients without dysplasia are recommended to undergo endoscopic surveillance every three years. Patients with

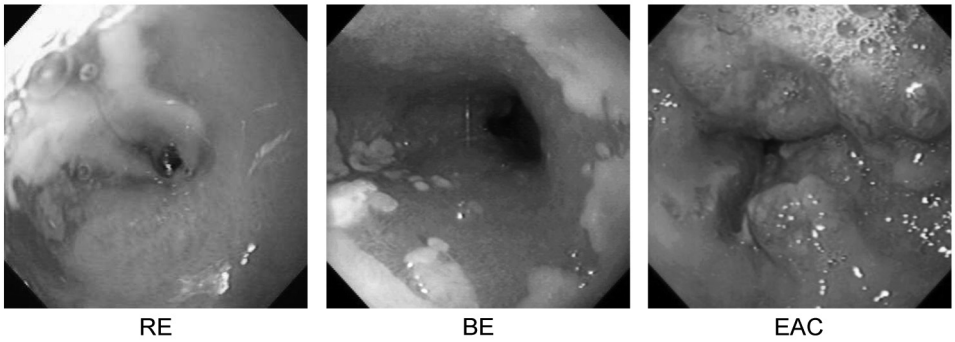

Figure 2. Endoscopic images of reflux esophagitis (RE), Barrett's esophagus (BE) and esophageal adenocarcinoma (EAC).

LGD are recommended to undergo endoscopic surveillance every year. For patients with HGD, surveillance every 3 months or endoscopic resection is advised. During endoscopy, four quadrant biopsies from every 2 cm of the BE segment should be taken to assess the presence of BE as defined by presence of IM containing goblet cells, and the presence of dysplasia. Dysplasia in BE is graded according to the Consensus Criteria of 1988, with adjustments as proposed in 2001.[25, 27, 29]

However, the benefit of surveillance in BE needs to be clarified in the absence of data from randomized controlled trials. It has been reported that BE patients in whom EAC was detected within a surveillance program had both earlier stage disease and a better survival than patients with EAC detected outside a surveillance program.[30] Nevertheless, there is little evidence that surveillance programs have prevented deaths from EAC[31, 32] as most patients with BE die from other causes than EAC.[33]

Similarly, surveillance in BE and the appropriate intervals are still under debate as the cost-effectiveness of surveillance is for a large part dependent on the incidence rate of EAC in BE.[34, 35] As stated above, the reported incidence rates of EAC vary between 0.2% and 3.5% per year.[10, 11] These differences could well be explained by several factors, with publication bias, selection bias and a retrospective design of various studies[32, 33, 36-40] being the most prominent ones leading to an overestimation of the cancer risk. Previous cost-effectiveness analyses of surveillance of BE have shown that an EAC incidence rate of 0.4% resulted in a cost-effective surveillance interval of 5 years in patients with BE and ND.[34] Another study, also using a model, showed that, when employing an incidence rate of 0.5%, surveillance in patients in BE does more harm than good.[35]

Hence, the benefit and efficacy of surveillance of patients with BE is still questionable.

Therefore, a cost-effectiveness analysis of surveillance based on real follow-up data instead of pooled literature data, is necessary to establish the real value of follow-up in BE.

AIM OF THIS THESIS

The aim of this thesis was to assess the incidence of neoplastic progression in patients with BE, to investigate risk factors involved in neoplastic progression, and to evaluate the value of these factors in identifying patients at high risk of progression. These data were used to determine the yield and cost-effectiveness of surveillance in patients with BE.

OUTLINE OF THIS THESIS

Chapter 2 consists of a systematic review and meta-analysis of studies on the incidence of EAC in patients with BE under surveillance, as well as the risk of mortality due to EAC. **Chapter 3** examines the role of promising biomarkers, i.e. p53, Ki67 and DNA ploidy, as markers of neoplastic progression in BE, using a case-control study design. **Chapter 4** explores easy to apply predictors for the development of progression in BE which could be used to identify BE patients with a high risk of neoplastic progression. **Chapter 5**, reports on the observed incidence rates of progression from ND to LGD, and from ND or LGD to HGD and EAC in a prospective multicenter BE cohort. **Chapter 6** reports on true progression rates from BE to EAC using multi-state Markov models based on a large prospective BE cohort. In **Chapter 7**, we evaluate the cost-effectiveness of different intervals of surveillance in BE and compare endoscopic interventions with esophagectomy. Finally, in **Chapter 8**, the results of this thesis are discussed and recommendations for future research are described.

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Chapter 2

Clinical Gastroenterology and Hepatology 2010; 8:235-44

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

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ABSTRACT

Background and aim: As the risk of esophageal adenocarcinoma (EAC) and mortality in patients with Barrett's esophagus (BE) are important determinants of the potential yield and cost-effectiveness of BE surveillance, clarification of these factors is essential. We therefore performed a systematic review and meta-analysis to determine the incidence of EAC and mortality due to EAC in BE under surveillance.

Methods: Databases were searched for relevant cohort studies in English language that reported EAC risk and mortality due to EAC in BE. Studies had to include patients with histologically proven BE, documented follow-up, and histologically proven EAC on surveillance. A random effects model was used with assessment of heterogeneity by the I^2-statistic and of publication bias by Begg's and Egger's tests.

Results: Fifty-one studies were included in the main analysis. The overall mean age of BE patients was 61 years; the mean overall proportion of males was 64%. The pooled estimate for EAC incidence was 6.3/1,000 pyrs (95%CI: 4.7-8.4) with considerable heterogeneity ($p<0.001$; $I^2=79\%$). Nineteen studies reported data on mortality due to EAC. The pooled incidence of fatal EAC was 3.0/1,000 pyrs (95%CI: 2.2-3.9) with no evidence for heterogeneity ($p=0.4$; $I^2=7\%$). No evidence of publication bias was found.

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

INTRODUCTION

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

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

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

METHODS

Search strategy

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

Study selection

Studies were included if they met the following criteria: (1) written in English; (2) histologically proven BE (columnar lined esophagus (CLE) or specialized intestinal metaplasia (SIM)); (3) documented follow-up data either in person-years (pyrs) or mean follow-up period; and (4) histologically proven EAC on surveillance. Studies were excluded if they were available as abstracts only because the abstracts did not allow full data extraction. Studies written in any foreign language were identified within the primary search strategy, but were eventually excluded, as we were not able to make a complete translation of the manuscripts. We also excluded studies if they lacked data on follow-up, or if they reported solely on patients who underwent endoscopic ablation or surgery. If serial studies from a single center reported cancer risk or mortality in the same cohort, only the most recent publication was included.

Data extraction

Two investigators (MS and PdJ) independently collected the following data from each study: country, year and type of study; definition of BE used; number of patients in the study with documented follow-up; mean follow-up period; person-years of follow-up; mean age at entering surveillance; sex ratio; number of prevalent and incident cancers;

number of prevalent and incident HGDs; number of patients who died during the study; and number of patients who died due to EAC. In addition, where available, data on the proportion of patients with SSBE, and low-grade dysplasia at baseline BE diagnosis were also extracted. Where possible, we excluded patients with baseline HGD for this analysis. In case of disagreement, a third independent investigator was asked for a review (EJK).

Data analysis

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

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

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

RESULTS

The search strategy yielded 7,200 abstracts, of which 190 were relevant to the review topic and subsequently reviewed. Following evaluation of the full text papers, 51 articles met the inclusion criteria and were included in the final analysis.[2, 11, 12, 14, 15, 17, 18, 29-69]

Study characteristics

Of the 51 studies included, 20 were from the United Kingdom, 16 from the United States, 13 from other European countries, and two from Australia. Baseline characteristics of the study cohorts are given in Tables 1 and 2. Forty studies provided data on mean age, the overall mean age was 61.3 years (range 40.0-70.0).[2, 11, 12, 14, 15, 17, 18, 20, 29-37, 39, 41, 46-53, 56-59, 61-67, 69-71]

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

First author	Year	Geography	Total no. under FU	Male (%)	Mean age (yrs)	SIM pos (%)	Baseline LGD (n)	Baseline HGD (n)	LSBE (%)	SSBE (%)	Pyrs of FU	Incident EAC (n)	Tot. mort. (n)	Due to EAC (n)	Due to other causes (n)
Spechler[29]	1984	USA	105	NA	58	100	35	10	NA	NA	350	2	16	1	15
Cameron[14]	1985	USA	104	67	59.6	NA	NA	NA	100	0	884	2	25	1	24
Robertson[30]	1988	UK	56	55	62	77	8	NA	100	0	162	3	4	0	4
Ovaska[31]	1989	Finland	26	NA	59.2	NA	NA	NA	100	0	166	3	NA	NA	NA
Hameeteman[2]	1989	Netherlands	50	60	59.3	68	6	1	100	0	260	5	NA	NA	NA
Miros[32]	1991	Australia	81	NA	63.3	NA	10	3	100	0	289	3	21	2	19
Williamson[33]	1991	USA	176	65	56	NA	20	0	100	0	497	5	NA	NA	NA
Iftikhar[34]	1992	UK	102	61	63	NA	2	NA	100	0	462	4	6	1	5
Attwood[35]	1992	UK	26	46	70	NA	NA	NA	100	0	90	1	NA	NA	NA
Ortiz[36]	1996	Spain	27	74	40	85	0	NA	100	0	127	1	NA	NA	NA
Wright[37]	1996	UK	166	65	58.5	NA	NA	NA	100	0	461	6	NA	NA	NA
Komorowski[38]	1996	USA	14	79	NA	79	7	NA	93	7	70	2	NA	NA	NA
Sharma[39]	1997	USA	32	98	63.1	91	5	0	0	100	99	1	NA	NA	NA
Younes[40]	1997	USA	61	NA	NA	100	25	NA	100	0	201	5	NA	NA	NA
Katz[41]	1998	USA	102	83	63	100	5	NA	100	0	563	3	19	2	17
Streitz[42]	1998	USA	136	NA	NA	NA	NA	NA	100	0	510	7	NA	NA	NA
Schoenfeld[43]	1998	USA	123	NA	NA	NA	0	NA	54	46	323	2	NA	NA	NA
Teodori[44]	1998	Italy	30	60	NA	100	NA	NA	100	0	350	4	NA	NA	NA
Wilkinson[45]	1999	UK	12	NA	NA	NA	1	NA	100	0	57	0	NA	NA	NA
MacDonald[46]	2000	UK	143	60	57	NA	0	NA	100	0	629	5	33	3	30
Reid[47]	2000	USA	327	81	62	100	122	76	100	0	979	9	NA	NA	NA
Srinivasan[48]	2000	USA	9	89	60	100	3	1	89	0	36	0	NA	NA	NA
Eckardt[49]	2000	UK	357	58	63	86	NA	NA	NA	NA	594	2	NA	NA	NA
Bani Hani [71]	2001	Germany	60	NA	61	0	0	NA	100	0	1293	12	11	0	11
Conio[50]	2001	USA	154	70	62.3	100	NA	NA	76	24	585	4	35	1	34
Rana[51]	2001	UK	44	73	58	68	NA	NA	100	0	418	2	20	2	18
Fitzgerald[52]	2001	UK	96	NA	65	71	6	NA	100	0	375	0	NA	NA	NA
Spechler[53]	2001	USA	108	NA	NA	100	NA	NA	100	0	1037	4	NA	NA	NA
Conio[11]	2003	Italy	166	81	59.9	100	16	NA	64	36	1100	5	18	3	15
Parilla[54]	2003	Spain	43	77	NA	100	3	NA	100	0	258	0	NA	NA	NA
Murray[55]	2003	UK	2969	57	NA	56	171	19	100	0	11068	29	NA	NA	NA

Table 1. Continued

First author	Year	Geography	Total no. under FU	Male (%)	Mean age (yrs)	SIM pos (%)	Baseline LGD (n)	Baseline HGD (n)	LSBE (%)	SSBE (%)	Pyrs of FU	Incident EAC (n)	Tot. mort. (n)	Due to EAC (n)	Due to other causes (n)
Anderson[20]	2003	UK	2373	58	58.2	54	NA	NA	100	0	7413	NA	253	12	241
Hurschler[56]	2003	Switzerland	207	NA	64.4	45	19	NA	100	0	966	10	NA	NA	NA
Hillman[57]	2003	Australia	353	71	59.2	100	50	NA	100	0	1588	9	NA	NA	NA
Hage[12]	2004	Netherlands	105	55	63.4	100	11	0	100	0	1329	6	72	4	68
Solaymani[58]	2004	UK	1656	61.6	63.6	NA	NA	NA	100	0	2615	13	111	13	98
Basu[59]	2004	UK	138	74	62.1	0	3	NA	88	12	405	1	NA	NA	NA
Meining[60]	2004	Germany	148	NA	NA	67	NA	NA	100	0	376	0	NA	NA	NA
Aldulaimi[15]	2005	UK	126	76	63	100	NA	NA	100	0	338	12	NA	NA	NA
Dulai[61]	2005	USA	575	99	60	100	134	NA	100	0	2775	2	164	3	161
Murphy[62]	2005	UK	178	71	57	100	33	NA	81	19	613	3	NA	NA	NA
Oberg[63]	2005	Sweden	140	74	57.3	100	NA	NA	100	0	946	3	NA	NA	NA
Gladman[64]	2006	UK	195	55	62.9	100	NA	NA	90	10	1068	4	21	1	20
Vieth[65]	2006	Germany	748	68	62.6	100	19	10	42	33	4875	15	NA	NA	NA
Sharma[66]	2006	USA	618	NA	60.9	100	101	NA	100	0	2546	12	NA	NA	NA
Cook[17]	2007	UK	502	55	58.8	86	NA	NA	100	0	5247	14	246	13	233
Olithselvan[67]	2007	UK	121	70	60.2	NA	NA	NA	100	0	424	2	NA	NA	NA
Gatenby[68]	2008	UK	807	NA	NA	NA	NA	NA	100	0	3912	23	NA	NA	NA
Musana[69]	2008	USA	216	76	62	100	45	7	52	25	691	4	NA	NA	NA
Moayyedi[18]	2008	UK	1272	63	66.6	100	NA	NA	100	0	5705	47	245	25	220
Martinek[70]	2008	Czech. Rep	135	76	59.4	100	NA	NA	36	64	700	2	NA	NA	NA

LSBE, long segment Barrett's esophagus; SSBE, short segment Barrett's esophagus; SIM, specialized intestinal metaplasia; LGD, low-grade dysplasia; HGD, high-grade dysplasia; FU, follow-up; pos, positive; tot. mort., total mortality; UK, United Kingdom; USA, United States of America; NA, non-applicable (data not available)

Sex ratio was reported in 37 studies and the overall male proportion was 64%.[2, 11, 12, 14, 15, 17, 18, 20, 29, 33-39, 41, 44, 46-49, 51, 54, 55, 57-59, 61-65, 67, 69, 70] Initial Barrett length was reported in 23 studies, rendering a mean length of 5.3 cm (range 1.5-8.1 cm).[11, 12, 31, 34-36, 39, 41, 43, 46, 48-52, 54, 59, 62, 63, 67, 69, 70, 72] In 49 studies, a length of 3 cm was used as a cut-off to classify patients as having LSBE or SSBE. Thirty-seven studies only included patients with LSBE which accounted for 13,948 patients. One study only included patients with SSBE (n=32). Eleven studies included both LSBE and SSBE patients (n= 1,229 and n=608 respectively). The definition of BE showed variation between studies. In 21 studies it was defined as SIM-positive[11, 12, 18, 29, 37, 39, 40, 43, 44, 48, 50, 52, 57, 59, 61-64, 66, 69, 70], in 6 studies as SIM-positive and CLE[15, 17, 20, 53-55], in 18 studies as CLE or SIM only[2, 14, 30-36, 41, 45-47, 49, 51, 67, 68, 72], and in 6 studies the definition of BE was unclear.[38, 42, 56, 58, 60, 65] In total, 9,897 (78%) patients were SIM-positive. Presence of baseline LGD was reported in 30 studies, with an overall prevalence of 11%.[2, 11, 12, 29, 30, 32-34, 36, 38-41, 43, 45-48, 52, 54-57, 59, 61, 62, 65, 66, 69, 71] Baseline HGD was reported in ten studies and could not be excluded from the baseline analysis. [2, 12, 29, 32, 33, 39, 47, 48, 55, 65, 69] The overall baseline prevalence of HGD was 3%.

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

Variable	Number of studies	Cumulative number of patients	Number of patients with selected variable	Overall percentage
Males	37	13,930	8,904	64
SIM positive	35	12,641	9,897	78.3
Baseline LGD	26	7,539	860	11.4
Baseline HGD	8	4,505	127	2.8
LSBE	11	1,837	1,229	67
SSBE			608	33
Only LSBE	37	13,948	13,948	100
Only SSBE	1	32	32	100

LSBE, long segment Barrett's esophagus; SSBE, short segment Barrett's esophagus; SIM, specialized intestinal metaplasia; LGD, low-grade dysplasia; HGD, high-grade dysplasia

Incidence of EAC

Fifty studies reported the incidence of EAC and were used in the analysis (Table 1). In total, these studies included 14,109 patients followed for 61,804 person-years. During this follow-up 344 incident EACs developed. A random effects models produced a pooled estimate for EAC incidence in BE of 6.3/1,000 pyrs (95%CI: 4.7-8.4) (Figure 1). There was however considerable heterogeneity in these incidence rates (χ^2=238.2; df=49; p<0.001; I^2=79%).

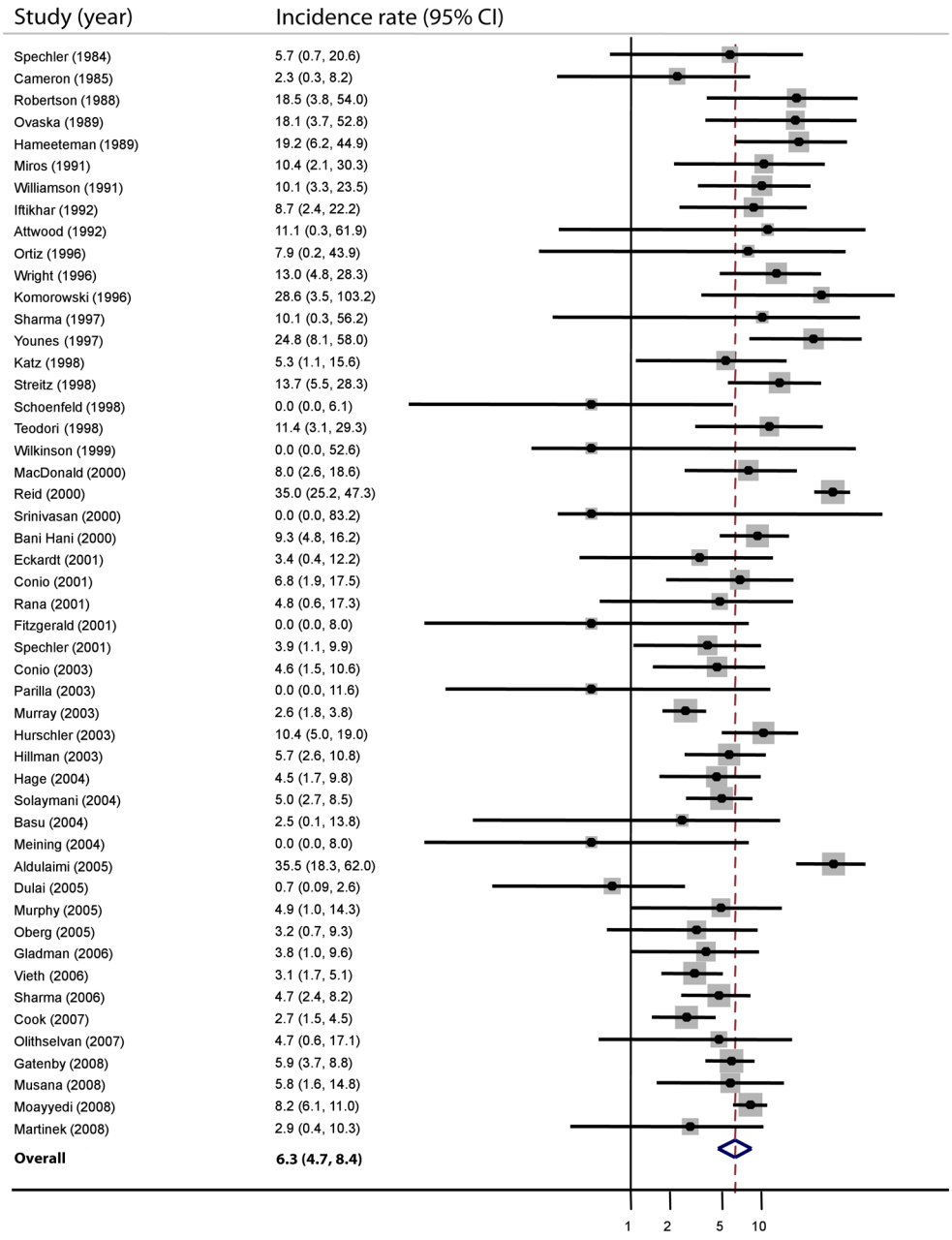

Figure 1. Forrest plot showing the overall incidence of EAC in 50 studies. The cancer incidence rate is a log scale on the x-axis (1, 2, 5 denote 1/1,000, 2/1,000 and 5/1,000 person-years of follow-up, respectively)

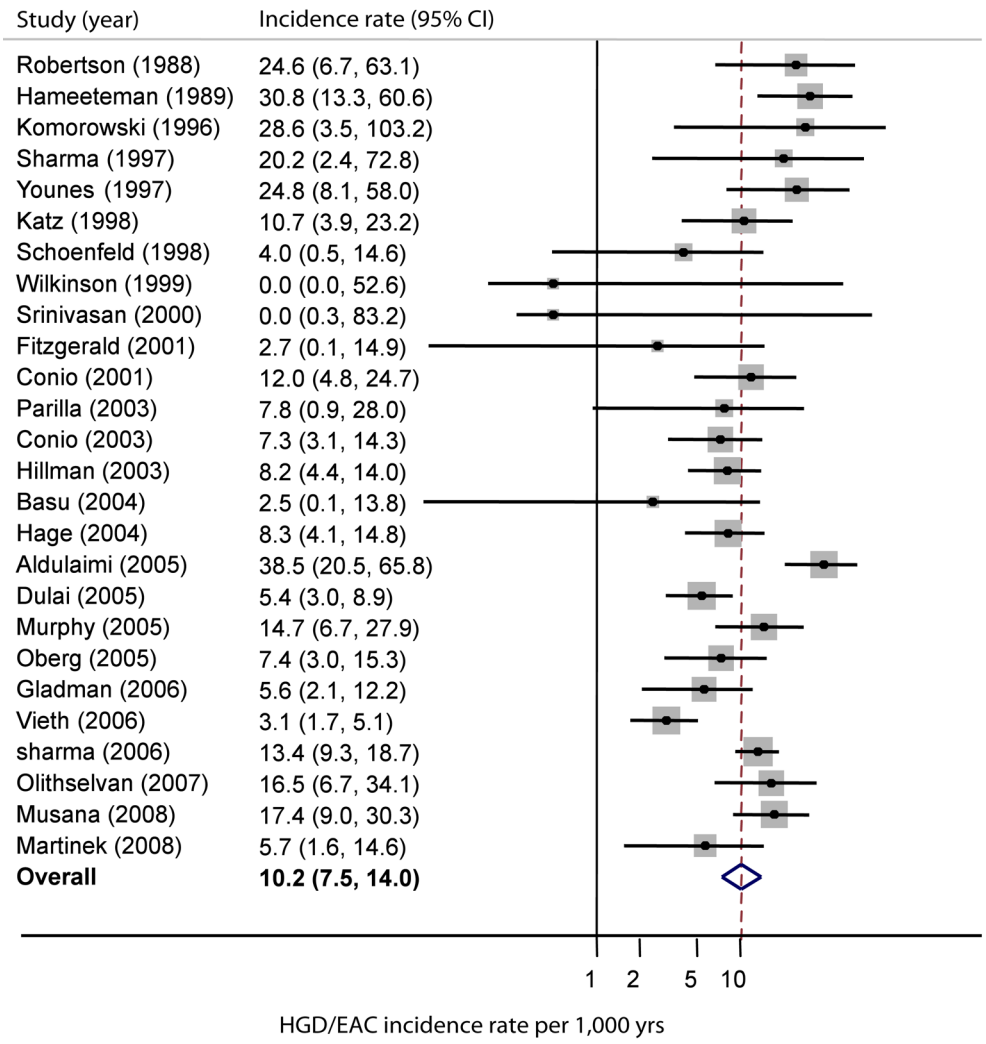

Figure 2. Forrest plot showing the overall incidence of HGD and EAC in 26 studies. The HGD/EAC incidence rate is a log scale on the x-axis (1, 2, 5 denote 1/1,000, 2/1,000 and 5/1,000 person-years of follow-up, respectively)

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

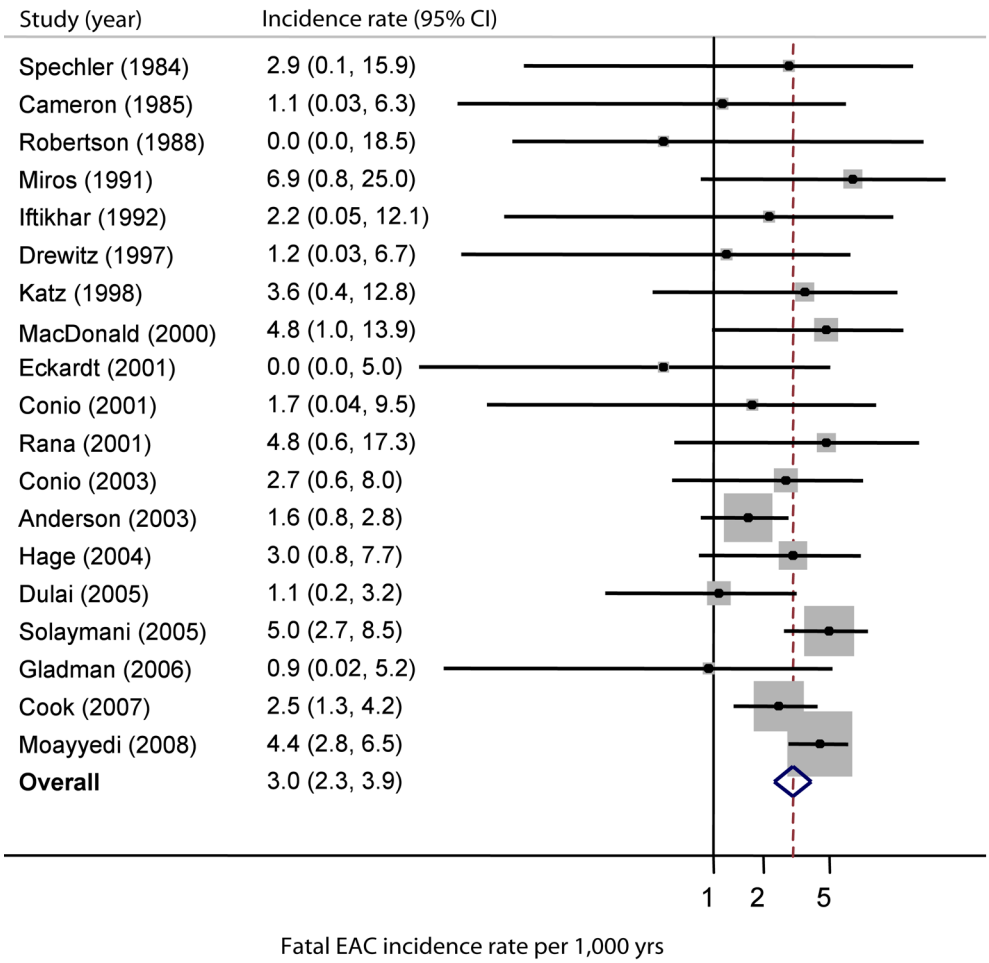

Figure 3. Forrest plot showing the overall incidence of fatal EAC in 19 studies. The fatal EAC incidence rate is a log scale on the x-axis (1, 2, 5 denote 1/1,000, 2/1,000 and 5/1,000 person-years of follow-up, respectively)

Exclusion of studies in which cases with HGD could not be separated from the study cohorts showed a pooled EAC estimate of 6.3/1,000 pyrs (95%CI: 4.9-8.2). As expected, considerable heterogeneity in these incidence rates was present (χ^2=98; df=39; p<0.001; I^2=60%). The pooled incidence rate of EAC in the analysis including the aforementioned 10 studies with HGD patients was similar, except for the fact that the heterogeneity was much larger in those fifty studies than in the forty used in the sensitivity analysis (50 studies I^2=79% vs. 40 studies I^2=60%).

Incidence of HGD and EAC

Twenty-six studies reported both on the incidence of HGD and EAC in their BE patients (Table 1).[2, 11, 12, 15, 30, 38-41, 45, 48, 50, 52, 54, 57, 59, 61-67, 69, 70, 72] In total, these studies included 4,528 patients followed for 22,559 pyrs, with 103 incident cases of EAC and 91 incident cases of HGD during follow-up. The pooled estimate of incidence of both EAC and HGD combined was 10.2/1,000 pyrs (95%CI: 7.5-14.0). Again, there was marked evidence of heterogeneity (χ^2=83; df= 25, p<0.001; I^2=70%) (Figure 2).

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

Mortality due to EAC

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

Cause-specific mortality

In 17 studies the total number of patients who died during surveillance was reported.[11, 12, 14, 17, 18, 20, 29, 30, 32, 34, 41, 46, 49-51, 61, 73] Only, 12 studies provided the cause-specific mortality.[11, 12, 14, 17, 18, 20, 29, 32, 46, 49, 50, 73] These studies included 4,207 patients followed for 24,959 pyrs, with 921 deaths. Sixty-four of 921 deaths (7%) were due to EAC and 857 (93%) due to other causes. The pooled estimate of the mortality rate due to other causes than EAC was 37.1/1,000 pyrs (95%CI: 31.6-43.6), with evidence of large heterogeneity (χ^2= 91.7; df =17; p<0.001; I^2= 82%). Figure 4 shows the cause-specific mortality in BE patients. Cardiovascular disease was the most common cause of death, with 320 deaths (35%) in patients with BE.

Publication bias

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

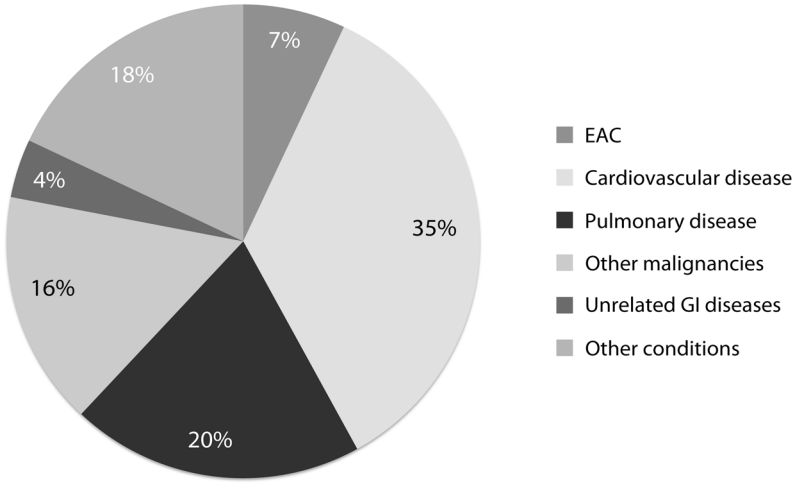

Figure 4. Mortality in BE: causes of death in patients with Barrett's esophagus

DISCUSSION

Both the incidence rate of progression to EAC and mortality due to EAC are critical factors for the cost-effectiveness of surveillance.[13, 19] Our meta-analysis showed that the overall estimate of the incidence of EAC in patients with BE was 6.3 cases per 1,000 pyrs of follow-up and that the overall incidence of EAC and HGD combined was 10.2/1,000 pyrs, which corresponded to an annual risk of 0.6% and 1.0%, respectively. Furthermore, the overall estimate of mortality due to EAC in patients with BE was 3.0/1,000 pyrs of follow-up. This is low, as expected, and correlates to one fatal case of EAC per 333 pyrs of follow-up. The mortality rate due to other causes than EAC was 12-fold higher with an estimate of 37 deaths per 1,000 pyrs of follow-up, as compared to the mortality rate due to EAC.

So far, four systematic reviews have been published on esophageal cancer risk in patients with BE.[16, 21-23] However, none of these estimated mortality rates in BE patients and two of them did not use HGD as an outcome.[16, 23] The most recent review included publications up to 2006.[21] Our review on EAC incidence was an update of that review with inclusion of studies up to October 2008. Our findings are in agreement with those reviews, reporting annual EAC risks ranging from 0.5% to 0.7%.[16, 21-23] In addition, two studies showed a decline in EAC incidence to 5/1,000 pyrs[22] and 4.4/1,000 pyrs[21] when small studies were excluded from the analysis, as was also the case in our study. The presence of geographic variation in BE cancer risk has previously been suggested by others.[5, 75]

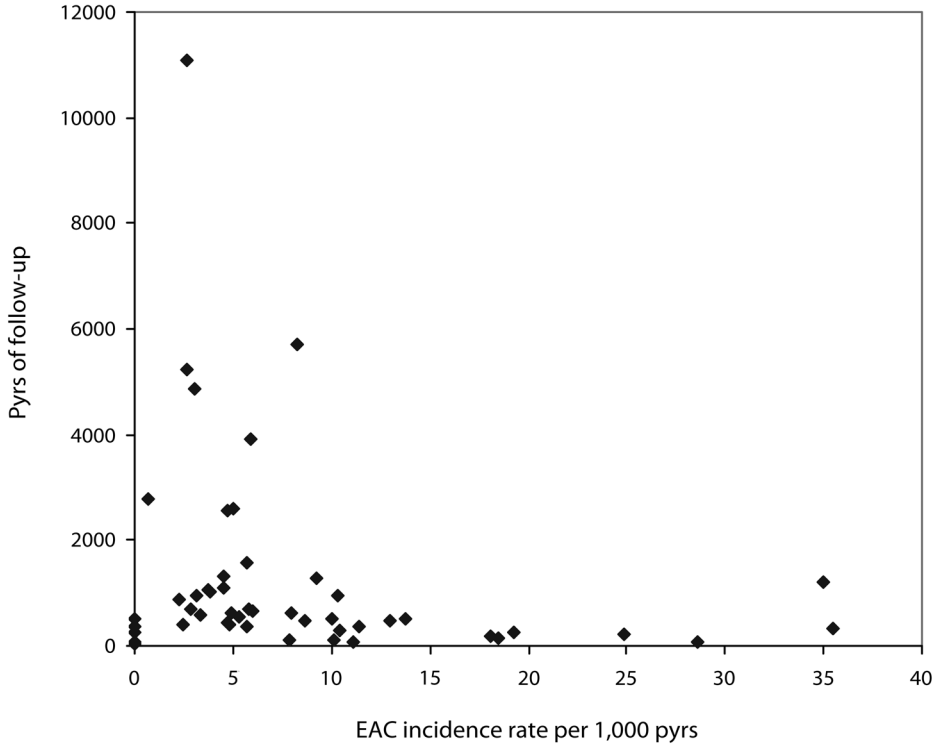

Figure 5. Funnel plot of EAC incidence rate against person-years of follow-up

There were very small differences in EAC incidence between different geographic regions, with only a slightly higher EAC incidence in the U.S. and U.K. compared to other European countries, which is in line with other studies.[21, 22]

To our knowledge, this is the first systematic review analyzing studies that report on mortality rates in patients with BE. Although the risk of EAC is clearly elevated in BE patients as compared to the general population, the majority will not develop EAC. Moreover, it has been suggested that few will die from it.[20, 74] The overall pooled estimate of fatal EAC in our review was 3.0 per 1,000 pyrs which corresponded to an annual risk of 0.3%. As there was no evidence for heterogeneity in this analysis, this is a reliable estimate of the mortality rate due to EAC for patients with BE under surveillance. When examining cause-specific mortality in BE patients, only 7% of the total number of patients died from EAC and 93% died due to other causes. Cardiovascular disease (including stroke) accounted for 34% of the total number of patients who died, followed by 20% due to pulmonary disease and 16% due to other malignancies. This emphasizes that EAC mortality in patients with BE

under surveillance is relatively low. From our analysis, we can only speculate whether the natural course of EAC in patients with BE is slow, or that other explanations for the observed low mortality are more important. One explanation could be length time bias. Another explanation could be that further progression to invasive EAC was prevented because early endoscopic or surgical treatment was performed. Unfortunately we lack exact data on the magnitude of the preventive effect of surveillance on EAC-related mortality. To our knowledge there are no randomized controlled trials comparing surveillance with no surveillance in BE patients. An alternative means to approach this issue is to look at series reporting on EAC-related mortality in BE patients with no surveillance. A study from our own center[3] reported a low incidence of EAC-related mortality in BE patients not under surveillance. Even if we assume that surveillance of BE patients leads to a complete prevention of EAC-related mortality, the impact of surveillance on overall mortality can not be larger than the total cumulative incidence of HGD and EAC in these patients. When we note that the overall incidence of these lesions is limited, this provides information on the maximal effect of surveillance, and thus also provides a lower border of cost-effectiveness estimates. A truly low risk of death due to EAC would undermine the cost-effectiveness of generalized BE surveillance.

The incidence of EAC estimated in this meta-analysis approximates the incidences used in published cost-effectiveness analyses on BE surveillance[13, 19] and confirms that the benefit of generalized BE surveillance is questionable. Our findings support the search for valid risk stratification tools to identify the minority of patients who are likely to benefit from surveillance.

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

Marked heterogeneity was present in the analyses on EAC incidence and the combined HGD/EAC incidence. This could not be clearly explained by publication bias, but might be due to differences in cohort compositions regarding age, gender and period of inclusion or differences in surveillance endoscopies and biopsy protocols. Another explanation could

be that in small studies selected patient groups with a high cancer risk were included. Also, not all EACs may have been identified in large studies. In this meta-analysis the results of assessment of publication bias were borderline significant. This implies that the EAC incidence may be lower than previously thought, which would imply that the benefit of costly surveillance programs is overemphasized. Mixing of heterogeneous estimates from large and small studies also resulted in inflated estimates of cancer risk.

Several limitations of our study need to be considered. Firstly, we did not include abstracts or reports in foreign languages in this analysis for reason of inability of full data extraction. As the total amount of extra studies was minimal (n=4), we do not think that exclusion of these studies from the analysis significantly altered our results. Secondly, as the majority of included studies did not accurately report on demographic and clinical patient characteristics, we were unable to adjust for confounding variables. Thirdly, the number of studies included in our mortality-analysis was rather small, even though we thoroughly searched the published literature to find all studies which reported on mortality due to EAC and all-cause mortality in BE patients. This supports the notion that more research is needed on this important issue. With regard to our analysis on mortality rates in BE patients, we were unable to compare these incidences with overall mortality rates in the general population. This limits the interpretation of the magnitude of this risk. Finally, causes of death due to other causes than EAC could have been misclassified, as ICD-classes or death certificates were not used in all studies reporting mortality.

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

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Chapter 3

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Aneuploidy and overexpression of Ki67 and p53 as markers for neoplastic progression in Barrett's esophagus: a case-control study

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ABSTRACT

Background: Surveillance of patients with Barrett's esophagus (BE) aims at early detection and treatment of neoplastic changes, particularly esophageal adenocarcinoma (EAC). Histological evaluation of biopsies has its limitations and biomarkers may improve early identification of BE patients at risk for progression to EAC. The aim of this study was to determine the predictive value of p53, Ki67 and aneuploidy as markers of neoplastic progression in BE.

Methods: Twenty-seven patients with BE with histologically proven progression to high-grade dysplasia (HGD) or EAC (cases) and 27 BE patients without progression (controls) were selected, matched for age, gender, length of follow-up. Dysplasia grade was determined in 212 biopsies obtained during surveillance endoscopies from cases and in 231 biopsies from controls. DNA ploidy status was determined by flow cytometry, whereas Ki67 and p53 expression was determined by immunohistochemistry. Hazard ratios (HR) were calculated by Cox regression adjusted for potentially confounding variables.

Results: Univariate analysis revealed that low-grade dysplasia (LGD) increased the risk of developing HGD/EAC compared with no dysplasia (HR 3.6; 95% CI: 1.6–8.1). Aneuploidy (HR 3.5; 95% CI 1.3-9.4), strong Ki67 overexpression (HR 5.2; 95% CI: 1.5-17.6) and moderate p53 overexpression (HR 6.5; 95% CI: 2.5-17.1) were also associated with an increased risk of developing HGD/EAC, independent from the histology result. Multivariable analysis showed that in the presence of LGD, p53 overexpression, and to a lesser extent, Ki67 overexpression remained important risk factors for neoplastic progression, whereas aneuploidy was no longer predictive.

Conclusion: P53 overexpression and, to a lesser extent, Ki67 overexpression could predict neoplastic progression in BE irrespective of the histology result. These markers may be useful to identify patients at an increased risk of developing EAC, either alone or used as panel.

INTRODUCTION

Barrett's esophagus (BE) is primarily caused by chronic gastroesophageal reflux (GERD) and characterized by the replacement of the normal stratified squamous epithelium lining the distal esophagus by columnar epithelium with specialized intestinal metaplasia (IM) containing goblet cells.[1, 2] The prevalence of both BE and GERD has increased in recent years.3 BE is a premalignant disorder associated with the development of esophageal adenocarcinoma (EAC). Over the past three decades, the incidence of EAC has dramatically increased in the Western world.[4, 5] Recently, the annual risk of developing EAC in patients with BE was estimated to be approximately 0.5%.[6] EAC carries a poor prognosis with a high mortality rate and the majority of patients with this malignancy presents at an advanced and often incurable stage.[7, 8] It is known that the development of EAC is a stepwise process that leads from IM with no dysplasia (ND), to low-grade dysplasia (LGD), high-grade dysplasia (HGD), and finally EAC.[9]

The American College of Gastroenterology (ACG) recommends surveillance of all BE patients by performing surveillance endoscopy with extensive biopsy sampling.[10] The goal of surveillance is to detect early neoplastic changes in order to start treatment before invasive EAC develops. However, histological evaluation of biopsies obtained during endoscopy has its limitations. Due to sampling error and substantial interobserver variation, it misses tools to unambiguously identify the subset of patients who require more frequent surveillance.[11-13] Therefore, there is a need for objective biomarkers to use in combination with histology to stratify individual patients according to the risk for progression to cancer.

Several studies have reported that DNA ploidy as determined by flow cytometry can be such an effective addition to histology for differentiating BE patients into those with a low or a high risk of developing EAC.[14, 15] Reid et al. have shown that flow cytometric abnormalities correlate with a histological diagnosis of dysplasia and EAC in BE.[15-17] Others have also demonstrated that aneuploidy measured by FC is able to predict progression, and thus may be an objective aid in identifying patients at increased risk.[17, 18] Another potential biomarker for neoplastic progression in BE is Ki67. This protein is exclusively present in proliferating cells (G1-, S-, G2- and M-phase) and is absent in resting cells.[19] Finally, the tumor suppressor gene p53 has been suggested to be a prognostic marker in BE as well.[20] P53 is involved in controlling cell proliferation and is able to inhibit cell transformation.[21] A stepwise overexpression of p53 has been demonstrated in the multistep process of esophageal carcinogenesis.[22] Overexpression of p53 and Ki67 in BE correlate with severity of dysplasia.[23]

In a retrospective, longitudinal study, we recently reported that the grade of dysplasia in BE correlated with aneuploidy, as well as with Ki67 and p53 overexpression. In that study, we determined ploidy status and expression of Ki67 and p53 in BE patients who progressed to HGD or EAC.[24] In the present study, we aimed to validate these findings by comparing the same group of BE patients who progressed to HGD or EAC with a matched group of BE patients who did not develop neoplastic progression after a similar period of follow-up.

METHODS

Patient selection

This retrospective case-control study was conducted in the BE patient cohort of the Erasmus MC-University Medical Center in Rotterdam. Cases and controls were selected from the same cohort of 355 BE patients who were under surveillance in our center between January 1994 and December 2006. Cases and controls were selected from the same surveillance cohort. Cases were BE patients that developed progression to HGD or EAC during surveillance. Controls were those without progression to HGD or EAC during a comparable surveillance period. Matching of cases and controls was done on a 1:1 basis. This meant that we identified one control per case, first with regard to gender and second with regard to age. Our aim was that the maximum age difference would be 3 years per case-control pair, but we did not succeed in some case-control pairs, for example the youngest pair. When multiple controls were available for one case, we selected the control with the longest surveillance follow-up time. Finally, the results were analyzed after adjusting for age and gender, thus taking any differences between cases and controls into account.

All available paraffin blocks with biopsies taken at different levels from the columnar-lined esophagus (CLE) during previous surveillance endoscopies between 1987 up to 2006 were retrieved and analyzed as described below. Since 2000, we are using a standardized biopsy protocol (four-quadrant biopsies every 2 cm). Before 2000 the biopsy protocol was not standardized.

Cases were determined to have HGD or EAC when this was present in biopsies obtained in the last follow-up visit. Biopsies prior to this time point could only show BE with or without LGD. Controls were defined as BE with or without LGD during follow-up.

Histology and immunohistochemistry

Three consecutive sections of 4 µm each from every available biopsy set were cut and used for stainings. The first slide was used for hematoxylin and eosin (H&E) staining and examined for the presence of BE as defined by presence of IM containing goblet cells.[1] H&E-slides were evaluated by an expert gastrointestinal pathologist (HvD). In line with the definition of BE according to the ACG guidelines, blocks with biopsies with CLE but without IM were excluded from this study. Dysplasia in BE was graded according to the Consensus Criteria of 1988, with adjustments as proposed in 2001.[11, 13]

The next two slides were stained by the streptavidin-biotin-peroxidase method using the primary antibody anti-human p53 protein (Clone DO-7, Dako) to assess p53 protein expression, and the primary antibody anti-human Ki67 antigen (clone MIB-1, Dako) to estimate the proliferation rate by labeling Ki67 antigen, as previously described.[24]

All slides were evaluated in a blinded fashion for nuclear Ki67 and p53 staining by two independent investigators (MS, PvS). Only moderate to intense brown (p53) or red (Ki67) nuclear staining was considered positive. The grading of positive cells was performed as previously described.[24, 25] For p53, <15% positive nuclei was regarded as normal expression (grade 0), 15-40% as moderate overexpression (grade 1) and >40% as strong overexpression (grade 2). For Ki67, the percentage of positive nuclei was determined in longitudinally sectioned crypts and villi, and a percentage of positive cells <20% was regarded as normal expression (grade 0), 20-50% as moderate overexpression (grade 1), and >50% as strong overexpression (grade 2).

Flow Cytometry

Biopsy samples from the paraffin blocks were processed for flow cytometry as previously described.[24] The DNA content of the isolated nuclei was then analyzed using a 4-color flow cytometer (FACScalibur, Becton Dickinson, San Jose, CA). Data analysis was performed using CellQuest software (version 2.0.2; Becton Dickinson).

The obtained histograms were independently interpreted by two investigators (MK, MS) who were blinded to the histological and immunohistochemical data of the samples. In line with previous studies, aneuploidy was defined as the presence of a second discrete peak on the histogram at >2.7N containing at least 2.5% of the nuclei. Similarly, tetraploidy was defined as the presence of a 4N fraction (range: 3.85N-4.1N) consisting >6% of the nuclei, as described previously.[18, 26] Finally, diploidy (normal DNA content) was defined as the presence of a large peak at 2N containing the majority of nuclei, while the remaining nuclei did not fulfill the criteria of aneuploidy or tetraploidy.

Statistical analysis

The variables age, gender, length of surveillance, number of endoscopies and number of biopsies were expressed as means with standard deviation (SD). T-tests and χ^2 - tests were used to assess differences between cases and controls.

A Cox proportional hazard regression model with HGD or EAC as outcome was used. For each patient the beginning of the follow-up period was the start of surveillance and it ended at the last endoscopy visit. Time-dependent covariates were included to calculate hazard ratios (HR) with 95% confidence intervals (CI) with adjustment for potentially confounding variables, such as sex and age. The fact that multiple biopsies per each patient were obtained, was considered as updated values in the time-dependent covariates. Subsequently, Cox regression analysis was performed with adjustment for histology results. This addressed the predictive power of the biomarkers in addition to histology. HGD and/or EAC outcome and censoring times were defined relative to the first endoscopy in this study. At each point in time, we used the result that was most different from normal among the biopsy samples.

We also examined a simple sum score for the predictive value of the prognostic factors considered (biomarkers and presence of LGD) for the development of neoplastic progression. When no dysplasia or no abnormal biomarkers were present, the score was set at 0. A patient could have a maximum of four abnormal results, or a maximum score of 4.

A p-value <0.05 was considered statistically significant, and a p-value between 0.05 and 0.10 as indicating a trend (borderline significant). Smoothing splines were used to visualize patterns over time. By using 2000 bootstrap samples, we calculated the corresponding 95%CI for four points in time before case definition. Statistical analyses were conducted using SPSS (version 11.0, Chicago, Illinois, USA) and R software (R Foundation for Statistical Computing, version 2.6.2).

RESULTS

Patient characteristics

Cases included 27 patients (24 males) with a mean (± SD) age of 59 ± 10 years (range 37-76 years) at the time of BE diagnosis. Controls included 27 patients (20 males) with a mean age of 56.2 ± 11.2 years (range 29-74 years) at the time of BE diagnosis (age and gender: p=NS). Mean duration of surveillance was similar in both groups (cases: 6.9 ± 4.2 years vs. controls: 7.9 ± 5.1 years, p=NS). A total of 167 upper GI endoscopies (mean: 6 ±

Table 1. Characteristics of patients with Barrett's esophagus

Variables	Cases (n = 27)		Controls (n = 27)		p†
	mean	range	mean	range	
Age (years)	58.8	36.6 - 76.2	56 .2	29.6-74.2	0.36
Gender (% male)	89		74		0.16
Follow up period (years)	6.9	0.4 - 16.3	7.9	1.9-18.9	0.41
Number of endoscopies	6.2	2 - 18	6.1	3-15	0.93
Number of biopsies during follow-up	7.9	2-25	8.6	3-20	0.67

†p-value from χ^2-tests/t-tests

4, range 2-18 per patient) were performed in cases in the period from BE diagnosis to the end of follow-up. This yielded 212 paraffin blocks containing biopsies from (CLE) with a histological diagnosis of BE. In controls, a total of 165 upper GI endoscopies (mean: 6 ± 3, range 3-15 per patient) were performed, which yielded 231 paraffin blocks with biopsies from CLE containing a histological diagnosis of BE (Table 1).

Histology

Biopsies from cases obtained during surveillance showed no dysplasia (ND) in 99 (47%), LGD in 69 (32%), HGD in 31 (15%) and EAC in 14 (6%) of 213 samples. In biopsy specimens from controls, ND was observed in 220 (95%) and LGD in 11 (5%) of 231 samples. During follow-up, eleven control patients showed LGD once. Prior to neoplastic progression, the fraction of samples with LGD increased in cases, whereas this was not observed in controls over time (Figure 1a).

Ki67 expression

In cases, normal Ki67 expression (grade 0) was found in 65/211 (31%), moderate (grade 1) overexpression in 81 (38%) and strong (grade 2) overexpression in 65 (31%) samples. In controls, normal (grade 0) expression was observed in 156/228 (68%), moderate (grade 1) overexpression in 61 (27%) and strong (grade 2) overexpression in 11 (5%) samples. Two samples from cases and three from controls could not be evaluated because not enough tissue was available.

Per endoscopy visit of the cases, normal Ki67 expression was present in 51/167 (30%), moderate Ki67 overexpression in 49 (30%) and strong overexpression in 49 (30%) follow-up visits. In controls, normal Ki67 expression was present in 98/164 (60%), moderate Ki67 overexpression in 48 (30%) and strong overexpression in 8 (5%) follow-up visits.

A gradual increase in the proportion of samples with Ki67 overexpression was seen

in cases and controls; however, this increase was more pronounced in cases (Figure 1b).

P53 expression

In cases, normal p53 expression (grade 0) was found in 96 (46%), moderate (grade 1) overexpression in 29 (14%) and strong (grade 2) overexpression in 85 (40%) of 210 samples. In controls, these fraction were normal (grade 0) in 217/226 (96%), moderate (grade 1) in 8/226 (3%) and strong (grade 2) in one sample (0.4%). Three samples from cases and five

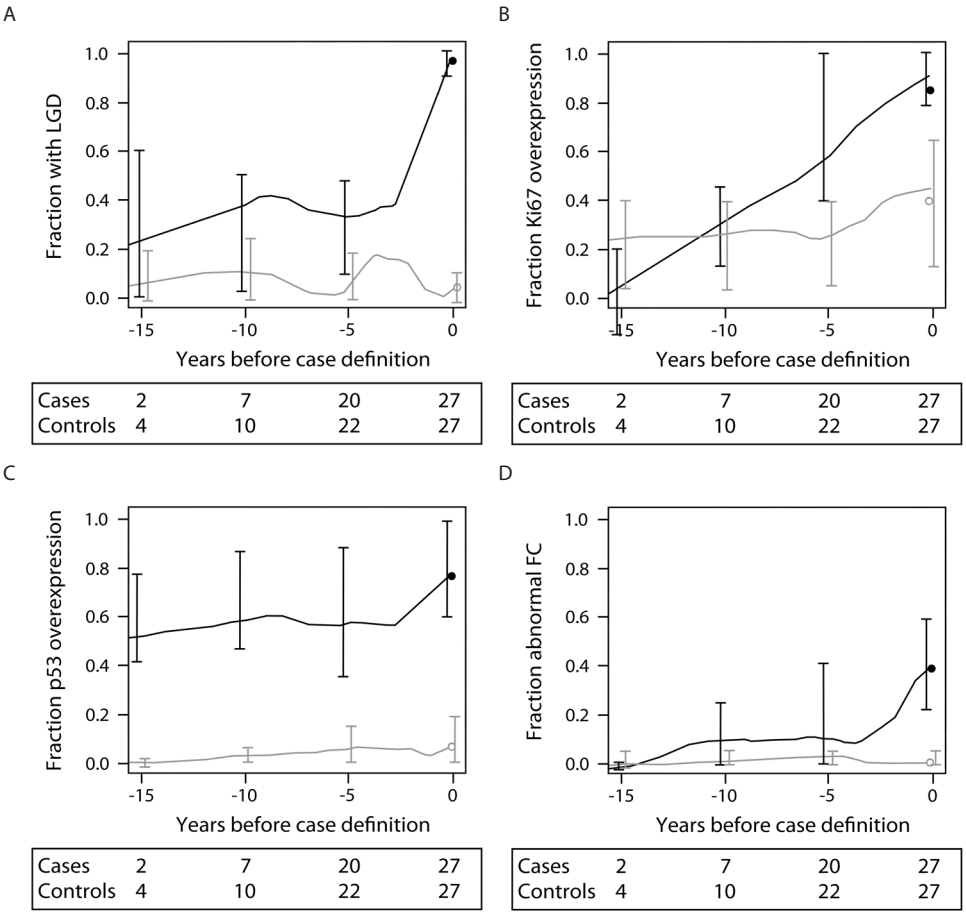

Figure 1. Fraction of patients with Barrett's esophagus with an abnormal result of a biomarker in biopsy samples over time until development of high-grade dysplasia (HGD) or esophageal adenocarcinoma (EAC) in cases and controls (—— cases; —— controls). The numbers under each figure represent the number of patients at that point of time. The black dot and the open dot at time point zero represent the fraction of patients at either time point with progression (cases n=27) or at last follow-up visit without progression (controls n=27). (A) Low-grade dysplasia (LGD) (B) Ki67 overexpression (C) p53 overexpression and (D) abnormal flow cytometry (FC) results.

from controls could not be evaluated because not enough tissue was available.

Per endoscopy visit of the cases, normal p53 expression was present in 56/167 (33%), moderate p53 overexpression in 22 (13%) and strong p53 overexpression in 70 (42%) follow-up visits. In controls, normal p53 expression was present in 144/164 (88%), moderate p53 overexpression in 7 (4%) and strong p53 overexpression in 1 (0.6%) follow-up visits.

No increase in the proportion of samples with p53 overexpression was seen in controls over time, whereas an increased fraction of p53 overexpression was already present up to 15 years before development of HGD or EAC in cases. This increased fraction of p53 overexpression remained stable in cases (Figure 1c).

DNA ploidy

In cases, a normal diploid DNA content was present in 175/210 (83%) biopsy samples, whereas in 35 (17%) samples aneuploidy or tetraploidy was detected. In controls, a diploid DNA content was found in 217/218 (99%) samples and aneuploidy in only one (1%) sample. Three (1%) samples from the cases and 13 (6%) from the controls could not be evaluated because not enough nuclei could be isolated.

Per endoscopy visit of the cases, a normal diploid DNA content was present in 115/167 (69%) follow-up visits and aneuploidy or tetraploidy was present in 33 (20%) follow-up visits. In controls, a normal diploid DNA content was present in 145/164 (88%) follow-up

Table 2. Predictive value of the different biomarkers for neoplastic progression in Barrett's esophagus according to Cox regression analysis with time-dependent covariates

Variable		Adjusted for age and sex		Adjusted for age, sex and LGD	
		HR (95% CI)†	p	HR (95% CI) ††	p
Histology	ND	1.0	0.002		
	LGD	3.6 (1.6-8.1)			
Flow cytometry	Diploidy	1.0	0.014	1.0	0.12
	Aneuploidy	3.5 (1.3-9.4)		2.3 (0.8-6.3)	
Ki67	0-20%	1.0	0.029	1.0	0.086
	20-50%	1.7 (0.7-4.0)		2.2 (0.9-5.1)	
	>50%	5.2 (1.5-17.6)		3.2 (0.9-11.2)	
p53	0-15%	1.0	<0.001	1.0	0.004
	15-40%	6.5 (2.5-17.1)		5.4 (2.0-14.5)	
	>40%	3.2 (1.3-8.2)		1.8 (0.6-5.2)	

HR, hazard ratio; CI, confidence interval; ND, no dysplasia; LGD, low-grade dysplasia

† HR's for histology, flow cytometry, Ki67 and p53 were all adjusted for age and gender

†† HR's for flow cytometry, Ki67 and p53 were all adjusted for age, gender and presence of LGD

visits and aneuploidy or tetraploidy was present in 2 (1%) follow-up visits.

Over time, no increase in the fraction of samples with aberrant FC result was observed in controls. In cases, the proportion of samples with aberrant FC result increased. This increase was most outspoken at the time close to development of HGD or EAC (Figure 1d).

Predictive value of the biomarkers

Adjusted for age and gender, the presence of LGD in BE significantly predicted progression towards HGD or EAC (HR 3.6; 95%CI 1.6-8.1). In addition, all three biomarkers studied, overexpression of p53 and Ki67, and FC abnormalities (aneuploidy and tetraploidy), were also associated with an increased risk of neoplastic progression in BE (Table 2). After adjustment for age, gender and the presence of LGD, overexpression of Ki67 showed a trend towards an association with an increased risk of progression to HGD or EAC, whereas overexpression of p53 was significantly associated with an increased risk of neoplastic progression in BE (Table 2).

The multivariable combination of more than 20% Ki67 overexpression, more than 15% p53 overexpression and the presence of LGD in BE revealed that these three markers were associated with an increased risk of developing HGD or EAC (Table 3).

The median sum of the number of abnormal prognostic factors was 1. In multivariable Cox regression analysis with adjustment for age and gender, the risk of developing neoplastic progression was significantly increased in patients with higher scores. For the scores of 1 to 4, the HR was 3.7, 4.7, 12 and 34, respectively (Table 4).

DISCUSSION

In this case-control study, we evaluated the clinical value of three biomarkers for progression of BE towards HGD or EAC. The selected cases and controls were similar with regard to age, gender and period of surveillance, and all originated from the same patient cohort.

Table 3. Association between Ki67, p53 and histology in biopsies from patients with Barrett's esophagus in multivariable Cox regression analysis with time-dependent covariates

Variables	HR (95%CI)[†]	p
Presence of LGD	2.6 (1.0-6.5)	0.047
> 15% p53 overexpression	2.6 (1.0-6.4)	0.043
> 20% Ki67 overexpression	2.1 (0.9-4.7)	0.084

HR, hazard ratio; CI, confidence interval; LGD, low grade dysplasia

[†] adjusted for age and gender

Table 4. Predictive value of total number of abnormal prognostic characteristics in Barrett's esophagus according to Cox regression analysis with time-dependent covariates

Score	Adjusted for age and gender		
	HR	95% CI	p
0	1.0		<0.001
1	3.7	0.99-14	
2	4.7	1.1-20	
3	12.0	2.5-57	
4	34.3	7.2-163	

HR, hazard ratio; CI, confidence interval
score 1 = one abnormal biomarker or low-grade dysplasia (LGD); score 2 = combination of two abnormal biomarkers or LGD; score 3 = combination of three abnormal biomarkers or LGD; score 4 = combination of four abnormal biomarkers or LGD

Our results showed that when LGD was diagnosed, the risk of neoplastic progression was 3.6 times higher than with a diagnosis of ND (Table 2). Over time, the proportion of samples with a histological diagnosis of LGD increased prior to the development of neoplastic progression in cases. This is in line with the proposed stepwise process of progression from non-dysplastic BE to LGD, HGD and finally EAC.[1, 27, 28] It has been demonstrated that 25% of patients with at least once a diagnosis of LGD will ultimately progress to HGD or EAC.[6] However, other studies showed lower percentages of patients with LGD progressing to HGD or EAC.[29, 30] Nonetheless, the risk of developing neoplastic progression in BE is considered to be relatively low with figures ranging from 2.1% to 7.5% per patient-year of follow-up.[17, 30-32] Therefore, the risk of patients with LGD to develop HGD or cancer needs to be further elucidated and this limits the use of LGD as the sole characteristic predicting progression to HGD or EAC in BE. Moreover, inter- and intraobserver variability in diagnosing the presence and grade of dysplasia in BE is a major problem, particularly for the diagnosis of LGD. This variability is likely to result in misclassifications in BE patients, with consequently over- or underestimating the risk of neoplastic progression. In the event of overdiagnosing LGD, this will lead to follow-up endoscopies at time intervals that are too frequent, while the opposite is true in case of underdiagnosing LGD.[12] This highlights the need for additional biomarkers. Preferably, these biomarkers should have limited or no observer variability to supplement the currently used histological examination.

Overexpression of p53 resulted in a 5-fold increased risk of progression to HGD or EAC, which was independent of the presence of LGD (Table 2). This is in agreement with previously reported findings[33], but in contrast to other studies, in which this was not found[34, 35], or in which high percentages of false negative and false positive tests were

reported.[36] This discrepancy can possibly be explained by variations in endoscopic sampling technique in different studies. We found that the proportion of samples with p53 overexpression was higher in cases than in controls at all time points (Figure 1c), which suggests that p53 overexpression occurs early in the malignant transformation of BE.[33, 37, 38]

Immunohistochemical staining for p53 has some marker-specific drawbacks which are grossly the same in different laboratories and are not dependent on the technique. The antibody directed to p53 not only stains mutant p53 but may also stain wild-type p53. Nevertheless, immunohistochemical staining of p53 is considered to be indicative for the presence of a mutant form of p53. This mutant p53 has a greater half-life (up to 200 min) than intact p53 (15-20 min) and is not degraded in the normal way, which will result in accumulation of nuclear p53 that is detectable by immunohistochemistry.[39, 40] About 30% of mutant p53 may be present without being expressed and this will be undetectable by immunohistochemistry.[41]

Overexpression of Ki67 resulted in a 2- to 3-fold increased risk of progression to HGD or EAC, which was independent of the presence of LGD (Table 2). We observed a linear increase in Ki67 overexpression over time, which was more pronounced in cases than in controls (Figure 1b). Expression of Ki67 has been demonstrated to be increased both in BE samples with LGD and in those with only reactive changes, with levels being higher in the former than in the latter.[22] This suggests that both in cases and in controls Ki67 expression can be increased in response to gastroduodenal reflux into the esophagus, with a further increase if neoplastic changes develop. Other studies have also suggested that Ki67 is a prognostic biomarker for neoplastic progression in BE with an increase in Ki67 overexpression closer to the point in time that either HGD or EAC is detected. These studies have also shown that, in the same way as for the p53 interpretation, the interobserver variation in the interpretation of Ki67 expression was lower compared to the histological interpretation.[23, 24] In addition, Ki67 staining pattern was reported to correlate with the histological presence and degree of dysplasia in BE.[42]

Independent of the presence of LGD, aneuploidy was found to be a predictor for neoplastic progression in BE (Table 2). The fraction of samples with an abnormal FC result in cases increased approximately three years prior to the time of detecting HGD or EAC. This reflects an increased frequency of DNA abnormalities in parallel with the occurrence of dysplastic and/or neoplastic changes in BE.[24, 43, 44] When the histological result, i.e. the presence of LGD, was also taken into account, aneuploidy was no longer of additional value in the prediction of neoplastic progression (Table 2).[24] These findings are however in contrast to other studies in which a predictive value of DNA abnormalities in addition to

histology has been reported.[17, 18, 45] We performed FC on paraffin-embedded tissue, which was available from these patients. Nonetheless, others have convincingly shown that FC results on paraffin-embedded tissue are nowadays comparable to those on fresh material [46, 47], mainly as a result of improvements in the methodology and analysis of FC on this type of material.[44, 48] Another explanation for our contradictory results could be that we analyzed the histograms through visual inspection rather than by using a more objective way such as a mathematical model.[44] Visual interpretation of histograms by at least 2 independent researchers should however be comparable to a more objective method.[17]

In our study, we found that a higher age, male gender, LGD, p53 overexpression, and Ki67 overexpression all were associated with an increased risk of neoplastic progression in BE (Table 3). A combination of these prognostic factors is required to clearly define subgroups of patients at an increased risk. We found that the presence of three or four abnormal prognostic factors clearly predicted the development of HGD or EAC over time with a 12- to 34-fold increased risk, respectively (Table 4). In clinical practice, these patients likely require a more frequent follow-up schedule. Our results also support other studies, in which it has been suggested that a panel of biomarkers is needed to identify BE patients with the highest risk of neoplastic progression.[49, 50] At this moment, however, changing surveillance strategies would be too premature and further validation of these markers in larger, prospective studies is required to confirm our findings and to find whether these and other biomarkers are indeed able to identify high risk BE patients for the development of HGD or EAC. Also, lead time bias has to be taken into account in future research when assessing the performance of these markers.

We found that changes in overexpression occurred both in patients who displayed neoplastic progression and in those without progression. Over time, genetic alterations develop in the Barrett's epithelium, resulting in disruption of biological processes at the cellular level. These alterations may lead to the development of neoplastic progression.[1] Yet, we do not know in which time frame this process of neoplastic progression occurs. This could well explain why some patients did show overexpression but not progressed to HGD or EAC. Still, it is possible that these patients will eventually also develop HGD or EAC. Another explanation for the overlap of overexpression of p53 and Ki67 in cases and controls could be the relatively low sensitivity and specificity of the markers. This also supports the use of a panel of biomarkers to increase the sensitivity as well as the specificity to predict which patient is at high risk of developing HGD or EAC.

To our knowledge, this is the first study investigating the predictive value of a combination of easy to apply biomarkers for the risk of neoplastic progression in a single cohort of BE patients. There are some limitations, as indicated above. Furthermore, we

used a relatively small number of cases and controls. This limits the power of our study, which is reflected in rather large confidence intervals. This may also explain why more than 50% overexpression of Ki67 and more than 40% overexpression of p53 had no predictive value for neoplastic progression in the presence of LGD. Another limitation is the fact that the multivariate model could be overfit and this could have affected our results. However, lower ratios than one predictor for each ten pairs have previously been used in etiologic research and are sometimes needed given the frequency of the condition and event under study and the exploratory character of the research question.[51] Therefore, we accepted to have a slightly lower ratio.

In conclusion, p53 overexpression and Ki67 overexpression predict neoplastic progression in BE irrespective of the histology result. If further studies corroborate our findings, these biomarkers may be used as parts of a risk stratification tool to identify patients at increased risk for developing HGD or EAC.

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Chapter 4

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Predictors for neoplastic progression in patients with Barrett's esophagus: a prospective cohort study

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ABSTRACT

Background and aims: Patients with Barrett's esophagus (BE) have an increased risk of developing esophageal adenocarcinoma (EAC). As the absolute risk remains low, there is a need for predictors of neoplastic progression to tailor more individualized surveillance programs. The aim of this study was to identify such predictors of progression to high-grade dysplasia (HGD) and EAC in patients with BE after 4 years of surveillance and to develop a prediction model based on these factors.

Methods: We included 713 patients with BE (≥2 cm) with no dysplasia (ND) or low-grade dysplasia (LGD) in a multicenter, prospective cohort study. Data on age, gender, BMI, reflux symptoms, tobacco and alcohol use, medication use, upper gastrointestinal endoscopy findings, and histology were prospectively collected. As part of this study, patients with ND underwent surveillance every 2 years, whereas those with LGD were followed on a yearly basis. Log linear regression analysis was performed to identify risk factors associated with the development of HGD or EAC during surveillance.

Results: After 4 years of follow-up, 26/713 (3.4%) patients developed HGD or EAC, with the remaining 687 patients remaining stable with ND or LGD. Multivariable analysis showed that a known duration of BE ≥ 10 years (RR 3.2; 95%CI: 1.3-7.8), length of BE (RR 1.11 per cm increase in length; 95%CI: 1.01-1.2), esophagitis (RR 3.5; 95%CI: 1.3-9.5) and LGD (RR 9.7; 95%CI: 4.4-21.5) were significant predictors of progression to HGD or EAC. In a prediction model, we found that the annual risk of developing HGD or EAC in BE varied between 0.3% and up to 40%. Patients with ND and no other risk factors had the lowest risk of developing HGD or EAC (< 1%), whereas those with LGD and at least one other risk factor had the highest risk of neoplastic progression (18-40%).

Conclusion: In patients with BE, the risk of developing HGD or EAC is predominantly determined by the presence of LGD, a known duration of BE ≥ 10 years, longer length BE and presence of esophagitis. One or combinations of these risk factors are able to identify patients with a low or high risk of neoplastic progression and could therefore be used to individualize surveillance intervals in BE.

INTRODUCTION

In patients with Barrett's esophagus (BE), the normal squamous epithelium is replaced by columnar epithelium harboring goblet cells, i.e., intestinal metaplasia (IM).[1] The major risk factor for the development of BE is chronic gastroesophageal reflux disease (GERD).[2] Both GERD and BE predispose to the development of esophageal adenocarcinoma (EAC).[3-5] This malignancy has a poor prognosis with a 5-yr survival rate of less than 20%.[6] The incidences of BE and EAC have both been rising in the past three decades in Western countries.[7-9] Although the incidence of EAC in patients with BE is higher than in the general population[10], only a minority of BE patients develop EAC with an estimated annual risk of 0.5%.[11-13]

Currently, all patients with BE are advised to undergo endoscopic surveillance with biopsy sampling.[14] Until now, a histologic diagnosis is used to determine the surveillance interval. However, there is no clear documentation of additional factors that could identify the subgroup of patients which will actually progress to high-grade dysplasia (HGD) and EAC. Consequently, the majority of patients with BE will undergo endoscopies that are not really indicated and are a major burden from the perspective of patients and endoscopy capacity, but are also impairing cost-effectiveness of the current surveillance guideline in patients with BE.[15,16]

Previous studies have shown that a hiatal hernia, a long BE segment and low-grade dysplasia (LGD) are associated with EAC development.[2, 17-22] Factors, such as male sex, advanced age and non-Hispanic white ethnicity are also associated with GERD, BE and EAC, but were not useful to discriminate between high and low risk patients because of their common prevalence in these patient groups.[17]

Hence, there is a need to identify factors which are able to predict which patients with BE have an increased risk of developing HGD and EAC. This would allow individualization of surveillance in patients with BE and improve cost-effectiveness of such a surveillance program.

In this study, the aim was to identify easy to apply predictors for the development of progression in BE from baseline, with either no dysplasia (ND) or LGD as histologic result, to HGD or EAC in order to tailor a more individual-based surveillance program.

METHODS

Study design

Between November 2003 and December 2004, we performed a prospective, multicenter cohort study in 3 university medical centers and 12 regional hospitals in The Netherlands and included 713 patients with BE in this study. In total, 142 of 713 (20%) of patients were from tertiary referral centers and 571 (80%) from primary referral centers. Patients were included when there was: 1) endoscopic evidence of BE of ≥ 2 cm in length at baseline endoscopy, 2) intestinal metaplasia with ND or LGD in the baseline biopsy, 3) no previous history of HGD or esophageal cancer.

As part of this study, endoscopic follow-up with biopsies was performed in patients with BE and baseline ND every 2 years and in patients with baseline LGD yearly. Patients who developed HGD or EAC during follow-up were advised to undergo endoscopic treatment or esophagectomy, as appropriate, and were excluded from further surveillance in this study. The primary outcome of our cohort study was the development of HGD or EAC during follow-up.

Data collection

At baseline, patients were asked to fill out a standardized questionnaire with regard to demographic factors (age, gender), anthropometric characteristics (length, weight), smoking habits, alcohol use, personal history of BE and GI symptoms, family history of BE and medication use (proton pump inhibitors (PPIs), histamine-2-receptor antagonists, non-steroidal anti-inflammatory drugs (NSAIDS), aspirin, COX-2 inhibitors) and these data were stored in a computerized database.

Endoscopic findings were prospectively collected in an individual case record form and included information on length of BE, presence of irregularities in BE (including nodules and ulcers), presence and size of a hiatal hernia, and presence and grade of esophagitis (Los Angeles classification[23]). Gastroenterologists were instructed to take four-quadrant biopsies every 2 cm over the whole length of the BE. In addition, targeted biopsies were taken from mucosal abnormalities, if present.

Histological findings were collected and processed in the database. All biopsy samples from BE patients were examined by the local pathologist in the hospital where the BE patient was identified or followed. Dysplasia in BE was graded according to the consensus criteria of 1988, with adjustments as proposed in 2001.[24, 25] The latter comprises LGD, HGD and EAC, with indefinite for dysplasia not being an option for the pathologists.[26] All biopsy specimens were sent to one member of a panel of five expert GI-pathologists (HvD,

GM, AM, JO, FtK). The expert pathologists were not aware of the diagnosis of the local pathologist. If there was disagreement between the local and the expert GI-pathologist on the histological diagnosis, another member of the panel, who was blinded to the previous findings, also reviewed the slides until a majority diagnosis was reached. In this study, endomucosal resection specimens were not used for histological diagnosis as HGD or EAC on biopsy samples were considered endpoints for this study.

Ethics

The study protocol was approved by the Medical Ethics Committee of the Erasmus MC University Medical Center Rotterdam and the local Medical Ethics Committees of the participating centers. Prior to baseline endoscopy, written informed consent was obtained from all patients.

Statistical analysis

Follow-up time was defined as the time from the date of baseline endoscopy in this study to the most recent surveillance endoscopy date or the endoscopy date that resulted in a diagnosis of HGD or EAC. For this study, we collected and validated data until January 31, 2009. Incident HGD or EAC was defined as the development of HGD or EAC at least 6 months after baseline endoscopy.

Demographic, endoscopic and histological characteristics were analyzed using the low risk category as the referent group. Continuous variables were age, length of hiatal hernia, length of BE and mean body mass index (BMI). Categorical variables were sex, BMI in categories (<20, 20-25, 25-30, >30), smoking status (never, former, current), alcohol use (never, former, current), reflux symptoms, prior history of BE (0-10yrs, >10yrs), familial history of BE, medication use, BE irregularities including erosions or nodules, esophagitis and grade of esophagitis (Los Angeles Classification), and grade of dysplasia.

Survival curves were constructed using the Kaplan-Meier method and the curves were compared using the log-rank test for equality. A generalized linear model was made to calculate risk ratios (RRs) with corresponding 95% confidence intervals (CIs).[27] Incident HGD or EAC was the outcome variable which was assumed to have a Poisson distribution. The model used a logarithmic link function and the natural logarithm of the follow-up time as offset variable. For each patient, consecutive intervals of follow-up were analyzed, with updating of covariate data from surveillance biopsy specimens. We adjusted for age and gender in all analyses focusing on single covariates. In the multivariable analysis, all statistically significant covariates were used to assess their combined influence, again adjusted for age and gender. Two-sided p-values <0.05 were considered to be statistically

significant.

Finally, we developed a prediction model for neoplastic progression, which was based on a male patient of 60 years, with internal validation by bootstrapping.[28, 29] Bootstrap estimates were used to derive the final predictive model by correcting the risk ratios for overoptimism.[27, 30] Statistical analyses were conducted using SPSS software (version 15.0, Chicago, Illinois, USA) and R software (R Foundation for Statistical Computing, version 2.8.1).

Table 1. Baseline characteristics of patients with Barrett's esophagus

Variable		N (%)/ mean (range)
No. of patients		713
Mean age (yrs)		60.5 (20-86)
Male gender		525 (74)
Mean BMI (kg/m^2)		27 (17-40)
Smoking	Current	145 (20)
	Former	319 (45)
	Never	237 (33)
Alcohol use	Current	542 (76)
	Former	65 (9)
	Never	92 (13)
PPI-use		642 (90)
NSAID-use		34 (5)
Aspirin-use		100 (14)
Familial history of BE		80 (11)
Prior history of BE	< 10 yrs	621 (87)
	≥ 10 yrs	92 (13)
	Median duration	3.0 (1.0-7.0)
Mean BE length (cm)		4.5 (2.0-16.0)
Hiatal hernia		617 (87)
	Mean length (cm)	3.6 (1.0-14.0)
BE irregularities		41 (6)
Esophagitis		73 (10)
Grade of esophagitis	A	27 (3.8)
	B	36 (5)
	C	8 (1.1)
	D	2 (0.3)
Histology	LGD	111 (16)
	ND	602 (84)

PPI, protonpump-inhibitor; NSAID, non-steroidal anti-inflammatory drugs; BE, Barrett's esophagus; LGD, low-grade dysplasia; ND, no dysplasia

RESULTS

Patient characteristics

The baseline demographic, endoscopic and histological characteristics of the included patients (n=713) are shown in Table 1. The mean age was 60.5 years (range 20-86) and 74% was male. During surveillance, 26 patients developed HGD or EAC. In those 26 patients (100%), the biopsy diagnosis was confirmed in the resected specimens. Of the 26 patients with progression to HGD or EAC, 58% had LGD at baseline and the mean BE length at baseline was 6.1 cm (range 2.0-16.0), and 81% was male. Regarding sex, 21 of 525 (4%) men and 5 of 188 (3%) developed progression. Regarding age, the age of patients with progression was also equally divided with a mean of 62.7 years (range 37.7-84.8). The mean follow-up time of the group that progressed to HGD or EAC was 2.1 years (range 0.5-4.6). The mean follow-up time of patients without progression was 3.6 years (range 0.9-5.2). The 4-yr cumulative incidence of HGD or EAC was 3.6% (95%CI: 2.2-5.0).

Predictive value of demographic, endoscopic and histological factors

The 4-yr cumulative incidence of HGD or EAC in patients with a known BE duration ≥ 10 yrs was 9.6% (95%CI: 2.2-17.0) compared to 3.1% (95%CI: 1.5-4.7) in patients with a known BE duration < 10yrs (Figure 1a), in patients with or without esophagitis 13.3% (95%CI: 4.5-22.5) vs.3.0% (95%CI: 1.4-4.6) (Figure 1b), in patients with or without BE irregularities 10.1% (95%CI: 0.7-19.5) vs. 3.2% (95%CI: 1.8-4.6) (Figure 1c) and in patients with LGD or ND 13.3% (95%CI: 6.4-20.2) vs. 1.9% (95%CI: 0.1-3.1) (Figure 1d).

Univariate analysis, adjusted for age and gender, confirmed that LGD at baseline or during surveillance was associated with an increased risk of neoplastic progression (RR 9.6, 95%CI: 4.3-21.0). This was also the case for the presence of esophagitis (RR 3.9, 95%CI: 1.6-9.6), irregularities in BE (RR 3.5, 95%CI: 1.2-10.3), a known BE duration ≥ 10 years (RR 2.5; 95%CI 1.03-6.0), and a longer length BE (RR 1.12 per cm increase, 95%CI: 1.02-1.2) (Table 2). Male sex, advanced age, GI symptoms, BMI, smoking habits, alcohol use or medication use had no predictive value for the development of neoplastic progression (Table 2).

In multivariable regression analysis, adjusted for age and gender, LGD (RR 9.7, 95%CI: 4.4-21.5), esophagitis (RR 3.5, 95%CI: 1.3-9.5), a known BE duration ≥ 10 years (RR 3.2, 95%CI: 1.3-7.8) and a longer length of BE (RR 1.11 per cm increase, 95%CI: 1.01-1.2) were independent predictors of progression to HGD or EAC (Table 3).

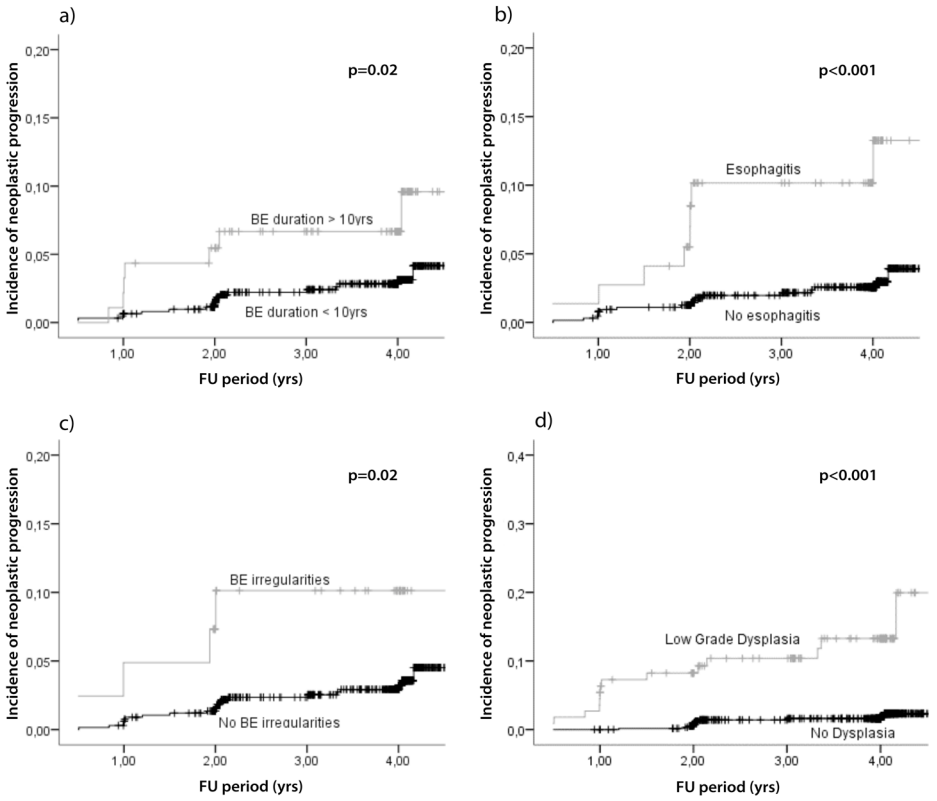

Figure 1. Cumulative incidence of HGD/EAC in patients with less or more than 10 years duration of BE (a), in patients with or without presence of esophagitis (b) or BE irregularities (including ulcers or nodules) (c) or in patients with a histological diagnosis of low-grade dysplasia or no dysplasia (d). Tick marks indicate time of last follow-up endoscopy for patients without HGD/EAC at last endoscopy.

Prediction model for annual risk of progression

Figure 2 shows the predicted annual risk of progression to HGD or EAC in patients with ND or LGD for different lengths of BE in combination with one or more of the above-reported predictors of neoplastic progression. The annual risk of developing neoplastic progression strongly increased in patients with LGD in combination with other risk factors compared to patients with ND or LGD and no other risk factors. The annual risk of developing HGD or EAC in patients with LGD and 2 other risk factors ranged from 18% for a BE length of 2 cm up to 40% for a BE length of 16 cm. In patients with ND and 2 risk factors, this risk varied between 4% and 7% per year. If apart from LGD, no other risk factors were present, the risk of progression varied between 3% and 6% per year and in patients with only ND between

Table 2. Risk ratios of clinical, endoscopic and histological factors for progression to high-grade dysplasia or esophageal adenocarcinoma (age- and gender-adjusted analysis)

Variable		Univariate analysis adjusted for age and gender	
		RR (95%CI)	p-value
Gender	Female	1.0 (reference)	0.298
	Male	1.7 (0.6-4.5)	
Age		1.02 (0.99-1.06)	0.260
Smoking	Never	1.0 (reference)	0.805
	Former	1.2 (0.5-3.2)	
	Current	1.4 (0.5-4.4)	
Alcohol use	Never	1.0 (reference)	0.235
	Former	3.2 (0.6-17.0)	
	Current	1.5 (0.3-6.7)	
BMI		0.99 (0.9-1.1)	0.801
Heartburn	No	1.0 (reference)	0.153
	Yes	1.8 (0.8-3.9)	
Regurgitation	No	1.0 (reference)	0.357
	Yes	1.5 (0.6-3.5)	
PPI-use	No	1.0 (reference)	0.800
	Yes	0.9 (0.3-2.9)	
NSAID-use	No	1.0 (reference)	0.953
	Yes	0.9 (0.1-7.0)	
Aspirin-use	No	1.0 (reference)	0.256
	Yes	0.4 (0.1-1.8)	
Duration of BE	< 10 yrs	1.0 (reference)	0.042
	≥ 10 yrs	2.5 (1.03-6.0)	
Length of BE	cm	1.12 (1.02-1.2)	0.013
Length of hiatal hernia	cm	0.9 (0.7-1.2)	0.821
Esophagitis	No	1.0 (reference)	0.004
	Yes	3.9 (1.6-9.6)	
BE irregularities†	No	1.0 (reference)	0.020
	Yes	3.5 (1.2-10.3)	
Histology	ND	1.0 (reference)	<0.001
	LGD	9.6 (4.3-21.0)	

RR, Risk ratio; CI, confidence interval; BMI, body mass index (kg/m^2); PPI, proton pump inhibitor; NSAID, nonsteroidal anti-inflammatory drugs; BE, Barrett's esophagus; ND, no dysplasia; LGD, low-grade dysplasia; †BE irregularities including ulcers and nodules

0.3% and 1.0%. Table 3 shows that the annual risk of progression should be multiplied with approximately 1.1, when the model includes a female of 60 years and not a male

Table 3. Risk ratios of endoscopic and histological factors associated with progression to high-grade dysplasia and esophageal adenocarcinoma (age- and gender-adjusted multivariable analysis)

Variable		Multivariable analysis adjusted for age and gender (n=713)	
		RR (95%CI)	p-value
Gender	Female	1.0 (reference)	0.876
	Male	1.1 (0.4-3.0)	
Age	per year	0.99 (0.96-1.04)	0.948
Duration of BE	< 10 yrs	1.0 (reference)	0.011
	≥ 10 yrs	3.2 (1.3-7.8)	
Length of BE	per 1 cm	1.11 (1.01-1.2)	0.038
Esophagitis	No	1.0 (reference)	0.013
	Yes	3.5 (1.3-9.5)	
BE irregularities†	No	1.0 (reference)	0.232
	Yes	2.0 (0.6-6.6)	
Histology	ND	1.0 (reference)	<0.001
	LGD	9.7 (4.4-21.5)	

RR, Risk ratio; CI, confidence interval; BE, Barrett's esophagus; ND, no dysplasia; LGD, low-grade dysplasia; †BE irregularities including ulcers and nodules

of the same age. The risk ratio for a patient with any age can be estimated by 0.99^(X (=age)-60) (Table 3) and then multiply this risk ratio with the annual risk obtained from the prediction model (Figure 2). Since both age and sex were no significant risk factors, the variations in risk of progression due to age and sex were only small.

DISCUSSION

In this prospective, observational cohort study, we identified LGD, a longer known duration of BE, longer length of BE and esophagitis as predictors for progression to HGD and EAC in BE. Until now, several studies have investigated risk factors for neoplastic development in BE.[2, 17, 21, 22, 31] In contrast to many studies, we excluded patients who already had HGD or EAC at baseline and only included patients with BE and baseline ND or LGD. We used strict and consistent criteria for the diagnosis of BE, LGD, HGD, and EAC. Moreover, follow-up time was taken into account in the statistical analysis.

In our study, LGD at baseline or during surveillance was the strongest independent predictor for progression to HGD or EAC, in line with previous reports.[21, 32-36] We limited the interobserver variation by using a panel of expert pathologists to evaluate the dysplasia grade. Nonetheless, other studies without an expert panel have also reported an increased risk of progression in patients with LGD.[18, 32] Remarkably, when we would have performed

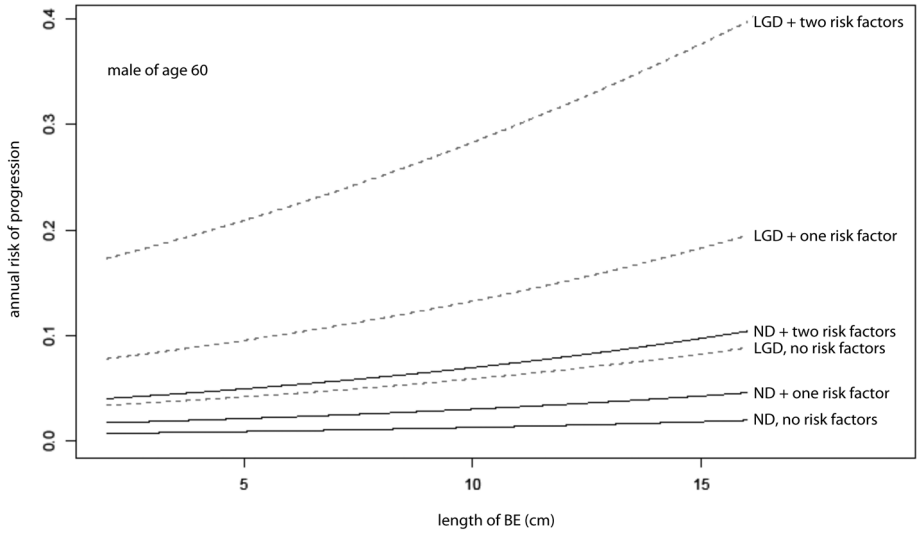

Figure 2. Predicted annual risk of progression to HGD or EAC in patients with BE and with or without low-grade dysplasia and with or without any risk factor plotted against the length of BE segment. The black lines represent BE patients with no dysplasia (ND) and 0, 1 or 2 risk factors (duration of BE of more than 10 years, presence of esophagitis). The dotted lines represent BE patients with LGD and 0, 1 or 2 risk factors. The prediction model was based on a male patient of 60 years.

the same analysis based on the histologic diagnosis of the local pathologist, the predictive effect of LGD would have been even stronger. This is due to the fact that many patients were downgraded from LGD to ND by the expert pathologists (unpublished results). It is well known that a histologic diagnosis of LGD has a low reproducibility.[25] The past few years, adjunct tools have been developed, such as quantitative pathologic analysis and use of biomarkers, that can be used to confirm the diagnosis of LGD.[35, 37] However, these tools still have not been validated in clinical practice and are only used in expert referral centers.[38] It is known that the proportion of patients with LGD that annually progresses to HGD or EAC has been reported to range from 2.1% to 7.5% per patient-year of follow-up.[34, 36, 39, 40] Although this is a relatively high proportion, it also implies that more than 90% of patients with LGD will not progress on a yearly basis. Although we recognize that the predictive value of LGD in identifying high-risk patients can be debated, we confirmed that LGD is a risk factor of progression to HGD or EAC in BE.[41]

Of all demographic factors studied, a longer known duration of BE had a predictive value for progression. Patients with a diagnosis of BE as long as 10 or more years prior to inclusion had a 3-fold increased risk to develop HGD or EAC compared to patients with a

shorter BE diagnosis. To our knowledge, this is the first time that it is shown that a longer established diagnosis of BE is associated with a higher risk of developing HGD or EAC. We used the first date that a BE diagnosis was endoscopically and histologically confirmed to calculate the duration of BE. It has been shown that a longer symptom duration is associated with higher grades of dysplasia.[42] We were not able to confirm this association, probably due to the fact that many patients with BE were asymptomatic as the majority (90% of patients) used PPIs at baseline. Alternatively, it could also be that patients in this study just underreported their symptoms. Nonetheless, our findings suggest that progression to EAC in BE may take at least 10 years showing that it is indeed a relatively slow process.[13]

Longer length BE was also associated with an increased risk of neoplastic progression. We showed that for each cm increase in BE length, there was an 11% increase in the risk of developing HGD or EAC. Other studies have also reported this association.[2, 19, 20, 39, 43] It should be noted that in patients with a longer BE segment more biopsy samples were taken (4 biopsies per 2 cm) compared to patients with a shorter segment, which may well increase the likelihood of detecting neoplastic progression in BE. The increased risk associated with a longer length of BE has been attributed to a larger surface area being at risk of neoplastic progression.[2]

Esophagitis was another predictor of progression to HGD or EAC. The risk of developing HGD or EAC was 3.5 times higher in esophagitis compared to the situation when this was not present. The underlying inflammation in esophagitis is likely to increase the risk of mutations leading to HGD or EAC.[44] However, we cannot rule out the possibility that inflammation was overinterpreted as dysplasia. We also graded the esophagitis. It was however not possible to find an association between the grade of esophagitis and the risk of developing neoplastic progression due to the relatively small number of patients with esophagitis. Eight of 26 (31%) patients who developed progression in this study had baseline esophagitis and 7/8 (88%) patients used PPIs. In total, 55/73 (75%) patients developed esophagitis despite the use of PPIs. It could well be that these patients used a PPI dose that was too low to completely block gastroesophageal reflux, or they may have been not compliant with PPI-therapy or unresponsive to it. By contrast, in the whole cohort, the use of PPI was correlated with the absence of esophagitis (data not shown). We were not able to detect a protective effect of PPI-use on neoplastic progression. This is in line with some previous studies.[45-47] It should be noted, however, that our study did not have enough discriminative power to show a preventive effect of PPIs on the risk of developing HGD or EAC as the vast majority of our patients were using PPIs (90%).

We did not find a protective effect of NSAIDs or aspirin on the prevention of HGD or

EAC as others have suggested.[48] In addition, more controversial risk factors for neoplastic progression in BE, such as alcohol use, smoking, high BMI were also not found to be associated with the development of HGD or EAC. This could be due to the small number of patients who developed progression and the moderate period of follow-up.

A combination of prognostic factors has been suggested to be required to define subgroups of patients at an increased risk of progression.[49] If applied in a predictive model, patients with LGD and all of the above mentioned risk factors were found to have an annual risk of progression ranging from 18% to 40% depending on an increasing BE length. These patients were clearly found to be at a higher risk of EAC development compared to patients with ND and the same risk factors or patients with LGD but none of these risk factors. Low-risk patients are those with ND and no other risk factors (Figure 2). Based on this, we conclude that the annual risk of progression in BE rapidly increases if more than one risk factor of progression is found in a patient. For clinical practice, we advise that these high-risk patients should undergo a more frequent endoscopic follow-up schedule as they probably have an annual risk of EAC of more than 1%. Future studies are definitely needed to validate this model. In addition, the exact interval of surveillance in high-risk patients needs to be determined.

Several limitations of this study need to be discussed. First, we did not use a validated questionnaire for demographic factors. We avoided however suggestive questions and asked the patients to reply with responses that were unambiguous. Second, it may well be that we missed patients who developed HGD or EAC despite the strict protocol for endoscopy, biopsy taking and histological evaluation. Third, due to a relatively low number of patients who developed HGD or EAC during surveillance and the relatively short follow-up time, we had a lower power for some of the analyses. Further validation of our findings is therefore needed. Finally, due to potential effect of more frequent endoscopies in the LGD group, lead time bias might have influenced our results.

In conclusion, our prospective study shows that LGD, a duration of BE of 10 years or more, a longer BE segment and esophagitis are predictive of an increased risk of developing HGD or EAC in BE. A combination of risk factors is able to identify patients with a high or a low risk of neoplastic progression. If further validated, these individualized risk estimates may well be useful to determine the frequency of surveillance endoscopies in patients with BE.

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Chapter 5

Submitted

The incidence of neoplastic progression in patients with Barrett's esophagus: a prospective multicenter study

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ABSTRACT

Background: Barrett's esophagus (BE) predisposes to esophageal adenocarcinoma (EAC). EAC develops from no dysplasia (ND) to low-grade dysplasia (LGD) and high-grade dysplasia (HGD). Reported incidence rates of EAC have shown considerable variation. Our aim was to determine the incidence rates of HGD or EAC in BE patients with baseline ND or LGD and to evaluate the 'regression' rate from LGD to ND during follow-up.

Methods: In this prospective, multicenter cohort study, we included patients with a BE segment of 2 cm or longer and histologically ND or LGD at baseline. Patients with BE and ND had follow-up endoscopies including biopsies every 2 years, whereas those with baseline LGD had yearly endoscopic follow-up. The main outcomes were the annual risks of progression towards LGD, HGD and EAC for ND and to HGD and EAC for LGD. These were analyzed with actuarial methods.

Results: We included 715 patients with BE (baseline ND: n=607; baseline LGD: n=108). During 2,598 person-years of follow-up, 16 patients developed HGD and 10 patients EAC, corresponding to an annual risk of HGD of 1.0% (95%CI: 0.6-1.4) and EAC of 0.4% (95%CI: 0.1-0.6). In patients with baseline ND, the annual risk of HGD was 0.6% (95%CI: 0.3-0.9) and of EAC 0.2 % (95%CI: 0.0-0.4). Patients with baseline LGD had higher annual risks, i.e., 3.4% (95%CI: 1.5-5.4) for HGD and 1.7% (95%CI: 0.3-3.1) for EAC. The annual risk of 'regression' from LGD to ND was 5.4% (95%CI: 4.5-6.2).

Conclusion: A relatively low incidence of HGD and EAC in BE was found during follow-up, with patients with LGD having a higher risk than those with ND. More studies are needed to identify predictors of neoplastic progression in BE and improve the cost-effectiveness of the currently employed surveillance protocol.

INTRODUCTION

Barrett's esophagus (BE) is a premalignant condition and predisposes to the development of esophageal adenocarcinoma (EAC). This development is a stepwise process from no dysplasia (ND) to low-grade dysplasia (LGD) and high-grade dysplasia (HGD).[1-3] In the past decades, the incidence of both BE[4] and EAC has been rapidly rising, as demonstrated by a 7-fold increased incidence of EAC between 1973 and 2006.[5] In general, patients with BE have a 30- to 125-fold higher risk of developing EAC than the general population.[2]

EAC is associated with a poor prognosis and a high mortality risk with a 5-year survival rate less than 20%.[6] As a result of this malignant potential, regular surveillance endoscopies are recommended in patients with BE. The goal is to detect neoplasia at an early stage, making curative treatment possible.[7] Over the past few years, it has been shown that HGD and early EAC can be treated endoscopically, avoiding an esophagectomy.[8-10]

Surveillance in BE is still debated as the cost-effectiveness of surveillance is in particular dependent on the incidence of EAC.[11, 12] The reported incidences of EAC show considerable variation ranging from 0.2% to 3.5% per year.[13, 14] These differences could well be explained by several factors, with publication bias, selection bias and a retrospective study design[15-22] being the most prominent ones leading to an overestimation of the cancer risk. Recent reviews have shown that the overall incidence rate of EAC is likely to be around 0.6% per year.[22, 23]

In order to accurately determine incidence rates in BE, we established a large cohort of patients with BE that was followed prospectively. The aims of this study were 1) to estimate the incidence rates of LGD, HGD and EAC in this cohort of patients with ND or LGD in BE and 2) to evaluate the 'regression' rate from LGD to ND during follow-up.

METHODS

Study design and data entry

We conducted a prospective multicenter cohort study in 3 university medical centers and 12 regional hospitals in the Netherlands. Between November 2003 and December 2004, 956 consecutive patients were found to be eligible for this study. We included all patients who met the following criteria: 1) endoscopic evidence of BE ≥ 2 cm at baseline endoscopy, 2) the presence of intestinal metaplasia with ND or LGD on histology at baseline endoscopy, 3) no HGD or EAC at baseline endoscopy, and 4) no previous history of HGD or esophageal cancer. Since there is no objective evidence for an association between the timing of the initial BE diagnosis (i.e. the time that a patient is known with a BE diagnosis)

and the development of progression[24], all patients with a diagnosis of BE according to the inclusion criteria qualified for this study. Patients with columnar mucosa but without IM on baseline biopsy (n=127) or with BE < 2 cm at baseline endoscopy (n=15), with severe co-morbidity (n=9) and patients with prevalent HGD or EAC (n=24) were excluded.

We monitored subsequent follow-up endoscopies after baseline endoscopy using a standardized case record form (CRF). In each participating center, CRFs were filled out by the endoscopist and sent to the study coordinator. The information in the CRFs was processed in a computerized database. Quality control of the data was guaranteed by prospective control of entered data, by patient contact if they had missed a follow-up endoscopy, and by regular meetings with all caregivers involved in the study.

The primary endpoint of the study was the incidence rate of HGD or EAC in patients with BE. The secondary endpoints were the incidence rate of LGD in patients with baseline ND and the incidence rate of regression from LGD to ND.

Patients

Seven hundred and eighty-one patients with BE were eligible for the study. Of these, 65 were excluded from the final analysis due to a lack of follow-up data because of: 1) refusal to participate (n=36), 2) death (n=11), 3) having moved to another area (n=9) and 4) other reasons (n=9). The remaining 718 patients were included in the analyses and 582 of 718 (81%) patients were known with a prior diagnosis of BE.

Endoscopy

During surveillance, each patient underwent endoscopy with biopsies of the BE segment. The examinations were conducted according to the study protocol. Prior to taking biopsies, endoscopic landmarks, such as the proximal margin of gastric folds and the length of the BE segment were identified and reported in the CRF. The Prague classification for BE was not used to describe the BE segment in a standardized way, as it was not available at the start of patient inclusion.

Four-quadrant biopsies were taken each 2 cm over the whole length of the BE with either a standard or jumbo biopsy forceps. In addition, targeted biopsies were taken from mucosal abnormalities, if present.

Endoscopic surveillance was only offered to patients who were potentially fit to undergo surgery. Patients with BE and baseline ND had follow-up endoscopy every 2 years, whereas patients with BE and baseline LGD underwent yearly endoscopy. Patients who developed HGD or EAC were excluded from further surveillance and offered endoscopic treatment or esophagectomy.

Histology

Biopsies were fixed in 10% buffered formalin, embedded in paraffin, serially sectioned, and then stained with hematoxylin and eosin. BE was defined as the presence columnar mucosa with IM containing goblet cells.[1] Biopsies from BE were first examined by the pathologist from the hospital where the patient was being followed. Subsequently, the biopsy specimens were sent to a GI-pathologist from a panel of five experienced GI-pathologists for review. If there was disagreement between the local and the expert GI-pathologist on the histological diagnosis, another member of the expert panel, who was blinded to the previous findings, was asked to review the slides as well. Only when a majority of pathologists agreed on the diagnosis (IM with goblet cells, with or without dysplasia or EAC), a final diagnosis was made. Dysplasia in BE was graded according to the consensus criteria of 1988, with adjustments as proposed in 2001 and according to the Vienna criteria.[25-28]

Ethics

The study was approved by the Institutional Review Boards of the Erasmus MC - University Medical Center Rotterdam and all participating centers. Written informed consent was obtained from all patients.

Statistical analysis

Follow-up time was defined as the time between the date of baseline endoscopy and the date of the most recent endoscopy or the date of endoscopy that resulted in a diagnosis of LGD, HGD or EAC. For this study, we collected and validated data until January 31, 2009. Incidence rates of LGD, HGD and EAC and regression rates from LGD to ND were calculated by dividing the number of incident cases by the total number of person-years (pyrs) of follow-up in the study sample or in subsets. Corresponding 95% confidence intervals (CI) were calculated assuming a Poisson distribution. Incident HGD or EAC was defined as the development of HGD or EAC at least 12 months after baseline endoscopy.

According to the sequence of neoplastic progression in BE[1-3, 29], we assumed that a patient who developed HGD also had gone through the stage LGD. Consequently, a patient who developed EAC also should have gone through the stages LGD and HGD. In case HGD was the outcome of a patient with baseline ND, the time to develop LGD was estimated to be half of the total follow-up time in that patient. In case EAC was the outcome of a patient with baseline ND, the time to develop LGD was estimated to be a third of the total follow-up time and the time to develop HGD was estimated to be two third of the total follow-up

time in that patient. When we observed LGD before progression to HGD or EAC, these assumptions were not applied. In patients with baseline LGD and progression to EAC, the time to develop HGD was estimated to be half of the total follow-up time of that patient.

Kaplan-Meier analysis was performed to evaluate the cumulative incidence rates of progression to HGD and EAC in patients with baseline ND or LGD and during follow-up. In the latter analysis, the time since last endoscopy was used instead of follow-up time since baseline endoscopy. The logrank test compared the 2 groups and $p<0.05$ was considered to be statistically significant. In addition, Cox regression analysis was performed to estimate hazard ratios (HR). The statistical analysis was conducted using SPSS-software (version 15.0 and 16.0, Chicago, Illinois, USA).

Table 1. Baseline characteristics of patients with Barrett's esophagus (BE) (n=715)

Variable			Range/IQR/%
Mean age (yrs)		60.6	19.5-86.0
Median BE length (cm)		4.0	2.0-6.0
Gender	Male	523	73%
Baseline histology	LGD	108	15%
	ND	607	85%
Reasons for loss	Refused participation	38	4.3%
	Death	14	2.0%
	Other	19	2.7%
Total number of endoscopies		2,390	
Median nr. of endoscopies		3.0	3.0-8.0
Mean period of FU (yrs)		3.6	0.7-5.2
Person years of follow-up	n=715	2,598	
	n=108	353	
	n=607	2,244	
Incident EAC	Total	10	
	baseline ND	4	
	baseline LGD	6	
Incident HGD	total	16	
	baseline ND	10	
	baseline LGD	6	
Neoplastic progression		26	

HH, hiatal hernia; PPI, proton-pump inhibitor; LGD, low-grade dysplasia; ND, no dysplasia, FU, follow-up; EAC, esophageal adenocarcinoma; HGD, high-grade dysplasia

RESULTS

Patient characteristics

In total, 144 of 718 (20%) of patients were included from tertiary referral centers and 574 (80%) from primary referral centers. Three of 718 patients developed progression to HGD (n=1) or EAC (n=2) within the first 12 months after inclusion and were excluded from further analysis. Of the remaining 715 patients, 607 (85%) had baseline ND and 108 (15%) patients baseline LGD (Table 1). The mean total follow-up time was 3.6 years (range 0.7-5.2 years), accounting for a total of 2,598 pyrs of follow-up. Patients with baseline ND had a total of 2,244 pyrs of follow-up and patients with baseline LGD a total of 353 pyrs. During surveillance, 10 patients with baseline ND and 6 with baseline LGD developed HGD. Four patients with baseline ND and 6 with baseline LGD developed EAC.

Observed transitions during surveillance

In patients with ND, follow-up 1, follow-up 2 and follow-up 3 correspond to 2, 4 and 6 years of follow-up, respectively, while for patients with LGD, follow-up 1, follow-up 2 and follow-up 3 correspond to 1,2 and 3 years of follow-up (Figure 1).

At the first follow-up moment, 564 (93%) of 607 patients with ND remained stable, 35 (6%) patients progressed to LGD, 4 (1%) patients to HGD and another 4 (1%) to EAC. In the group with baseline LGD, 82 (76%) of 108 patients regressed to ND, 20 (19%) remained stable, 2 (2%) progressed to HGD and 4 (4%) to EAC. After this first endoscopy, 646 patients were diagnosed with ND.

Five hundred-fifty-four (84%) of these 646 patients continued surveillance and 42 (7%) were lost to follow-up due to death (n=10), refusal of further participation (n=22) or other reasons (n=11). Until now, 49 (8%) of the 646 patients had not undergone the second follow-up endoscopy. In the LGD group, 52 (95%) of 55 patients continued surveillance and 3 (5%) patients were lost to follow-up. One patient died and 2 patients had another reason to stop participating.

After the second follow-up endoscopy, 523 (94%) of 554 patients with ND remained stable, 27 (5%) progressed to LGD and 3 (1%) to HGD. In the group with LGD, 13 (25%) of 52 patients remained stable, 34 (65%) regressed to ND, 4 (8%) progressed to HGD and one (2%) to EAC. At the start of the third follow-up endoscopy, 557 patients had ND and 40 LGD. In patients with ND, 182 (33%) of 557 patients continued surveillance, and 363 (65%) of 557 patients had not yet undergone the third follow-up endoscopy. Another 12 (2%) were lost to follow up due to death (n=1), refusal to participate further (n=8) or other reasons (n=3). In the LGD-group, 18 (45%) of 40 patients continued surveillance and

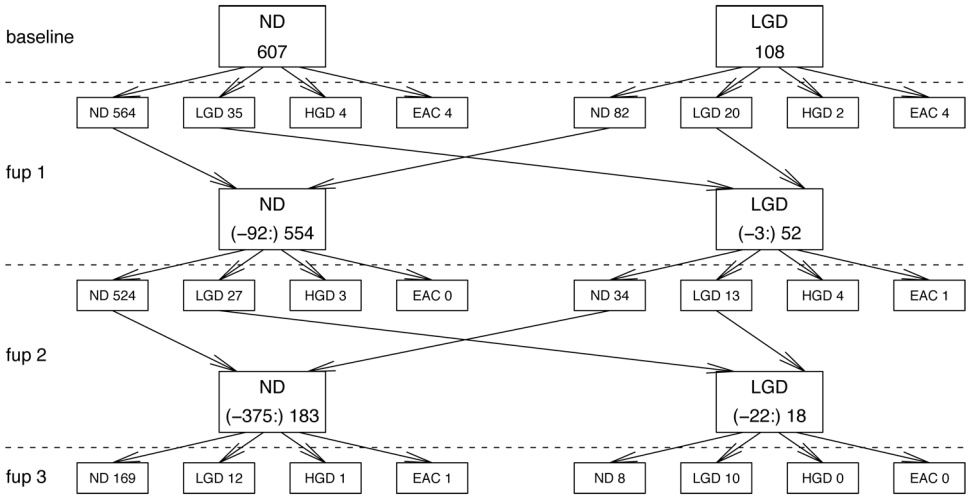

Figure 1. A simplified schematic overview of the observed histologic transitions in a cohort of 607 patients with baseline no dysplasia (ND) and 108 patients with low-grade dysplasia (LGD). The frequency of follow-up was yearly for patients with LGD and 2-yearly for those with ND. For patients with ND, follow-up 1 (fup1), follow-up 2 (fup2) and follow-up 3 (fup3) correspond to 2, 4 and 6 years of follow-up, respectively, whereas for patients with LGD, follow-up 1, follow-up 2 and follow-up 3 correspond to 1,2 and 3 years of follow-up, respectively. Patients who developed high-grade dysplasia (HGD) or esophageal adenocarcinoma (EAC) were excluded from further follow-up. In the larger boxes, numbers between brackets account for lost-to-follow-up patients or those who still had to undergo follow-up endoscopy. After follow-up 3 (fup3), surveillance was still proceeding but is not shown. During follow-up 4 and 5, 2 patients developed HGD. In total 16 patients developed HGD and 10 EAC during a mean follow-up of 3.6 years.

19 (48%) had not undergone the third follow-up endoscopy. Three patients were lost to follow-up due to death (n=1) or other reasons (n=2).

In the following follow-up endoscopies, another 2 patients also developed HGD (not shown in Figure 1). In total, 16 patients progressed to HGD and 10 to EAC. Table 2a and 2b show all observed histologic diagnoses and the follow-up times in patients who developed progression.

Incidence rates of neoplastic progression in BE

During surveillance, 10 (1.4%) of 715 patients progressed to EAC over a total of 2,598 pyrs of follow-up, corresponding to one case of EAC per 260 pyrs of follow-up. In patients with baseline ND, the incidence of EAC was one case per 561 pyrs of follow-up and in patients with baseline LGD, one case per 59 pyrs of follow-up.

Sixteen (2.2%) of 715 patients developed HGD during surveillance. The 10 patients who developed EAC were interpolated in these incident HGDs (see Methods). The overall

Table 2a. Observed histologic diagnoses in patients with Barrett's esophagus who developed high-grade dysplasia (HGD) during follow-up. The first column shows the exact follow-up time (in years), and the second column the observed histology

Patient	Baseline histology	FU-1† histology	FU-2 histology	FU-3 histology	FU-4 histology	FU-5 histology	FU-6 histology	FU-7 histology
1	ND	2.0 ND	2.0 HGD					
2	ND	2.0 LGD	0.4 HGD					
3	ND	2.0 HGD						
4	ND	1.2 ND	0.9 ND	0.9 HGD				
5	ND	1.9 HGD						
6	ND	2.0 HGD						
7	ND	2.0 LGD	2.1 HGD					
8	ND	2.0 ND	2.3 HGD					
9	ND	1.0 LGD	0.9 HGD					
10	ND	2.0 HGD						
11	LGD	0.7 LGD	0.5 ND	0.5 LGD	0.5 LGD	0.6 LGD	1.2 LGD	0.6 HGD
12	LGD	1.0 HGD						
13	LGD	1.0 HGD						
14	LGD	1.2 ND	0.9 ND	0.9 ND	0.9 HGD			
15	LGD	1.0 LGD	1.0 HGD					
16	LGD	1.0 ND	1.1 HGD					

†FU-1, first endoscopy visit; FU-2, second endoscopy visit etc.
ND, no dysplasia; LGD, low-grade dysplasia; HGD, high-grade dysplasia

Table 2b. Observed histologic diagnoses in patients with Barrett's esophagus who developed esophageal adenocarcinoma (EAC) during follow-up. The first column shows the exact follow-up time (in years), and the second column the observed histology

Patient	Baseline histology	FU-1† histology	FU-2 histology	FU-3 histology
1	ND	2.0 EAC		
2	ND	2.0 EAC		
3	ND	1.2 EAC		
4	ND	2.1 EAC		
5	LGD	1.0 EAC		
6	LGD	1.0 LGD	1.3 ND	1.0 EAC
7	LGD	1.0 LGD	1.1 EAC	
8	LGD	1.0 EAC		
9	LGD	1.0 EAC		
10	LGD	1.0 EAC		

†FU-1, first endoscopy visit; FU-2, second endoscopy visit etc.
ND, no dysplasia; LGD, low-grade dysplasia; EAC, esophageal adenocarcinoma

Table 3. Observed crude incidence rates during surveillance of 715 patients with Barrett's esophagus

Progression		Cases			Pyrs of FU	Annual risk (%)	95%CI	
From	To	Observed†	Interpolated‡	Analyzed††			Lower limit	Upper limit
ND	LGD	80	26	106	2,564	4.1	3.3	4.9
ND	HGD	10	4	14	2,242	0.6	0.3	1.0
ND	EAC	4	-	4	2,244	0.2	0.0	0.4
LGD	HGD	6	6	12	350	3.4	1.5	5.4
LGD	EAC	6	-	6	353	1.7	0.3	3.1
ND/LGD	HGD	16	10	26	2,592	1.0	0.6	1.4
ND/LGD	EAC	10	-	10	2,598	0.4	0.1	0.6
LGD	ND	139	-	139	2,598	5.4	4.5	6.2

ND, no dysplasia; LGD, low-grade dysplasia; HGD, high-grade dysplasia; EAC, esophageal adenocarcinoma; pyrs, personyears of follow-up; CI, confidence interval

†Observed cases means observed during surveillance

‡Interpolated cases means assumed to be present according to the sequence of carcinogenesis in BE

††Analyzed cases means the sum of observed and interpolated cases and used for the analysis of incidence rates

incidence of HGD was one case per 100 pyrs of follow-up. In the group with baseline ND, 14 (10 incident and 4 interpolated) patients developed HGD corresponding to one case of HGD per 160 pyrs of follow-up. In the group with baseline LGD, 12 (6 incident and 6 interpolated) patients developed HGD corresponding to one case of HGD per 29 pyrs of follow-up.

During follow-up, 80 patients progressed from ND to LGD. In total, 106 patients progressed to LGD yielding an annual risk of 4.1% (95%CI: 3.3-4.9). Regression from LGD to ND was observed in 82 of 108 (76%) patients with baseline LGD at the first endoscopy. In total, 139 patients developed ND after a previous diagnosis of LGD (Table 3).

Cumulative incidence of neoplastic progression

Patients with baseline LGD had a significantly higher cumulative incidence of progression to HGD and EAC than patients with baseline ND (Figure 2a, logrank: $p<0.001$, HR 5.4; 95%CI: 2.5-11.7). The prognostic effect of LGD was even stronger if analyzed per follow-up interval (Figure 2b, $p<0.001$, HR 15.7; 95%CI: 6.8-36).

DISCUSSION

The majority of the neoplastic lesions were detected within 2 years of follow-up. In addition, it was found that patients with baseline ND may have HGD or EAC detected at the next endoscopy. On the other hand, the incidence of HGD or EAC was higher in

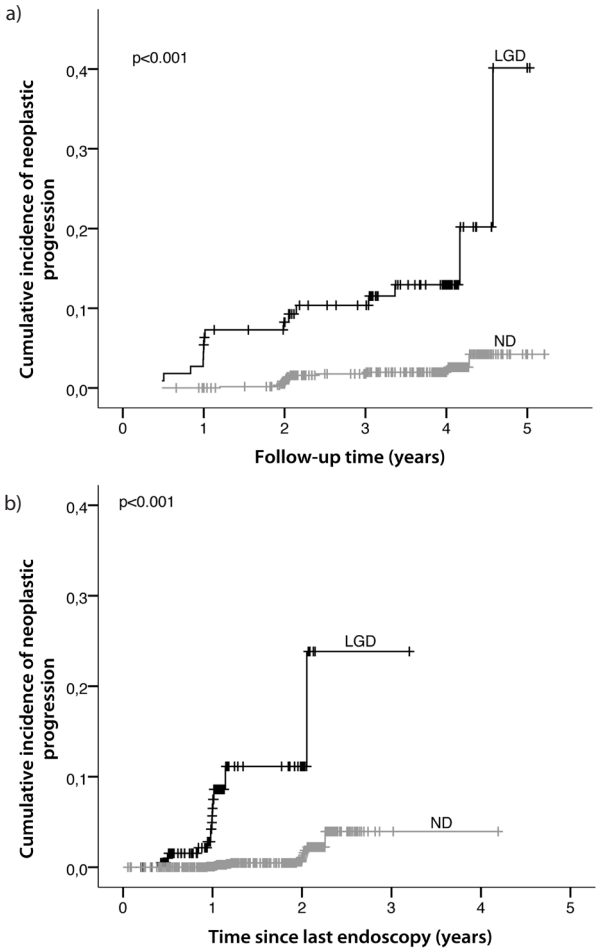

Figure 2. Cumulative incidence of neoplastic progression in a) patients with baseline no dysplasia in Barrett's esophagus (ND) (grey line) or baseline low-grade dysplasia (LGD) (black line), and b) ND or LGD at the start of a follow-up interval. The ticks represent the censoring in this analysis (development of HGD/EAC, start of a new interval of follow-up or lost-to-follow-up)

patients with baseline LGD compared to baseline ND, with overall annual incidence rates of EAC of 0.4% and of HGD of 1.0%.

It should be noted that the observed incidence rate of EAC in our study was in the lower range compared to previously published incidences of EAC.[13, 14] We found a higher incidence rate of HGD than of EAC alone, which is due to the fact that we interpolated 'missed' HGDs when patients were diagnosed with EAC. A number of factors, including publication bias[30], a retrospective study design, inclusion of a small number of patients

and a shorter duration of follow-up, may contribute to the differences in incidence rates of HGD and EAC between our cohort and other studies. The strengths of our study are however the prospective study design, the exclusion at baseline of cases with HGD or EAC at the initial endoscopy or within the first 12 months after inclusion, the use of a standardized endoscopy and biopsy protocol, and a well defined histologic definition of BE and dysplasia, the latter based on a majority opinion including the opinion of at least one member of a panel of expert GI-pathologists.

As the cost-effectiveness of surveillance in BE is dependent on the incidence of EAC, our lower overall estimate of this risk may well have important implications for surveillance. A previously reported mathematical model of surveillance in BE showed that employing an EAC incidence of 0.4% resulted in a cost-effective surveillance interval of 5 years in patients with BE and ND.[11] Another study, also using a decision analytic model, showed that surveillance in patients with BE was under no circumstances cost-effective.[12] This is in contrast to the currently used guidelines for surveillance in BE recommending endoscopy every 3 years in patients with BE and ND and yearly in patients with LGD.[7] Retrospective studies have suggested that patients with EAC found in a surveillance program were detected at an earlier stage EAC resulting in a better survival compared to patients with EAC detected outside such a program.[31] So far, no randomized trials have established the efficacy of surveillance in BE. Our results suggest that surveillance is worthwhile to consider as the majority of patients with neoplastic progression in this study had indeed "only" HGD or early-stage EAC (carcinoma in situ, T-1m). However, we have no long-term survival data of these patients and longer follow-up is needed.

Another important finding was the observed variation in diagnosis of ND and LGD during follow-up. A substantial fraction of patients with baseline LGD (76%) was downgraded to ND during follow-up, similar to previous studies.[18, 32, 33] This was likely not true regression of dysplasia. Plausible explanations include sampling error, resolution of associated inflammation, or interobserver variation. In a previous study we showed that the interobserver agreement in LGD diagnosis between two pathologists, being either expert or non-expert GI pathologists, was poor, similar to another study.[34, 35] This study also showed that more than 50% of LGD cases were downgraded to ND by expert GI-pathologists.[35] Another recent study demonstrated that overdiagnosing of LGD in community practices is rather common.[33] Furthermore, they only accepted a LGD diagnosis, if it was indeed confirmed by a second expert GI-pathologist, as we did in our study. As a result, the authors of the above-mentioned study concluded that a diagnosis of LGD if confirmed by an expert is a sign that a Barrett's segment is at risk of neoplastic progression.[33, 36]

Although it is accepted that patients with LGD have a higher risk of progression, it is unclear whether this risk changes over time.[17, 29, 37, 38] It may well be that it is impossible to determine the lifetime risk of neoplastic progression in BE based on one single follow-up visit, but, instead, an estimate of this risk should be done after each follow-up endoscopy. The cumulative incidence of HGD or EAC was significantly higher in patients with LGD at baseline or during follow-up than in patients with ND at baseline or during follow-up. In addition, the prognostic value of LGD during follow-up was also higher than the prognostic value of baseline LGD. This confirms that the annual risk of neoplastic progression in BE varies over time. On the other hand, the usefulness of LGD as a sole predictor of neoplastic progression can be debated due to the high variation in histological grading and observed likelihood of regression of LGD to ND. In our opinion, other predictors of progression, including biomarkers, are needed to more clearly identify BE patients at an increased risk of progression.[17, 38-40]

Some limitations of this study need to be discussed. First, the follow-up time in this study may have been too short to assess the real risk of neoplastic progression. Secondly, only a relatively small number of BE patients developed neoplastic progression. Third, we might have overestimated the incidence of LGD and HGD by the assumptions that we made. Without the interpolation of 'missed' HGDs, the overall incidence of HGD would decline to 0.6% per year. Finally, misclassification, due to sampling error or interobserver variation, could have influenced our results also leading to an overestimation of the neoplastic progression risk in patients with BE.

In conclusion, we found a relatively low overall incidence of HGD and EAC in BE during follow-up, with patients with histologically LGD having a considerably higher risk than those with ND. In addition, one in 20 BE patients with LGD was downgraded to ND. Our results question the cost-effectiveness of the currently employed surveillance protocol in BE and suggest that more studies are needed to identify predictors of neoplastic progression in an effort to improve cost-effectiveness of surveillance in BE.

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Chapter 6

Progression rates in patients with Barrett's esophagus estimated by a multi-state Markov model based on a large prospectively followed cohort

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ABSTRACT

Introduction: Observed transitions in the development from Barrett's esophagus (BE) to esophageal adenocarcinoma (EAC) are subject to diagnostic errors which may result in misclassification. In order to provide more accurate details on the natural history of progression in BE, we aimed to determine true transition rates of progression in patients with BE to EAC.

Methods: We calculated observed incidence rates from empirical data of a multicenter, prospective, cohort study (n=715; mean follow-up 3.6 years). At baseline, patients had either no dysplasia (ND) or low-grade dysplasia (LGD). The true progression rates from ND to LGD, high-grade dysplasia (HGD) and EAC were statistically estimated by a multi-state Markov (MSM) model. Misclassification rates used to overcome diagnostic errors were based on the literature as well as internally estimated from our cohort. Our assumption was that true regression (for example from LGD to ND) was not possible .

Results: Data-driven misclassification rates were very similar to those reported in the literature, with the lowest misclassification rate for ND but inferior misclassification rates for LGD, HGD and EAC. Using literature-based misclassification rates, the true progression rate from ND to LGD was 4/1,000 person years of follow-up (pyrs), from LGD to HGD 88/1,000 pyrs and from HGD to EAC 307/1,000 pyrs. Corresponding annual progression risks were 0.4%, 7.2% and 26%, respectively. Data-driven misclassification rates were found to result in slightly higher true progression rates, with annual risks of 0.9%, 10% and 27%, respectively.

Conclusion: The MSM modeling technique is recommended when observed rates are subject to errors and estimation of true rates is required. By using misclassification rates, underlying true transition rates of progression in BE can be calculated. These provide a more accurate insight in the natural history of BE, i.e., progression rates, which is essential for further decision-analytic modeling to determine cost-effectiveness of surveillance in BE.

INTRODUCTION

Barrett's esophagus (BE) is characterized by the presence of intestinal metaplasia (IM) in the distal esophagus. It is a premalignant disorder predisposing to the development of esophageal adenocarcinoma (EAC). During a multistep process, BE with no dysplasia (ND) may progress to low-grade dysplasia (LGD), high-grade dysplasia (HGD) and finally EAC.[1, 2] The prevalence of BE is rapidly increasing in the Western world.[3] Endoscopic surveillance is recommended in patients with BE aiming to detect and treat HGD and EAC at an early stage and thus preventing further progression to invasive EAC.[4]

Endoscopic surveillance is however debatable due to the burden to patients of regular upper GI endoscopies[5, 6] and its related high financial costs.[7] So far, no randomized trials have been performed to establish the cost-effectiveness of surveillance in patients with BE. Only mathematical models based on pooled literature data have been used to estimate the cost-effectiveness of surveillance using different surveillance strategies.[8, 9] These mathematical models were highly sensitive to the annual incidence of EAC that was used.[10, 11]

Reported annual incidences of EAC in BE show a wide variation ranging from 0.2 up to 3.5% per year.[12] Recent reviews have shown an annual incidence of EAC of 0.6% with considerable heterogeneity.[12, 13] This variation in the published incidences of EAC is probably due to publication bias[14], the retrospective design of multiple studies, and insufficient numbers of patients or follow-up time. Moreover, diagnostic errors, including poor performance of endoscopy, biopsy sampling error, and interobserver variation in determining dysplasia grade, impair the determination of the true health state of an individual patient. In order to correct for such diagnostic errors or misclassification, multi-state Markov (MSM) models can be used and extended by applying misclassification rates to estimate true progression rates in BE.[15, 16]

In this study, we aimed to determine the true transition rates of progression in BE using MSM models based on follow-up data from a large prospective BE cohort. In addition, we evaluated the differences in the determination of true progression rates by applying documented misclassification rates compared to data-driven misclassification rates.

METHODS

Observed incidence rates of progression in BE

We estimated incidence rates of neoplastic progression in BE based on a prospective, multicenter cohort study. For that purpose, 715 patients with a BE segment of ≥ 2 cm with baseline ND or LGD were included. We excluded patients with HGD or EAC at baseline, as well as those with a prior history of HGD or EAC. The included patients underwent regular endoscopic surveillance with biopsy sampling. The interval was based on the dysplasia grade, with bi-annual endoscopy in patients with ND, and annual endoscopy in those with LGD. Four-quadrant biopsies were taken every 2 cm over the complete length of the BE segment. In addition, targeted biopsies were taken from mucosal abnormalities, if present. The biopsy samples were reviewed by a panel of 5 expert gastrointestinal-pathologists to achieve a majority diagnosis. The primary end point of the study was the incidence of HGD and EAC in patients with baseline ND and LGD.

The total follow-up time was measured in person-years of follow-up (pyrs). Observed incidence rates of LGD, HGD, and EAC were calculated by dividing the number of incident cases by the total number of pyrs of follow-up in the full study sample and in subsets.

According to the sequence of neoplastic progression in BE[1, 2, 17], we assumed that a patient who developed HGD after an initial diagnosis of ND had also passed through the stage of LGD irrespective of whether this LGD had been observed in previous biopsies. Similarly, a patient who developed EAC was assumed to have passed through the stages of LGD and HGD. In subjects who developed HGD, the follow-up time to develop LGD was estimated to be the half of the total follow-up time in that patient. In case EAC was the outcome of a patient, the follow-up time to develop LGD was estimated to be a third of the total follow-up time, whereas the follow-up time to develop HGD was estimated to be two thirds of the total follow-up time for that patient.

Patients who developed HGD or EAC were excluded from further follow-up. As a result we were not able to observe the transition rate from HGD to EAC. For this reason, we added one patient who progressed from HGD to EAC to the observed data. In line with an annual incidence rate of 6%, we assumed a sojourn time of 16 years.[18, 19]

Multi-state Markov model

Based on the characteristics of BE, actual transitions from one stage to another can not be observed. As a result, we only have observed states at discrete moments based on pre-defined follow-up intervals. True progression rates were estimated using a multi-state Markov (MSM) model. In our model, we assumed that true histological regressions

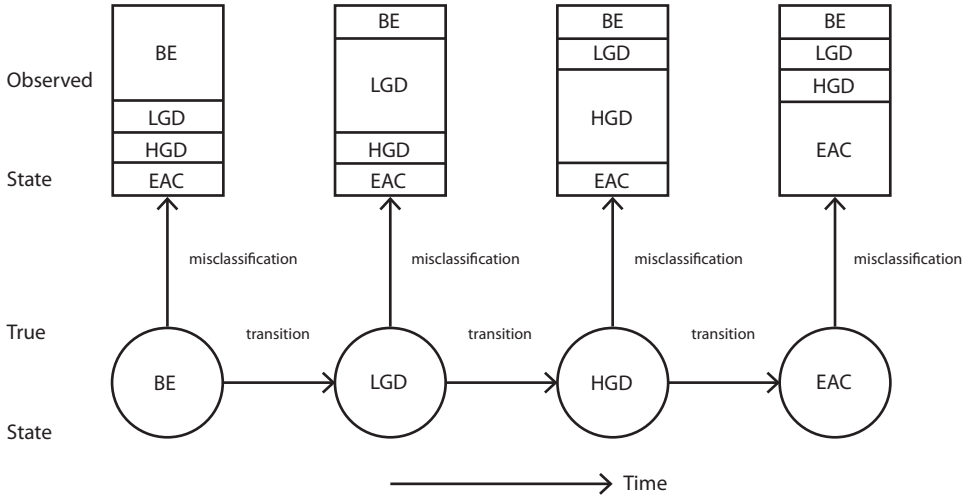

Figure 1. Multi-state Markov model
BE, Barrett's esophagus without dysplasia; LGD, low-grade dysplasia; HGD, high-grade dysplasia; EAC, esophageal adenocarcinoma

were not possible (Figure 1). We furthermore assumed that the annual progression rates to EAC follow a step-by-step natural history and were therefore restricted to progression from one state to the next, for example from LGD to HGD and not directly to EAC (even when this was actually observed). The probability of entering each of these true states was dependent on the time between observations and the progression rates. The observed state depended on the true state and misclassification rates. As always in Markov models the risk of transition was not dependent on the dwelling time in the current state.

Analyses

We performed an external and an internal analysis of the misclassification rates. In the external analysis, we incorporated recently published misclassification rates as fixed parameters to estimate true progression rates in patients with BE. These recently published misclassification rates were based on previously published observed incidences of progression to EAC in patients with BE[20], as well as on author consensus. In the internal analysis, the misclassification rates were data-driven, based on our prospectively collected data. All analyses were conducted using R-software (R Foundation for Statistical Computing, Vienna, Austria; version 2.10, using the MSM-package[21]).

Table 1. Baseline characteristics of a prospective cohort of patients with BE (n=715)

Variable		N/mean	IQR/percentage
Mean age (yrs)		61	53 - 69
Mean BE length (cm)		4.4	2.0 - 6.0
Sex	Male	523	73
Baseline histology	LGD	108	15
	ND	607	85
Mean period of follow-up (yrs)		3.6	3.5 - 4.1

IQR, interquartile range: 25th percentile – 75th percentile; BE, Barrett's esophagus; LGD, low-grade dysplasia; ND, no dysplasia

Table 2. Observed incidence rates in 715 prospectively followed patients with BE

Progression		Cases			Pyrs of FU	Risk per 1,000 pyrs	95%CI
From	To	Observed*	Interpolated†	Analyzed‡		of follow-up	
ND	LGD	80	26	106	2,564	41	33 - 49
LGD	HGD	6	6	12	350	34	15 - 54
ND+LGD	HGD	16	10	26	2,592	10	3 – 31
ND+LGD	EAC	10	-	10	2,598	4	1 - 6
LGD	ND	139	-	139	2,598	54	45 - 62

ND, no dysplasia; LGD, low-grade dysplasia; HGD, high-grade dysplasia; EAC, esophageal adenocarcinoma; pyrs, personyears of follow-up; 95% CI, 95% confidence interval

*Observed cases means observed during surveillance

†Interpolated cases means assumed to be present according to the sequence of carcinogenesis in BE

‡Analyzed cases means the sum of observed and interpolated cases and used for the analysis of incidence rates

RESULTS

Observed incidence rates of progression in BE

We included 715 patients with a mean age of 60.5 years (Table 1). At baseline, ND was found in 607 patients (85%) and LGD in 108 (15%). A total of 16 incident HGD cases and 10 EAC cases were observed during 2,598 pyrs of follow-up. The observed number of transitions from ND to ND was 1,365, from ND to LGD 84, from ND to HGD 9 and from ND to EAC 5. From LGD to ND the observed number of transitions was 140, from LGD to LGD 55, from LGD to HGD 8 and from LGD to EAC 7. The corresponding annual incidence rates of progression are shown in Table 2.

Misclassification rates

If ND was truly present, this would have been observed in 90% of cases based on the

Table 3. Misclassification rates based on the literature[20] ('external') and on follow-up of 715 patients with BE ('internal')

		Analysis	Observed health state			
			ND	LGD	HGD	EAC
True health state	ND	External	83.5%	14.5%	1.0%	1.0%
		Internal	90% (75-97%)	8.8% (7.5-10%)	0.5% (0.2-1.1%)	0.5% (0.2-0.9%)
	LGD	External	17.5%	69.2%	8.3%	5.0%
		Internal	17% (5-46%)	76% (1.2-100%)	6.3% (1.0-78%)	1.1% (0.02-38%)
	HGD	External	0%	11.5%	77.5%	11.0%
		Internal	0.1% (0.0-100%)	19% (0-100%)	60% (0-100%)	20% (0-100%)
	EAC	External	0%	5%	17.5%	72.5%
		Internal	0.2% (0.0-100%)	2.7% (0-100%)	37% (0-100%)	60% (0-100%)

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

internal misclassification rates (prospectively collected data), compared to 84% based on the external misclassification rates (literature data) (Table 3). The risk that the observed health state would be HGD or EAC was low in both analyses. For a true state of LGD, the observed state would be ND in 17% of cases based on both internal and external analyses. The chance that a truly state of LGD would be observed as HGD or EAC was low in both analyses. If HGD was present, the risk that the observed health state was LGD was 19% in the internal analysis, compared to 12% in the external analysis. In both analyses, the risk that a true EAC state would be diagnosed as ND or LGD was highly unlikely (Table 3).

True progression rates

Using external misclassification estimates, the rate of progression from ND to LGD was low (0.004, 95%CI: 0.002-0.011). True progression rates were increasingly higher for the subsequent histological states, i.e. from LGD to HGD 0.088 (95%CI: 0.007-1.09) and from HGD to EAC 0.307 (95%CI: 0.005-20, Table 4). These rates corresponded to annual risks of 0.4% for ND to LGD, 7.2% for LGD to HGD and 26.4% for HGD to EAC. For example, a patient with ND in a particular year had a 99.6% chance of still having ND by the end of the year and in a patient with LGD this chance was 92% (Table 5).

Using internal, data-driven misclassification rates, the true progression rates were slightly higher (Table 4), with annual risks of 0.9%, 10% and 27%, respectively (Table 5).

Table 4. True progression risks per 1,000 personyears (pyrs) of follow-up in patients with BE

		Risk per 1,000 pyrs of follow-up	95% CI
ND → LGD	External	4	2 - 11
	Internal	10	5 - 20
LGD → HGD	External	88	1 - 1,090
	Internal	128	13 - 1,300
HGD → EAC	External	307	5 - 2,030
	Internal	317	1 - 7,440

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

DISCUSSION

Our MSM-model allowed us to make estimations of misclassification rates and true progression rates based on a large cohort of prospectively followed patients with BE. The internal, data-driven misclassification rates were very similar to the previously reported misclassification rates.[20] As we suspected, the true progression rates differed substantially from the observed progression rates. As shown, the observed progression rate of ND to LGD was 41/1,000 pyrs of follow-up compared to the much lower true progression rate from ND to LGD of 10/1,000 pyrs of follow-up.

Although we observed regressions from LGD to ND, similar to other observational studies[22-24], this can most likely be attributed to misclassification since the generally accepted assumption is that only progression is possible in BE.[1, 25, 26] Accordingly, observed data are often incomplete and subject to misclassification due to sampling error or incorrect assessment of the disease state.[15] To accommodate for these errors, Markov models can be applied by using misclassification rates. For this, we used previously documented (external) and estimated data-driven (internal) misclassification rates, which only slightly affected the estimated true progression rates. Consequently, estimated misclassification rates corresponded well with documented misclassification of disease states. Of note, the confidence intervals were smaller in the external misclassification rate analysis since these were assumed to be fixed and not derived from the same data set. On the other hand, the bias in progression rates may be lower with internally estimated misclassification rates. Although it can be debated whether either external or internal misclassification rates are preferable, we propose to use the latter, since the risk of bias is likely to be more important than the expected loss in precision.

Previously, it has been demonstrated that MSM-models are important for simulating clinical situations that involve a repeated risk over time.[27] For example, MSM models have

Table 5. Annual risks of true progression in patients with BE based on external and internal misclassification rates

		Analysis	Health state after 1 year			
			ND	LGD	HGD	EAC
Health state at start of interval	ND	External	99.6%	0.4%	0.02%	0.002%
		Internal	99%	0.9%	0,06%	0.001%
	LGD	External	0%	91.6%	7.2%	1.2%
		Internal	0%	88%	10.2%	1.8%
	HGD	External	0%	0%	73.6%	26.4%
		Internal	0%	0%	72.8%	27.2%
	EAC	External	0%	0%	0%	100%
		Internal	0%	0%	0%	100%

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

demonstrated a more accurate picture of the natural history of disorders such as breast cancer and bronchiolitis obliterans.[16, 28-30] With regard to the former, these models have suggested a more rapid progression rate with respect to tumor size and node status and were shown to be useful for the evaluation of non-randomized programs of screening for breast cancer.[28-30] For HIV, MSM models have presented a more complete description of the natural history of this infection.[31]

Until now, the true progression rates in BE are unknown. Based on our MSM analysis, we confirm that the risk of progression increases from ND to EAC. This correlates well with the fact that neoplastic progression in BE is a gradual process[1, 25] in which the accumulation of (epi)genetic changes, which will result in disruption of different biological processes at a cellular level, increases for each subsequent step in the neoplastic process. The latter is known to be associated with a higher risk of progression.[32] In this way, our true progression rates provide a more accurate look on the natural course of progression in BE and the risk of transition. Estimates of true progression rates are essential to have accurate information on the natural history, i.e. risk of progression in BE which should be incorporated in a decision-analytic model in order to determine the true cost-effectiveness of surveillance in BE. Previously, cost-effectiveness analyses were based on pooled data from the literature and not on true progression rates from prospectively followed cohorts of BE patients.[8, 9]

There are some limitations to our study. Our internal analysis was based on empirical data from a prospectively followed cohort of BE patients, but the duration of follow-up of this cohort might not have been long enough to accurately assess the risk of neoplastic progression.[33] Secondly, only a small number of patients with BE progressed to HGD or EAC, which broadened the confidence intervals for our assumptions for transition to those

stages. Furthermore, we were not able to observe the transition rate from HGD to EAC, since we considered HGD to be an endpoint in the study.

In conclusion, misclassification rates are an important bias in the assessment of progression rates in BE. The MSM modeling technique can be used when observed rates are considered to be subject to some errors and estimation of true rates is needed. By using misclassification rates, underlying true transition rates of progression in BE can be calculated. These provide a more accurate insight in the natural history of progression in BE, which is essential for further decision-analytic modeling to determine the cost-effectiveness of surveillance in BE.

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Chapter 7

Surveillance of patients with Barrett's esophagus with baseline no dysplasia: a cost-effectiveness analysis

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ABSTRACT

Background and Aim: Guidelines recommend endoscopic surveillance in patients with Barrett's esophagus (BE). Moreover, when high-grade dysplasia (HGD) is detected, an endoscopic intervention is increasingly performed as alternative to esophagectomy. The aim of this study was to evaluate the cost-effectiveness of different surveillance intervals and to compare endoscopic interventions for HGD with esophagectomy for HGD or esophageal adenocarcinoma (EAC), based on a prospectively followed BE cohort.

Methods: In a prospective, multicenter cohort study, 715 BE patients were included with ND (n=607) or low-grade dysplasia (LGD, n=108) at baseline. Patients with BE underwent surveillance endoscopy according to the dysplasia grade present. We used a Multi-State-Markov model to calculate misclassification rates and true progression rates from ND to LGD, HGD and finally EAC, based on this cohort. These progression rates were incorporated in a decision-analytic model, which included quality of life data and estimates of real costs. In patients with baseline ND, we evaluated strategies with different intervals of surveillance (including no surveillance), with radiofrequency ablation (RFA) and/or endomucosal resection (EMR) when HGD was diagnosed and esophagectomy if EAC was diagnosed. We calculated the incremental cost-effectiveness ratios (ICER) for each strategy in terms of costs per quality adjusted life year (QALY).

Results: The true annual progression rate for ND to LGD was low (0.010), but was increasingly higher for LGD to HGD (0.128) and for HGD to EAC (0.317). Surveillance every 5 years with RFA for HGD and esophagectomy for EAC had an ICER of €16,348 per QALY gained compared to no surveillance. Strategies with shorter surveillance intervals or other treatment procedures provided higher costs with similar or more costs per QALY. The most critical variables for this analysis were the true progression rates.

Conclusions: Based on a willingness-to-pay threshold of €20,000 per QALY, endoscopic surveillance at intervals of 5 years combined with endoscopic therapy for HGD and esophagectomy for EAC is the preferred strategy for the management of patients with BE without dysplasia at baseline. As the critical factor in this model is the true progression rate in BE, markers to identify patients with an increased risk of neoplastic progression in BE are needed.

INTRODUCTION

Patients with Barrett's esophagus (BE) have an increased risk of developing esophageal adenocarcinoma (EAC). The development of EAC is assumed to follow the sequence of no dysplasia (ND) to low-grade dysplasia (LGD), high-grade dysplasia (HGD) and finally EAC.[1-3] EAC is associated with a poor prognosis and a high mortality rate with a 5-year survival rate less than 20%.[4, 5] Therefore, endoscopic surveillance in BE patients is recommended to detect neoplastic progression at an early and therefore curable stage.[3, 6-8]

Until now, cost-effectiveness analyses of surveillance in BE have been based on pooled literature data and not on prospectively collected results. In recent reviews, the overall annual risk of EAC in patients with BE was estimated to be 0.6%.[9, 10] The generally accepted assumption is that these EAC incidence rates are overestimating the true incidence rate of EAC in BE due to, amongst others, publication bias, selection bias, a retrospective study design, and/or sampling error.[9-12] In order to overcome these errors, true progression rates in BE can be calculated using a Multi-state Markov model. True progression rates obviously represent the natural history of BE more accurately.

Previous cost-effectiveness studies have used esophagectomy when HGD or EAC was detected and this has led to contradicting results.[13, 14] It is currently generally well accepted that endoscopic treatment is the preferred strategy for the management of HGD and for early stage (T1mucosal) EAC. Therefore, endoscopic interventions should be incorporated in cost-effectiveness analyses.[15] The currently most promising endoscopic interventions include endomucosal resection (EMR) and/or radiofrequency ablation (RFA).[5, 16] Recent studies concluded that RFA is a cost-effective strategy in the management of BE with HGD.[17, 18]

The aim of the current study was to evaluate the cost-effectiveness of different intervals of surveillance in BE with ND and to compare endoscopic interventions with esophagectomy for HGD or EAC, based on a prospectively followed BE cohort.

METHODS

Patients and observed progression rates in BE

We conducted a prospective, multicenter cohort study in 3 university and 12 regional hospitals in The Netherlands. We included 715 patients with a BE segment of 2 cm or more. BE was defined as the presence of specialized intestinal metaplasia.[1, 3] At baseline, BE with ND was detected in 607 patients and LGD in 108 patients. The included patients underwent surveillance with biopsy sampling according to the dysplasia grade present.

Four-quadrant biopsies were taken every 2 cm from the most distal part of the BE segment to the most proximal part of the BE segment. The biopsy samples were reviewed by a local pathologist and by a member of a panel of expert GI pathologists until a majority diagnosis was reached. The primary end point of that study was the incidence of HGD and EAC in patients with baseline ND and LGD.

Observed incidence rates of LGD, HGD, and EAC were calculated by dividing the number of incident cases by the total number of personyears (pyrs) of follow-up in the full study sample or subsets. The mean follow-up period was 3.6 years accounting for 2,598 pyrs of follow-up. According to the sequence of neoplastic progression in BE[1, 2, 19], the assumption was made that a patient who developed HGD also should have passed through the stage of LGD if this was not observed during follow-up. Similarly, a patient who developed EAC also should have passed through the stages of LGD and HGD if this was not observed during follow-up. In case HGD was the outcome in a patient, the follow-up time to develop LGD was estimated to be half of the total follow-up time for that patient. In case EAC was the outcome in a patient, the follow-up time to develop LGD was estimated to be a third of the total follow-up time and the follow-up time to develop HGD was estimated to be two third of the total follow-up time for that patient (Table 1).

True progression rates

The observed incidence rates (Table 1) are subject to misclassification due to several factors including sampling error and interobserver variation and therefore do not represent the true diagnosis or the actual state.[12, 20] Misclassification rates are needed to convert the observed rates in true progression rates. These true progression rates can be estimated using a multistate Markov (MSM) model.[21, 22] Although we observed histological regression in some patients, we assumed that this was not possible for true progressions.[1,23]

The MSM-model was used to calculate true health states and to estimate true rates of progression from ND to LGD, LGD to HGD and finally HGD to EAC in patients with BE (Figure 1, Table 2). In this analysis, the misclassification rates were estimated by the model based on observed data. All analyses were conducted using R-software (R Foundation for Statistical Computing, Vienna, Austria; version 2.10, using the MSM package[24]).

Model

We modified a previously published decision-analytic Markov model which was constructed in Windows Decision Maker (Beta Test version 2010).[13] We developed a computer cohort simulation with a base case of a 55-yr old male with BE with ND. It was assumed that all patients could be candidates for diagnostic and therapeutic procedures.

Table 1. Observed incidence rates in 715 prospectively followed patients with Barrett's esophagus (BE)

Progression		Cases					95% CI	
From	To	Observed*	Interpolated†	Analyzed‡	Pyrs of FU	Annual rate (%)	Lower limit	Upper limit
ND	LGD	80	26	106	2,564	4.1	3.3	4.9
LGD	HGD	6	6	12	350	3.4	1.5	5.4
ND+LGD	HGD	16	10	26	2,592	1.0	0.6	1.4
ND+LGD	EAC	10	-	10	2,598	0.4	0.1	0.6
LGD	ND	139	-	139	2,598	5.4	4.5	6.2

ND, no dysplasia; LGD, low-grade dysplasia; HGD, high-grade dysplasia; EAC, esophageal adenocarcinoma; pyrs, personyears of follow-up; 95% CI, 95% confidence interval

*Observed cases means observed during surveillance

†Interpolated cases means assumed to be present according to the sequence of carcinogenesis in BE

‡Analyzed cases means the sum of observed and interpolated cases and used for the analysis of incidence rates

The natural history of the cohort was modeled to examine the costs and effects (quality-adjusted life-year, QALY) of no surveillance. The model compared no surveillance with various surveillance strategies with different intervals and interventions as have been suggested for patients with BE.

Structure of the Analysis

Sixteen surveillance strategies were evaluated for this cohort. In the first surveillance strategy, endoscopy was only performed when patients had symptoms, such as new or worsening dysphagia or severe pyrosis, and esophagectomy was performed if HGD or EAC was diagnosed ('no surveillance' strategy). In the other 15 surveillance strategies, endoscopic surveillance was performed every 1-5 years with (i) esophagectomy if HGD or EAC was diagnosed ('surveillance and surgery' strategy); (ii) EMR followed by RFA in 90% if HGD was diagnosed and esophagectomy if EAC was diagnosed ('surveillance and EMR/RFA' strategy); and (iii) RFA if HGD was diagnosed and esophagectomy if EAC was diagnosed ('surveillance and RFA' strategy). In the strategies using endoscopic interventions, surveillance was assumed to be indicated after RFA treatment and was conducted based on the grade of dysplasia diagnosed in the biopsies taken during endoscopy. The endoscopic ablation therapy was modeled with a stepwise ablation procedure using the HALO ablation system (Barrx Medical, Sunnyville, California, USA).

The simulation began after baseline endoscopy and the model was run until death. The cycle length was three months. The true progression rates from BE with ND to LGD, to HGD and finally to EAC, and the misclassification rates were incorporated in the model. The annual progression rates were converted to 3-monthly probabilities. The model also

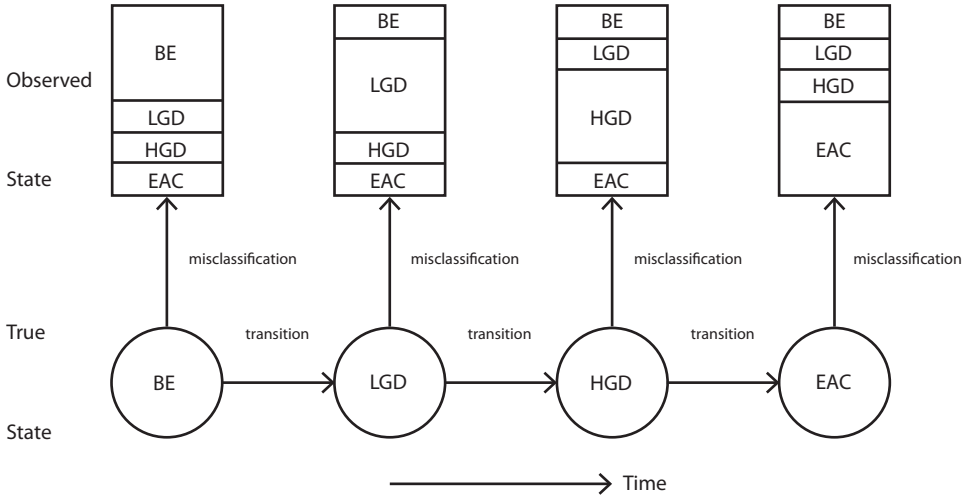

Figure 1. Multi-state Markov model
BE, Barrett's esophagus without dysplasia; LGD, low-grade dysplasia; HGD, high-grade dysplasia; EAC, esophageal adenocarcinoma

included long-term postsurgical states for prognosis of patients after surgery and rates of complications and morbidity after endoscopic interventions. In addition, death from causes other than EAC was assumed to be possible in any state and this was modeled as a time-dependent variable, related to patient age.

Costs and quality of life data

Real costs, not charges, were used in this model. Actual variable costs for endoscopic and surgical procedures and costs for inpatient postoperative care were obtained using the cost of the 2008 Diagnosis Treatment Combinations (DBC) from the NzA (Dutch Healthcare Tariff Board). A DBC can be defined as a predefined average package of care (diagnosis plus treatment) which in most cases has a fixed price. It accounts for all specific diagnoses made. However, not all interventions are encoded in a DBC and these prices were obtained from expert opinion.

Different utilities for different health states were derived from the published literature[5, 13, 16] and were used to convert absolute life-years experienced by cohort members to quality-adjusted life-years (QALYs). Costs and utilities were discounted at an annual rate of 3%. We also performed an analysis using a discount rate of 5%, which allowed us to compare our results with other published analyses[25] (see Appendix).

Analysis

The primary outcome of the study was the incremental cost per QALY between competing surveillance strategies, also known as the incremental cost-effectiveness ratio (ICER). The ICER is defined as the difference in costs when moving from one strategy to another strategy, divided by the change in QALYs between the same two strategies. Policy makers can use these ratios to compare cost-effectiveness of treatments or diagnostic protocols, in this case surveillance of patients with BE, to that of other commonly applied medical practices, depending on the willingness- to-pay threshold.[26]

In one-way sensitivity analyses, certain assumptions for the model were varied over a wide range of values. Ranges were based on published data. In the absence of these, baseline rates were halved and doubled to examine the effect.

RESULTS

True progression rates

Using data-driven, misclassification rates, the annual progression rate of ND to LGD was low (0.010, 95%CI: 0.005-0.02). True progression rates were increasingly higher for increasing histological states, i.e., from LGD to HGD 0.128 (95%CI: 0.013-1.3) and from HGD to EAC 0.317 (95%CI: 0.001-74.4) (Table 2). These rates corresponded to annual progression risks of 0.9% for LGD, 10% for HGD and 27% for EAC.

Natural history

Using baseline assumptions, the model calculated an overall annual incidence of EAC of 0.45% among 55yr-old male patients with BE and ND, which corresponds to 1 case per 222 pyrs of follow-up. This correlates well with the overall observed EAC incidence in the prospectively followed cohort of BE patients (0.4%).

Base case results

The costs of the strategy 'no surveillance' were € 5,048 for 15.61 discounted QALYs (Table 3). Compared to 'no surveillance', the strategy 'surveillance every 5 years with RFA' extended life by 0.3 QALYs (15.92 vs. 15.61) at an additional cost of € 4,977 (€10,025 vs. € 5,048). Thus, the ICER for this strategy was € 16,348 per QALY gained (€ 4,977 / 0.3 QALYs). Compared to 'surveillance every 5 years with RFA', the strategy 'surveillance every 4 years with RFA' increased effects by 0.02 QALY at an additional cost of € 1,205. Hence, the ICER for this strategy was € 60,554 per QALY gained. Assuming a maximum of willingness-to-

Table 2. Variables used as input in the cost-effectiveness analysis of surveillance in Barrett's esophagus (BE)

Variables		Base value	
True transition rates (per 3 months)			
ND → LGD		0.003	
LGD → HGD		0.03	
HGD → EAC		0.076	
Misclassification rates (per 3 months)			
True state	Observed state		
ND	LGD	0.088	
ND	HGD	0.005	
ND	EAC	0.005	
LGD	HGD	0.063	
LGD	EAC	0.011	
HGD	LGD	0.194	
HGD	EAC	0.202	
EAC	LGD	0.027	
EAC	HGD	0.374	
Costs (€)			a)
Costs of endoscopy		600	
Costs of endoscopy with complication		1,800	
Cost of perforation requiring surgery		5,773	
Costs of RFA		5,000	
Costs of EMR		865	
Costs of esophagectomy		18,755	
Costs of hospice care/year		31,025	
Costs of annual follow-up postesophagectomy		903	
Discount rate (costs and effects)		0.03	
Mortality in unresectable cancer		1.12	13
Short- and long term morbidity			
Short term			
Endoscopy		1 day	13, b)
Endoscopy with complication		1 week	13, b)
Elective surgery		2 weeks	13
Emergency surgery		4 weeks	13
EMR		3 days	b)
RFA		3 days	5, 16
Long term			
Quality of life postesophagectomy		0.97	13

Table 2. Continued

Variables	Base value	
Endoscopic and surgical procedures		
Endoscopy		[13]
Complications	0.0013	
Mortality	0.0192	
Perforation requiring surgery	0.657	
Surgical mortality		[13]
Elective esophagectomy	0.04	
Repair of esophageal perforation	0.02	

ND, no dysplasia; LGD, low-grade dysplasia; HGD, high-grade dysplasia; EAC, esophageal adenocarcinoma; RFA, radiofrequency ablation; EMR, endomucosal resection;
a), based on Diagnosis Treatment Combinations from NzA or expert opinion;
b), author consensus

pay threshold of € 50,000 per QALY[26] this strategy is not a cost-effective alternative for BE surveillance.

Compared to 'surveillance every 5 years with RFA', the strategy 'surveillance every 5 years with EMR and RFA' had similar effects (QALYs). However, the additional costs for this strategy were € 45 higher (Table 3). Consequently, the strategy 'surveillance and EMR/ RFA' was dominated by the strategy 'surveillance and RFA'.

Although the strategy 'surveillance and surgery' extended life expectancy over 'no surveillance', this strategy provided fewer QALYs at higher additional costs than the strategy 'surveillance and RFA' (Figure 2).

Sensitivity analysis

The most critical variables were the true progression rates in BE. If progression rates were halved, no strategy was cost-effective compared to 'no surveillance' assuming a maximum willingness-to-pay threshold of €50,000 per QALY[26]. In contrast, if these progression rates were doubled, 'surveillance every three years with RFA' and 'surveillance every two years with RFA' were still cost-effective assuming a willingness-to-pay threshold of €20,000 and 50,000 per QALY, respectively (ICERs of € 14,923 per QALY gained and € 27,358 per QALY gained respectively). In addition, if we decreased the utility of quality of life (QoL) value post-esophagectomy to 0.90, the ICERs increased. However, surveillance every 5 years with RFA for HGD remained cost-effective (ICER: €17,094). For post-esophagectomy QoL values < 0.70, 'no surveillance' is the preferred strategy.

Table 3. Base case analysis of cost-effectiveness of surveillance in patients with Barrett's esophagus (BE) (Discount rate 3%)

Strategy	Costs (€)	QALYs	ICER (€)
No surveillance	5,048	15.61	
Surveillance every 5 years with RFA	10,025	15.92	16,348
Surveillance every 5 year with EMR+90% RFA	10,070	15.92	x
Surveillance every 4 years with RFA	11,230	15.94	60,554
Surveillance every 4 year with EMR+90% RFA	11,278	15.94	x
Surveillance every 3 years with RFA	13,052	15.95	122,245
Surveillance every 3 year with EMR+90% RFA	13,104	15.95	x
Surveillance every 5 years with esophagectomy	14,855	15.85	x
Surveillance every 2 years with RFA	16,105	15.96	1,327,543
Surveillance every 2 year with EMR+90% RFA	16,163	15.96	x
Surveillance every 4 years with esophagectomy	16,490	15.87	x
Surveillance every 3 years with esophagectomy	18,784	15.87	x
Surveillance every 1 years with RFA	22,140	15.93	x
Surveillance every 1 year with EMR+90% RFA	22,209	15.93	x
Surveillance every 2 years with esophagectomy	22,347	15.86	x
Surveillance every 1 years with esophagectomy	29,833	15.82	x

RFA, radiofrequency ablation; EMR, endomucosal resection; QALYs, discounted quality adjusted life-years; ICER, incremental cost-effectiveness ratio; x, dominated strategy

DISCUSSION

Our study shows that for BE patients with baseline ND, surveillance with endoscopic interventions is a better cost-effective strategy compared to endoscopic surveillance with surgery for HGD or EAC. Compared to previous studies, the strength of our study is that it is based on true progression rates estimated from a prospectively followed cohort of BE patients instead of using a pooled incidence rate of EAC.[13, 14, 17, 27, 28] In this way, we limited the possibility of overestimating the progression rates by a more accurate simulation of the natural history of progression from BE to EAC.

Endoscopic therapy is increasingly being performed as the management of HGD.[5, 15, 29] Therefore, we incorporated endoscopic interventions, apart from esophagectomy, in this cost-effectiveness analysis. Until now, the most promising endoscopic interventions in BE are EMR and RFA. EMR is performed when mucosal irregularities suspect for neoplastic progression are present.[29, 30] An EMR specimen allows evaluation of tissue invasion.5 Previous studies have shown that RFA leads to complete eradication of BE in cases of HGD and is also used as an additional treatment after EMR with a high rate of complete

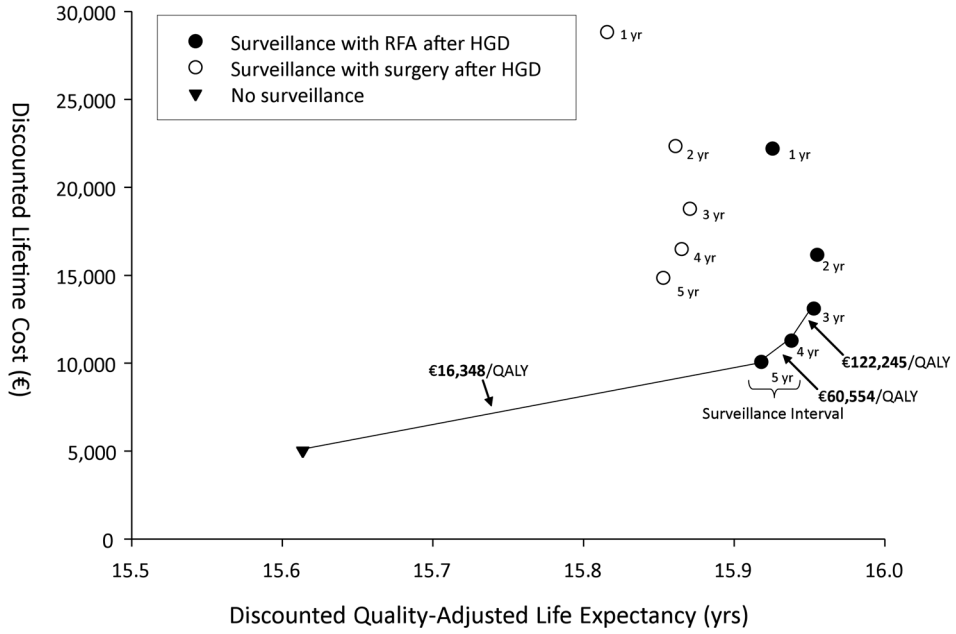

Figure 2. Cost-effectiveness analysis (discount rate 3%). The horizontal axis displays discounted quality-adjusted life expectancy in years for several surveillance strategies and the vertical axis displays the average discounted life-time cost per patient (discount rate 3%). Open circles represent the results for the endoscopic surveillance strategies every 1-5 years with 'surgery after high-grade dysplasia (HGD)/ esophageal adenocarcinoma (EAC)'. Black circles represent the results for the endoscopic surveillance strategies every 1-5 years with 'Radiofrequency ablation (RFA) after HGD (including esophagectomy for EAC)'. The triangle represents the strategy 'no surveillance'. The incremental cost-effectiveness ratio of moving from one strategy to the other is shown with the arrows.

eradication of BE. RFA has a low complication rate compared to other ablation techniques.[5, 16, 31] Previously, it has been demonstrated that in patients with baseline HGD, endoscopic ablation for HGD is a cost-effective strategy.[17] Due to uncertainty on the long term cancer risk after treatment with RFA, we chose to model different intervals of surveillance and combined this with ongoing endoscopic surveillance when RFA was completed.

The cost-effectiveness of a strategy does however not only depend on the additional benefit and additional costs, i.e. the ICER, but also on the willingness-to-pay threshold.[13] In the Netherlands, the threshold for a cost-effective screening or surveillance strategy is €20,000 per QALY gained.[26] Given this threshold, surveillance every 5 years combined with RFA for HGD and esophagectomy for EAC should be considered cost-effective. We also showed that the effectiveness of the strategy using EMR before RFA was similar, with little variation in costs. These two endoscopic intervention strategies are therefore comparable

especially when costs and benefits of these techniques are taken into account. Our results are in line with a previously performed cost-effectiveness analysis, which only used esophagectomy in case HGD or EAC was observed.[13] If we compare the cost-effectiveness of BE surveillance in the Netherlands, we found it however to be a less favorable medical strategy compared to other strategies (e.g. cervix cancer screening €12,500/QALY and breast cancer screening €4,000/QALY).

The progression rates from ND to EAC are the most critical variables for decision making regarding surveillance. If these rates are higher, more frequent surveillance strategies will become more cost-effective. The incidence of EAC in our model was very similar to the observed incidence rate of EAC in various meta-analyses.[10, 32-34] Our model provides a realistic look at the estimates of progression risks and surveillance in BE. Hence, some key variables of the model are subject to uncertainty such as the efficacy and durability of the effects of endoscopic interventions and the prognosis of patients treated with RFA. Long-term follow-up data of the above-mentioned ablation studies is therefore warranted.

In our prospective cohort study we observed that in the majority of patients who progressed to HGD or EAC this occurred within 2 years after the start of the study. This finding corroborates the generally accepted opinion that the risk of progression is highest in the first 2 years after commencing surveillance. However, there is no clear documentation of this pattern in the literature. However, if we would apply the strategy of surveillance every 5 years, neoplastic progression could well have been missed in patients with BE. We therefore suggest to repeat upper endoscopy 2 years after the first endoscopy in patients with BE with ND. If this second endoscopy reveals the same histology, i.e. BE with ND, surveillance can be extended to every 5 years. Furthermore, if mucosal irregularities in BE are found during endoscopy, EMR before RFA should be performed. As we have no clinical evidence yet for this assumption, future research elucidating these issues is required.

Our study has some limitations. As we do not know the true state at the start of the model, we assumed this was no dysplasia. Our results are therefore biased conservatively. Second, although we calculated true progression rates based on a prospective cohort study, the follow-up period was relatively short and the number of events was low. Moreover, we did not have data on early-stage (T1mucosal) EAC during follow-up. We therefore restricted the use of endoscopic therapy to patients who developed HGD. In the future, with longer follow-up data, more reliable estimates of true progression rates in BE can be made. Third, we have not modeled a strategy of surveying patients with baseline LGD. Fourth, the results of treatment with EMR, RFA and surgery were based on different studies from the literature, with variations in study design. Finally, it is generally accepted

that only a (small) subgroup of patients with BE will develop neoplastic progression.[9-11] Until now, factors that are able to identify high-risk patients are insufficiently validated in larger studies and could therefore not be used to determine risk-specific surveillance. We recognize that our model needs to be refined and updated when specific risk factors and/or biomarkers become available for a more individualized surveillance strategy.

In conclusion, endoscopic surveillance every 5 years combined with endoscopic therapy for HGD and esophagectomy for EAC was found to be the preferred strategy for the management of patients with BE with ND at baseline. Future studies are needed to determine the long-term effects of endoscopic therapy. Similarly, risk factors need to be identified and validated in order to support an individualized surveillance protocol in patients with BE and improve the cost-effectiveness of surveillance in BE.

APPENDIX

Table 1. Base case analysis of cost-effectiveness of surveillance in patients with Barrett's esophagus (BE) (Discount rate 5%)

Strategy	Costs (€)	QALYs	ICER (€)
No surveillance	3,613	12.66	
Surveillance every 5 years with RFA	7,611	12.84	21,357
Surveillance every 5 year with EMR+90% RFA	7,644	12.84	x
Surveillance every 4 years with RFA	8,525	12.85	76,179
Surveillance every 4 year with EMR+90% RFA	8,561	12.85	x
Surveillance every 3 years with RFA	9,919	12.86	160,210
Surveillance every 4 year with EMR+90% RFA	9,959	12.86	x
Surveillance every 5 years with esophagectomy	11,024	12.80	x
Surveillance every 4 years with esophagectomy	12,266	12.80	x
Surveillance every 2 years with RFA	12,293	12.86	X
Surveillance every 2 year with EMR+90% RFA	12,339	12.86	X
Surveillance every 3 years with esophagectomy	14,028	12.81	X
Surveillance every 2 years with esophagectomy	16,820	12.80	X
Surveillance every 1 years with RFA	17,157	12.84	X
Surveillance every 1 year with EMR+90% RFA	17,211	12.84	X
Surveillance every 1 years with esophagectomy	22,115	12.76	X

RFA, radiofrequency ablation; EMR, endomucosal resection; QALYs, discounted quality adjusted life-years; ICER, incremental cost-effectiveness ratio; x, dominated strategy

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Chapter 8

General discussion and conclusion

M. Sikkema

INTRODUCTION

The incidence of esophageal adenocarcinoma (EAC) is still increasing in the Western world.[1-3] In the Netherlands, 1,167 patients were newly diagnosed with EAC in 2008 compared to 830 new cases in 2002.[4] Barrett's esophagus (BE) is a premalignant condition that is associated with an increased risk of developing EAC compared to the general population. Therefore, patients with BE are recommended to undergo endoscopic surveillance with the aim to detect neoplasia at an early and therefore curable stage.[5] Currently, the interval of surveillance is based on the histological evaluation of randomly taken biopsy samples despite limitations such as sampling error and interobserver variation in grading dysplasia in histological slides.[6, 7] Furthermore, the efficacy of surveillance in BE is disputed due to the contradictory results of the impact of surveillance on cancer incidence and mortality. In addition, due to ambiguity about the natural history of BE and the varying progression rates to EAC, cost-effectiveness analyses of surveillance of and interventions for BE have reported conflicting results.[8, 9] A way to improve the effectiveness of surveillance is by risk stratification of different patient categories. However, this is currently not feasible as the known risk factors are commonly present in the community and predictors such as biomarkers are not yet validated and ready for clinical use.[10]

The aim of this thesis was to assess the incidence of neoplastic progression in patients with BE, to investigate risk factors involved in neoplastic progression, and to evaluate the role of these factors in identifying patients at a high risk of progression. These data were used to determine the yield and cost-effectiveness of surveillance in patients with BE.

INCIDENCE RATES OF PROGRESSION IN BARRETT'S ESOPHAGUS

Despite the fact that the reported annual incidence of EAC in BE patients have shown considerable variation, the generally accepted incidence of EAC is around 0.5% per year.[11-13] However, biases may have occurred due to several factors including publication bias and selective reporting[13], a retrospective study design, a limited size of study populations and a relatively short duration of follow-up in some studies. In addition, EAC-specific mortality rates in BE patients have shown contrasting results in various studies.[14, 15] Finally, it has been reported that the overall mortality in BE patients is increased compared to the general population[15, 16], whereas others were not able to confirm this.[9] In an effort to combine all these reported incidence and mortality rates, a meta-analysis could provide detailed information on the incidence and mortality rates of EAC in patients with BE. In **Chapter 2**, the results of a meta-analysis on incidence and mortality rates of EAC in BE patients are reported. It was shown that the overall pooled incidence of EAC in patients

with BE was 6.3 cases per 1,000 pyrs of follow-up and that the overall pooled incidence of EAC and HGD combined 10.2/1,000 pyrs, which corresponded to annual risks of 0.6% and 1.0%, respectively. Our findings are in agreement with previously published meta-analyses on the risk of developing EAC in BE.[13, 17-19] Similarly, we showed a decline in EAC incidence to 0.5%/yr when studies with less than 500 pyrs of follow-up were excluded.[18, 19] This emphasizes the importance of study size for the determination of the cancer incidence in BE. Furthermore, the overall pooled mortality due to EAC in patients with BE was even lower, i.e., 0.3%/yr. This emphasizes that EAC-related mortality in BE under surveillance is low compared to a 12-fold higher mortality rate due to other causes than EAC. Unfortunately, we can only speculate whether this is due to a preventive effect of surveillance on EAC-related mortality or to the fact that the natural history of progression in BE is indeed low. Previous long-term follow-up data from the Rotterdam cohort without surveillance in the first two decades provided also support for the latter argument.[20]

In order to determine accurate progression rates in BE, a prospective multicenter cohort study was performed in which 715 patients were included with 607 patients with no dysplasia (ND) and 108 with low-grade dysplasia (LGD) at baseline **(Chapter 5)**. The observed overall incidence rate of EAC was 0.4% per year, ranging from 0.2% per year in patients with baseline ND up to 1.7% per year in patients with baseline LGD. For HGD and EAC, the observed overall annual incidence rate was 1.0% (baseline ND: 0.6%/year; baseline LGD: 3.4%/year). We found a higher incidence rate for HGD than for EAC alone. This is due to the interpolation of 'missed' HGDs when patients were diagnosed with EAC. In this study, we observed relatively low overall incidence rates of HGD and/or EAC[21] in BE during follow-up, with patients with baseline LGD having a considerably higher risk than those with ND. The low incidence rate of EAC is close to the rate used in previously reported cost-effectiveness analyses.[8, 9] These studies indicated that the cost-effectiveness of the currently employed surveillance protocol in BE is questionable.

The observed progression rates reported in the previous chapters were subject to misclassification, due to sampling error or interobserver variation. This likely may lead to overestimation of the true progression rates in patients with BE. In order to correct for such diagnostic errors, multi-state Markov (MSM) models can be used that apply misclassification rates to estimate true progression rates in BE.[22, 23] In **Chapter 6**, such a model was used to calculate true progression rates in BE, using our cohort as well as external data for input. The true progression rates differed substantially from the observed progression rates. The observed progression rate of ND to LGD was 41.3/1,000 pyrs of follow-up compared to the much lower true progression rate of ND to LGD of 10/1,000 pyrs of follow-up. True progression rates were increasingly higher for each subsequent

histological transition. This correlates well with the natural history of neoplastic progression in BE as the accumulation of (epi)genetic changes, resulting in disruption of different biological processes at a cellular level, increase per subsequent step in tumor development.[24] Therefore, estimations of true progression rates are useful in providing a more accurate idea of the natural history of BE. Subsequently, these true rates can be incorporated in a decision-analytic model to determine the cost-effectiveness of surveillance in BE more precisely.

Until now, it can be concluded that the observed incidence of EAC in BE is approximately 0.5-0.6% per year based on our review including all previously published studies[13, 18, 19, 25] and the cohort study that we performed. As the rate of progression to EAC in BE appears to be low, surveillance in patients with BE might well be disputable.

One of the remaining gaps in the knowledge about BE is the prevalence of BE in the general population. One Scandinavian study reported a prevalence of 1.6%[26] which implies that the number of BE patients in the population is rather large. This could make surveillance of BE from a practical point of view complicated.[27] Nonetheless, in clinical practice, the overall prevalence of BE is largely unknown and only the group of patients who are symptomatic or undergo upper endoscopy for other reasons is currently known. Another, more important gap in our knowledge is the identification of BE patients at increased risk of progression. It is well recognized that only a subgroup of patients with BE will develop progression to HGD or EAC. Therefore, risk factors to identify patients who are at the highest risk of neoplastic progression in BE are needed.

PREDICTORS OF PROGRESSION IN BARRETT'S ESOPHAGUS

Due to the low annual risk of progression to EAC in BE patients, the majority of patients will not benefit from an endoscopic surveillance program. Identification of risk factors is needed to predict which patients with BE are at an increased risk of developing HGD or EAC. This risk stratification would allow individualization of surveillance in patients with BE, reduce patient burden and endoscopic demand, and improve cost-effectiveness of BE surveillance programs.

Several biomarkers have been examined that could be used in combination with histology to stratify individual patients according to the risk of neoplastic progression. A ideal biomarker should show variation in expression depending of the stage of progression and this should be detectable at an early stage in this process.[28] In Table 1, an overview is given of the currently known biomarkers that can be used in the surveillance of Barrett's esophagus.

Table 1. Overview of biomarkers for identifying BE patients with an increased risk of progression to HGD or EAC

Biomarker	Technique	Findings	Conclusion	Ref.
DNA content abnormalities				
Aneuploidy	Flow cytometry	Predictive value has been shown for fresh frozen samples. Very expensive and technically challenging Data on paraffin embedded tissue are warranted	Not easy to apply in clinical setting	35, 39
	Image cytometry	Emerging technique Less expensive and easier to apply	Validation studies are needed	40, 41
SNP	Genome wide assay	Promising results for SNP-based genotyping	Large validation studies are needed	42
Tumor suppressor genes				
P16 LOH	PCR	No evidence between silencing of p16 and dysplasia grade	Not useful	43, 44
P53 LOH	PCR	Most promising biomarker Very expensive and technically challenging	Not easy to apply in clinical setting	36, 39, 45
P53 staining	IHC	Promising biomarker Overexpression does not always correlate with mutations	Easy to apply and cheap	31, 46, 47
Proliferation markers				
MCM-2	IHC	Aberrant surface expression might predict progression.	Large validation studies are needed	48
Ki67	IHC	Controversial results on predictive value Cheap and easy to apply Useful in combination with other markers	As single marker not useful as biomarker	34, 47, 49
Cell cycle markers				
Cyclin A	IHC	Promising results	Prospective studies are needed to determine the usefulness	50
Cyclin D	IHC	Controversial results		46, 51
Epigenetic changes				
Hypermethylation	Methylation specific PCR	Promising biomarker Technically challenging and time consuming	Not easy to apply in clinical setting	52-55
Clonal diversity measures	Flow cytometry, PCR	Robust predictive value Technically challenging and time consuming	Not useful as single marker and needs to be combined in a panel	56

LOH, loss of heterozygosity; SNP, single nucleotide polymorphism; PCR, polymerase chain reaction; IHC, immunohistochemistry

In a case-control study **(Chapter 3)**, we evaluated the clinical value of three biomarkers for the detection of neoplastic progression in BE, i.e., p53, Ki67 and aneuploidy.[29] Overexpression of p53, a tumor suppressor gene, resulted in a 5-fold increased risk of

progression to HGD or EAC, which is in line with previous studies.[30-32] Overexpression of Ki67, exclusively present in proliferating cells, resulted in a 2- to 3-fold increased risk of progression to HGD or EAC as was demonstrated in other studies.[33, 34] DNA ploidy had no additional value in the prediction of neoplastic progression which is in contrast to previous studies.[35] In line with other studies that suggested that a panel of biomarkers is needed to identify BE patients with the highest risk of neoplastic progression[29, 36], we developed a simple sum score. As a result, if 3 to 4 abnormal prognostic factors were present, the development of HGD or EAC over time could be predicted with 12- to 34-fold increased risks, respectively. In clinical practice, patients with an increased risk should probably undergo more frequent follow-up endoscopy. It is currently too premature to actually change current surveillance strategies and further validation of these markers in larger, prospective studies is required to confirm our findings.

Apart from biomarkers, identification of easy-to-apply risk factors could aid in identifying patients at an increased risk of neoplastic progression. In line with other studies, we confirmed that the presence of LGD and a longer length of BE were predictors of progression in BE **(Chapter 4)**.[37, 38] To our knowledge, this is the first study that clearly demonstrated that a longer known duration of BE and the presence of esophagitis were also predictors of progression to HGD and EAC in BE. Other clinical factors such as male gender, high body mass index and presence of a hiatal hernia were not predictive for progression. If applied in a prediction model, patients with LGD and all of the above mentioned significant risk factors were found to have the highest annual risk of progression. Patients with ND and the same risk factors or patients with LGD without presence of these risk factors had a moderate increased risk of neoplastic progression. Patients with ND and no other risk factors had the lowest risk of progression. If further validated, these individualized risk estimates may aid in individualizing surveillance in patients with BE.

In conclusion, several biomarkers and other risk factors are known to be associated with neoplastic progression in BE. Unfortunately, these factors are not yet ready for clinical use. Based on our population, we were able to define the group of patients with the highest risk of progression, which should undergo more frequent surveillance endoscopy, but also the group of patients with the lowest risk of progression. Yet, this is based on observational data. We are still missing validated (bio)markers that are able to precisely identify high-risk patients. More research is needed on genetic susceptibility markers and on the use of genetic polymorphisms to identify the subgroup of patients at the highest risk of EAC. Ideally, a Barrett's risk score[10] should be developed based on combinations of clinical and biomarker variables.

SURVEILLANCE IN BARRETT'S ESOPHAGUS

Until now, randomized controlled trial data on the effectiveness of surveillance in BE in reducing EAC-related morbidity and mortality is lacking. Nonetheless, endoscopic surveillance is already widely performed in patients with BE. The only way to assess the effectiveness of surveillance in BE is by performing cost-effectiveness analyses. Previously published cost-effectiveness models have shown inconsistent results and have also used different therapeutic interventions.[8, 9, 57]

We performed a cost-effectiveness analysis based on true progression rates and compared endoscopic interventions and esophagectomy with esophagectomy alone in patients with BE **(Chapter 7)**. The true progression rates were based on a prospectively collected data set of BE patients instead of using a pooled incidence rates of EAC, as was done previously.[8, 9, 57-59] In this way, we limited the possibility of overestimating progression rates and we assumed that this resulted in a more accurate simulation of the natural history of progression from BE to EAC.

As endoscopic therapy is currently the preferred strategy for the management of HGD[60-62], we incorporated endoscopic interventions for HGD in this cost-effectiveness analysis. Until now, the most promising and commonly performed endoscopic interventions in BE include endomucosal resection (EMR) and radiofrequency ablation (RFA). EMR is performed to resect mucosal irregularities in BE that are suspected of containing already early malignancy[62, 63], whereas RFA is performed to ablate diffusely present HGD in BE.[61, 64, 65] In a study from the USA, it has previously been demonstrated that endoscopic ablation with endoscopic surveillance afterwards is a cost-effective strategy in patients with HGD.[57]

A strategy is called cost-effective if the additional benefit obtained from that strategy is worth the additional cost. It is also dependent on a threshold of willingness-to-pay for a strategy. In the Netherlands, the willingness-to-pay threshold is around €20,000 per Quality Adjusted Life Year (QALY) gained.[66] We demonstrated that surveillance endoscopy every 5 years combined with RFA for HGD and esophagectomy for EAC was cost-effective. The strategy of using EMR before RFA was comparable to RFA alone, with little variation in costs. Our results are in line with a previously performed cost-effectiveness analysis from the USA, that included esophagectomy as intervention for HGD or EAC.[8] Compared to other cost-effective strategies that are considered to be acceptable in the Netherlands such as screening for cervix cancer screening (€12,500/QALY) and breast cancer (€4,000/QALY)[66], the strategy of surveillance every 5 years for BE was found to be less favorable.

In order to improve the cost-effectiveness of surveillance of BE, the long-term effects of endoscopic therapy, i.e., RFA and EMR, should be determined, particularly with regard

to the decision whether long term endoscopic surveillance is indeed indicated after RFA or can be stopped after some time, for example 3-5 years after the end of treatment. Another way to improve cost-effectiveness of surveillance is to imply risk stratification (high-risk vs. low-risk groups for neoplastic progression) as was discussed above.

In **Chapter 5**, we observed that the majority of patients with BE and baseline ND developed progression to HGD or EAC within the first 2 years after inclusion. Therefore, we recommend to perform the first surveillance endoscopy in patients with BE and ND within 2 years after the initial endoscopy. If the second endoscopy again shows BE without dysplasia, surveillance can be extended to 5 years. In contrast, in patients with baseline LGD, the most optimal surveillance strategy still needs to be determined as this was not yet clear using our decision-analytic model and further work needs to be done in this regard. Apart from this, further studies are needed to confirm and implement our findings.

CONCLUSIONS AND FUTURE DIRECTIONS

EAC shows a rapidly increasing incidence in the Western world and is associated with a poor prognosis and high mortality. BE is a premalignant condition predisposing to the development of EAC via the metaplasia-dysplasia-carcinoma sequence. For this reason, all patients with BE are recommended to undergo surveillance according to the guidelines of the American College of Gastroenterology. Nonetheless, it should be recognized that only a subgroup of patients will develop neoplastic progression. Until now, no randomized controlled trials have demonstrated the value of endoscopic surveillance in BE and, in addition, the results from previous cost-effectiveness analyses are controversial. Currently, the interval of surveillance is based on the histologic diagnosis in BE, i.e., the grade of dysplasia.[67, 68] In order to improve the effectiveness of surveillance in BE, risk stratification that is not only based on histology is needed as well as an increasing application of endoscopic interventions for HGD and early stage EAC to reduce the risk of morbidity and mortality that is associated with esophagectomy.

This thesis shows that the observed annual incidence of EAC in a prospectively followed cohort is 0.4%, which is in line with previous studies. We also concluded from a meta-analysis of the literature that the mortality due to EAC is low in patients undergoing surveillance. As we know that the observed progression rates in BE are subject to misclassification and diagnostic errors, we estimated true progression rates based on a MSM-model. Using this model, we were able to describe the natural history of progression from BE to EAC in a more accurate way. Based on these true progression rates, we

demonstrated that surveillance every 5 years in patients with BE and baseline ND was a cost-effective strategy if endoscopic treatment for HGD and surgery for EAC is employed.

However, surveillance according to this scheme remains expensive. In an effort to reduce costs, endoscopy demand, and patient burden, risk stratification is needed which should be based on the recognition of high risk patients who should follow an individualized more frequent surveillance program. We found that length of BE, presence of esophagitis, a longer duration of BE and presence of LGD were such risk factors. In addition, the use of biomarkers should also be considered as they can be used as tools for predicting the risk of neoplastic progression. Particularly the biomarkers Ki67 and p53 were found to be useful in identifying high risk patients. Our preliminary prediction models were able to identify the group of patients with the highest risk of progression to HGD or EAC.

Future research is needed to describe the progression rates in the pathway from BE with ND to finally EAC more precisely. This will require longer follow-up data. If present, a more accurate knowledge of the natural history of the BE-EAC sequence will be available. In addition, long-term results of endoscopic ablative and resection techniques are needed as it is currently unknown whether endoscopically treated patients with BE are indeed cured with no remaining risk of recurrence of developing BE and/or malignancy. In addition, more research is needed to identify reliable biomarkers or a panel of markers which can used to identify patients at the highest risk of developing EAC. In this regard, promising results have been reported for clonal diversity measures.[56] Another way to assess genetic factors associated with the development of EAC are genome wide association studies which should be performed in large cohorts of patients with BE. Also, a reliable animal model to investigate the development of neoplastic progression in BE is needed as until now only cell lines and ex vivo cultures are available. If an animal model is available, mechanisms of BE formation and neoplastic progression is BE can be studied. In addition, new potential chemopreventive agents can be tested that are able to delay or even halt neoplastic progression in BE.

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Chapter 9

Summary
Samenvatting

SUMMARY

Barrett's esophagus (BE) is a premalignant condition predisposing to the development of esophageal adenocarcinoma (EAC). This malignancy has a poor prognosis with a high mortality rate and its incidence has been increasing during the past decades. For this reason, patients with BE are recommended to undergo endoscopic surveillance to detect neoplastic changes at an early and therefore curable stage. The interval of surveillance is only based on histologic interpretation despite its limitations. So far, the efficacy of surveillance has not been shown. In addition, the incidence of EAC in patients with BE shows considerable variation in reported studies. Hence, it is established that only a subgroup of BE patients will develop progression to EAC. For these reasons, data on the natural course of progression in BE are needed as well as identification of predictors of progression to distinguish between low and high risk patients and to individualize surveillance intervals. In this way, cost-effectiveness of surveillance in BE could be improved and mortality due to EAC reduced.

The aims and outline of this thesis are described in the first chapter of this thesis.

In **Chapter 2**, we calculated an overall incidence of EAC of 6.3/1,000 personyears (pyrs) of follow-up (95%CI: 4.7-8.4). This was based on 50 studies comprising 14,109 patients followed up for a total of 61,804 pyrs. The heterogeneity between these incidence rates was considerable (χ^2=258.2; df=49; p<0.001; I^2=79%). A sub-analysis of a total of 19 studies with mortality as endpoint, including 7,930 patients followed up for 33,022 pyrs, revealed an overall pooled incidence of EAC of 3.0/1,000 pyrs of follow-up (95%CI: 2.2-3.9), with no evidence of heterogeneity (χ^2=19.3; df=18; p=0.4; I^2=7%). The overall pooled mortality due to other causes than EAC was 12-times higher. We concluded that patients with BE are at low risk of malignant progression and predominantly die from other causes than EAC.

In order to identify risk factors of progression in BE, we evaluated 3 promising biomarkers next to histology in **Chapter 3**. This case-control study included 27 BE patients with histologically proven progression to high-grade dysplasia (HGD) or EAC and 27 BE patients without progression. Multivariable analysis showed that low-grade dysplasia (LGD) (HR 2.6; 95%CI: 1.0-6.5), p53 overexpression (HR 2.6; 95%CI: 1.0-6.4), and to a lesser extent, Ki67 overexpression (HR 2.1; 95%CI: 0.9-4.7) were risk factors for neoplastic progression in BE, whereas aneuploidy was not predictive for progression. Applied in a simple sum score, we showed that the presence of three or more risk factors was associated with a ≥12 times increased risk of progression in BE.

In **Chapter 4**, we reported on easy-to-apply predictors for the development of progression in patients with BE which could be used to identify patients with a high risk

of neoplastic progression. The presence of LGD (RR 9.7; 95%CI: 4.4-21.5), esophagitis (RR 3.5; 95%CI: 1.3-9.5), a known duration of BE ≥ 10 years (RR 3.2; 95%CI: 1.3-7.8), and longer length of BE (RR 1.11 per cm increase in length; 95%CI: 1.01-1.2) were found to be predictors of progression to HGD or EAC. After incorporating these risk factors in a prediction model, we found that patients with ND and none of the above-mentioned risk factors had the lowest annual risk of developing HGD or EAC (< 1%), whereas those with LGD and two or more risk factors had the highest annual risk of neoplastic progression (18-40%). In conclusion, these markers, either biomarkers or clinical factors, are useful to identify patients with a high risk of neoplastic progression, preferably used in a panel to improve specificity and sensitivity.

In order to determine the progression rates in BE more accurately, we performed a prospective, multicenter cohort study in which 715 patients with BE were included of which 607 had baseline no dysplasia (ND) and 108 baseline LGD **(Chapter 5)**. During 2,598 person-years (pyrs) of follow-up, 16 patients developed HGD and 10 patients EAC, corresponding to an annual risk of HGD of 1.0% (95%CI: 0.6-1.4) and EAC of 0.4% (95%CI: 0.1-0.6). In patients with baseline ND, the annual risk of HGD was 0.6% (95%CI: 0.3-1.0) and EAC 0.2 % (95%CI: 0.0-0.4). Patients with baseline LGD had higher annual risks, i.e., 3.4% (95%CI: 1.5-5.4) for HGD and 1.7% (95%CI: 0.3-3.1) for EAC. The annual risk of regression from LGD to ND was 5.4% (95%CI: 4.5-6.2).

As we know that misclassification is an important bias in the assessment of progression from BE with ND to EAC, we statistically estimated true progression rates from ND to EAC by multistate Markov (MSM) modeling **(Chapter 6)**. We used misclassification rates that were based on the literature as well rates internally estimated from our cohort of patients with BE. Using literature-based misclassification rates, the true progression rate from ND to LGD was 4/1,000 pyrs of follow-up, from LGD to HGD 88/1,000 pyrs and from HGD to EAC 307/1,000 pyrs. Corresponding annual risks of progression were 0.4%, 7.2% and 26%, respectively. Data-driven misclassification rates slightly increased the true progression rates, with annual risks of 0.9%, 10% and 27%, respectively. The latter rates provide a more accurate view on the natural course of progression in BE and should be used in cost-effectiveness analysis of surveillance in patients with BE.

In **Chapter 7**, we evaluated the cost-effectiveness of different intervals of surveillance in BE with baseline ND and compared the most promising endoscopic interventions such as radiofrequency ablation (RFA) and endomucosal resection (EMR) with esophagectomy. Therefore, we calculated the incremental cost-effectiveness ratios (ICER) for each strategy in terms of costs per quality adjusted life year (QALY). Our analysis showed that surveillance every 4 and 5 years with RFA for HGD and esophagectomy for EAC had ICERs

of €60,554 and €16,348 per QALY gained, respectively. Strategies with shorter surveillance intervals or other treatment procedures provided higher costs with similar or more costs per QALY gained. Regarding the generally accepted willingness-to-pay threshold in the Netherlands of €20,000 per QALY gained, surveillance every 5 years combined with RFA for HGD and surgery for EAC would be the preferred strategy for surveillance in patients with BE and baseline ND.

In **Chapter 8**, the main findings of this thesis are discussed and directions for further research in BE and the associated neoplastic progression to EAC are presented.

SAMENVATTING

Barrett slokdarm is een premaligne afwijking die gepaard gaat met een verhoogde kans op het ontstaan van adenocarcinoom van de slokdarm (AC). Deze maligniteit heeft een slechte prognose en hoge mortaliteit. De incidentie van AC is de laatste decennia sterk toegenomen en stijgt nog steeds. Om deze reden wordt geadviseerd om patiënten met een Barrett slokdarm endoscopische surveillance te laten ondergaan met als doel om vroegtijdig neoplastische veranderingen te kunnen ontdekken en deze te behandelen om slokdarmkanker te voorkomen. De frequentie van endoscopische surveillance wordt op dit moment alleen bepaald door de histologische beoordeling van biopten ondanks de beperkingen hiervan. Tot nu toe is de effectiviteit van surveillance van patiënten met een Barrett slokdarm niet aangetoond. Tevens vertoont de incidentie van AC bij patiënten met Barrett slokdarm variatie in de tot nu toe gepubliceerde studies. Kennis van het natuurlijk verloop van progressie van Barrett slokdarm tot AC en het identificeren van risicofactoren voor het ontwikkelen van neoplastische progressie zou kunnen leiden tot het onderscheiden van patiënten met een laag en een hoog risico op slokdarmkanker. Op deze manier zou de kosten-effectiviteit van surveillance van Barrett patiënten verbeterd kunnen worden en uiteindelijk de mortaliteit ten gevolge van AC verlaagd kunnen worden.

In **Hoofdstuk 1** worden de doelen en de achtergronden van dit proefschrift beschreven.

Door een meta-analyse uit te voeren berekenden wij in **Hoofdstuk 2** een samengestelde incidentie van AC in patiënten met een Barrett slokdarm van 6,3/1.000 persoonsjaren (95% betrouwbaarheidsinterval (BI): 4,7-8,4). Dit was gebaseerd op 50 studies waarin 14.109 patiënten met 61.804 persoonsjaren follow-up waren geïncludeerd. Bij deze analyse was sprake van een aanzienlijke heterogeniteit tussen de studies (χ^2=258,2; df=49; p<0,001; I^2=79%). Negentien studies met een totaal van 7.930 patiënten en 33.022 persoonsjaren, werden geïncludeerd in een analyse met mortaliteit ten gevolge van AC als eindpunt. De samengestelde mortaliteit was 3,0/1.000 persoonsjaren (95% BI: 2,2-3,9) zonder heterogeniteit tussen de studies (χ^2=19,3; df=18; p=0,4; I^2=7%). De mortaliteit door andere oorzaken dan slokdarmkanker was 12 keer hoger. Wij concludeerden hieruit dat het risico op neoplastische progressie in patiënten met een Barrett slokdarm laag is en dat deze patiënten vooral overlijden aan andere oorzaken dan slokdarmkanker.

Om risicofactoren op het ontwikkelen van progressie in Barrett slokdarm te identificeren werden naast histologie drie veelbelovende biomarkers onderzocht in **Hoofdstuk 3**. In deze case-control studie werden 27 Barrett patiënten geïncludeerd met

histologisch bewezen progressie naar hooggradige dysplasia (HGD) of AC en 27 Barrett patiënten zonder progressie. Multivariabele regressie analyse toonde dat laaggradige dysplasie (LGD) (HR 2,6; 95%CI: 1,0-6,5), overexpressie van p53 (HR 2,6; 95%CI: 1,0-6,4) en in mindere mate, overexpressie van Ki67 (HR 2,1; 95%CI: 0,9-4,7) belangrijke risicofactoren waren voor het ontwikkelen van neoplastische progressie in de Barrett slokdarm. Aneuploïdie bleek geen voorspeller te zijn voor het ontwikkelen van HGD of AC. Als we deze factoren gebruiken in een gesommeerde score, vonden we dat het hebben van 3 of meer risicofactoren geassocieerd is met een ≥ 12x verhoogd risico op progressie naar HGD of AC in patiënten met een Barrett slokdarm.

In **Hoofdstuk 4** werden risicofactoren die eenvoudig te gebruiken zijn, bestudeerd die geassocieerd zouden kunnen zijn met een verhoogd risico op neoplastische progressie bij patiënten met een Barrett slokdarm. LGD (RR 9,7; 95%BI: 4,4-21,5), oesophagitis (RR 3,5; 95%BI: 1,3-9,5), een duur van bekend zijn met Barrett slokdarm ≥ 10 jaar (RR 3,2; 95%BI: 1,3-7,8), en een langere lengte van het Barrett segment (RR 1,11 per cm toename in lengte; 95%BI: 1,01-1,2) waren zulke voorspellers voor progressie naar HGD of AC in Barrett patiënten. In een predictiemodel vonden wij dat patiënten zonder dysplasie (ND) en geen enkele risicofactor het laagste jaarlijkse risico op het ontwikkelen van HGD of AC hadden (<1%), terwijl patiënten met LGD met 2 andere risicofactoren het hoogste jaarlijks risico hadden (18-40%). Uit deze 2 studies kan worden geconcludeerd dat deze factoren, zowel biomarkers als klinische factoren, nuttig kunnen zijn bij het identificeren van Barrett patiënten met een hoog risico op progressie. Bij voorkeur zou een panel van factoren gebruikt moeten worden.

Om de kans op progressie in Barrett slokdarm nauwkeuriger te kunnen bepalen, werd in **Hoofdstuk 5** een prospectieve, multicenter cohort studie uitgevoerd waarin 715 patiënten met een Barrett slokdarm werden geïncludeerd waarvan 607 patiënten ND hadden bij inclusie en 108 patiënten LGD. Gedurende 2.598 persoonsjaren ontwikkelden 16 patiënten HGD en 10 AC wat overeenkomt met een jaarlijks risico op HGD van 1,0% (95%BI: 0,6-1,4) en op AC van 0,4% (95%BI: 0,1-0,6). Patiënten met ND bij inclusie hadden een jaarlijks risico op HGD van 0,6% (95%BI: 0,3-1,0) en op AC van 0,2% (95%BI: 0,0-0,4). Patiënten met LGD bij inclusie hadden een hoger jaarlijks risico, namelijk 3,4% (95%BI: 1,5-5,4) voor HGD en 1,7% (95%BI: 0,3-3,1) voor AC. Het jaarlijks risico op regressie van LGD naar ND was 5,4% (95%BI: 4,5-6,2).

Zoals bekend is misclassificatie een belangrijke bias bij het onderzoeken van overgangskansen van Barrett slokdarm zonder dysplasie naar kanker. Om deze reden hebben wij echte overgangskansen van Barrett slokdarm zonder dysplasie tot AC berekend door een multistate Markov model te gebruiken **(Hoofdstuk 6)**. Daarvoor

werden misclassificatie kansen gebruikt die zowel op de bestaande literatuur gebaseerd waren als intern werden geschat door het model gebaseerd op de geobserveerde data van ons eigen patiëntencohort. Als we de op de literatuur gebaseerde misclassificatie kansen gebruikten, zagen we dat de echte overgangskans van Barrett slokdarm met ND naar LGD 4/1.000 persoonsjaren was, van LGD naar HGD 88/1.000 persoonsjaren en van HGD naar AC 307/1.000 persoonsjaren. De corresponderende jaarlijkse overgangsrisico's waren respectievelijk 0,4%, 7,2% en 26%. Als we de misclassificatie kansen gebaseerd op eigen data gebruikten, zagen we dat deze overgangskansen iets hoger waren met jaarlijkse risico's van 0,9%, 10% en 27%. Deze echte overgangskansen geven het natuurlijk verloop van progressie van Barrett slokdarm naar AC nauwkeuriger weer en zouden beter gebruikt kunnen worden in kosten-effectiviteit analyses van surveillance bij patiënten met een Barrett slokdarm.

In **Hoofdstuk 7** hebben wij de kosten-effectiviteit geëvalueerd van verschillende frequenties van surveillance bij patiënten met een Barrett slokdarm en werden endoscopische interventies zoals radiofrequente ablatie (RFA) en endomucosale resectie (EMR) vergeleken met slokdarmresectie. Hiervoor werden de incrementele kosten-effectiviteit ratio's (ICER) berekend voor elke strategie. Onze analyses toonden dat surveillance elke 4 of 5 jaar gecombineerd met RFA voor HGD en slokdarmresectie voor AC een ICER hadden van respectievelijk €60.554 of €16.348 per gewonnen levensjaar. Strategieën met een korter surveillance interval of met andere behandelingen zijn duurder bij gelijke of minder effecten per gewonnen levensjaar. Gelet op de geaccepteerde drempel voor surveillance of screening programma's in Nederland van €20.000 per gewonnen levensjaar, is surveillance elke 5 jaar met RFA voor HGD en chirurgie voor AC de voorkeursstrategie voor surveillance bij patiënten met een Barrett slokdarm.

In **Hoofdstuk 8** worden de belangrijkste bevindingen van dit proefschrift beschreven en aanwijzingen voor toekomstig onderzoek besproken.

Chapter 10

Dankwoord
Curriculum vitae
Portfolio

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Het is mooi, het is klaar, het is goed, het is gedaan…..

Roosbeef

APPENDIX

CYBAR-studie: deelnemende centra

Erasmus MC, Rotterdam	
Afdeling Maag-Darm-Leverziekten:	M. Kerkhof, M. Sikkema, F. Kastelein, P.D. Siersema, E.J. Kuipers
Afdeling Pathologie:	H. van Dekken
IJsselland Ziekenhuis, Capelle aan den IJssel	
Afdeling Maag-Darm-Leverziekten:	W.A. Bode, H. Geldof, F. Bekkering
Afdeling Pathologie:	H. van der Valk
Ikazia Ziekenhuis, Rotterdam	
Afdeling Maag-Darm-Leverziekten:	R.J.Th. Ouwendijk, C. Leunis
Afdeling Pathologie:	R.W.M. Giard
VU Medisch Centrum, Amsterdam	
Afdeling Maag-Darm-Leverziekten:	E.C. Klinkenberg
Afdeling Pathologie:	G.A. Meijer, M. Broeckaert
Albert Schweitzer Ziekenhuis, Dordrecht	
Afdeling Maag-Darm-Leverziekten:	W. Lesterhuis, R. Beukers
Afdeling Pathologie:	R.J. Heinhuis
Deventer Ziekenhuis, Deventer	
Afdeling Maag-Darm-Leverziekten:	F. ter Borg
Afdeling Pathologie:	J.W. Arends
Streekziekenhuis Midden-Twente, Hengelo	
Afdeling Maag-Darm-Leverziekten:	G. Tan
Afdeling Pathologie:	J. van Baarlen
Rijnstate Ziekenhuis, Arnhem	
Afdeling Maag-Darm-Leverziekten:	R.A. de Vries, P. van Embden
Afdeling Pathologie:	A.H. Mulder

Sint Franciscus Gasthuis, Rotterdam
Afdeling Maag-Darm-Leverziekten: A.J.P. van Tilburg
Afdeling Pathologie: H. van der Valk

Medisch Spectrum Twente, Enschede
Afdeling Maag-Darm-Leverziekten: J.J. Kolkman
Afdeling Pathologie: J. van Baarlen

Maasland Ziekenhuis, Sittard
Afdeling Maag-Darm-Leverziekten: L. Engels
Afdeling Pathologie: W. Vos

Universitair Medisch Centrum Groningen, Groningen
Afdeling Maag-Darm-Leverziekten: F.T.M. Peters
Afdeling Pathologie: A. Karrenbeld

Isala Klinieken, Zwolle
Afdeling Maag-Darm-Leverziekten: B.E. Schenk, F. van Veen
Afdeling Pathologie: F. Moll

De Heel Medisch Centrum, Zaandam
Afdeling Maag-Darm-Leverziekten: R. Loffeld
Afdeling Pathologie: M.J. Flens

Franciscus Ziekenhuis, Roosendaal
Afdeling Maag-Darm-Leverziekten: H. van Roermund
Afdeling Pathologie: F. Lockefeer

CURRICULUM VITAE

Marjolein Sikkema werd geboren op 8 maart 1980 te Brielle. Zij volgde het middelbaar onderwijs aan het Maerlant College te Brielle. In juni 1998 behaalde zij haar gymnasiumdiploma. Vervolgens is zij datzelfde jaar begonnen met de studie geneeskunde aan de Universiteit van Antwerpen te België. In juni 2005 behaalde zij met Grote Onderscheiding haar artsendiploma. Vanaf augustus 2005 was zij gedurende 1 jaar werkzaam als ANIOS Interne Geneeskunde in het IJsselland Ziekenhuis te Capelle a/d IJssel (Dr. H.R.A. Fischer). Vanaf september 2006 begon zij aan haar promotie-onderzoek op het laboratorium van de afdeling Maag-, Darm- en Leverziekten van het Erasmus MC onder begeleiding van Prof. Dr. E.J. Kuipers, Prof. Dr. P.D. Siersema en Prof. Dr. E.W. Steyerberg (afdeling Maatschappelijke Gezondheidszorg). Op 1 mei 2009 startte zij met de vooropleiding Interne Geneeskunde in het UMC Utrecht (opleider Dr. M.M.E. Schneider). Op 1 mei 2011 zal zij starten met de opleiding tot Maag-Darm-Leverarts in het UMC Utrecht (opleider Prof. Dr. P.D. Siersema).

PHD PORTFOLIO SUMMARY

Name PhD student:	Marjolein Sikkema
Erasmus MC Department:	Gastroenterology and Hepatology
PhD period:	September 2006 – April 2009
Promotores:	prof. dr. E.J. Kuipers
	prof. dr. P.D. Siersema
	prof. dr. E.W. Steyerberg (dept. of Public Health)

(Inter)national Conferences: oral presentations

2007 October — *Annual meeting of the Dutch Society of Gastroenterology and Hepatology (NVGE), Veldhoven, The Netherlands*
- High expression of p53 and Ki67 and aneuploidy predict neoplastic progression in Barrett Esophagus

November — *Symposium "Focus op 't Sticht", 2007, Utrecht, The Netherlands*
- CYBAR-study

2008 May — *Digestive Disease Week, San Diego, USA*
- Flow CYtometry in BARret esophagus (CYBAR study): a prospective cohort study
- Prospective multicenter study on the incidence of neoplastic progression in Barrett's esophagus patients

October — *United European Gastroenterology Week, Vienna, Austria*
- Flow CYtometry in BARret esophagus (CYBAR study): a prospective cohort study
- Prospective multicenter study on the incidence of neoplastic progression in Barrett's esophagus patients

2009 March — *Annual meeting of the Dutch Society of Gastroenterology and Hepatology (NVGE) Veldhoven, The Netherlands*
- Risk of esophageal adenocarcinoma and mortality in patients with Barrett's esophagus: a systematic review and meta-analysis

2009 March *Annual meeting of the Dutch Society of Gastroenterology and Hepatology (NVGE) Veldhoven, The Netherlands (continued)*

- Overexpression of p53 and Ki67 and aneuploidy as markers for neoplastic progression in Barrett's esophagus: a nested case-control study
- Surveillance in a prospectively followed cohort of patients with Barrett's esophagus in the Netherlands: a cost-effectiveness analysis

May *Digestive Disease Week, Chicago, USA*

- Predictors for Neoplastic Progression in Patients with Barrett's Esophagus: A Prospective Cohort Study
- Surveillance in a Prospectively Followed Cohort of Patients with Barrett's Esophagus in the Netherlands: A Cost-Effectiveness Analysis

International conferences: poster presentations

2008 May *Digestive Disease Week, San Diego, USA*

- High expression of p53 and Ki67 and aneuploidy predict neoplastic progression in Barrett's Esophagus

2009 May *Digestive Disease Week, 2009, Chicago, USA*

- Risk of Esophageal Adenocarcinoma and Mortality in Patients with Barrett's Esophagus: A Systematic Review and Meta-Analysis
- Overexpression of p53 and Ki67 and Aneuploidy As Markers for Neoplastic Progression in Barrett's Esophagus: A Nested Case-Control Study

Courses

Introduction in data-analysis, NIHES, August 2007
English course, January 2008

Membership

Dutch Society of Gastroenterology (NVGE)