Advances in Barrett's Esophagus Surveillance and Improved Prediction of Prognosis and Therapy Response in Patients with Esophageal Adenocarcinoma

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Vooruitgang in Barrett slokdarm surveillance en verbeteringen in het voorspellen van prognose en behandelresponse bij patiënten met een adenocarcinoom van de slokdarm

Proefschrift

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General introduction and outline of the thesis

S.H. van Olphen

Barrett's esophagus

Barrett's esophagus (BE) is a condition caused by chronic gastro-esophageal and biliary reflux in which the squamous epithelium of the distal esophagus is replaced by columnar epithelium containing goblet cells [1]. BE can be recognized during endoscopy by red discoloration of the normally vale pink mucosa. Patients with BE have an increased risk to develop esophageal adenocarcinoma (EAC) with an estimated incidence of 0.1 to 0.4% per year [2, 3, 4]. The progression from BE to EAC is a gradual process, in which intestinal metaplasia evolves via low-grade dysplasia (LGD), to high-grade dysplasia (HGD) and finally to EAC, a cancer with an overall 5-year survival of less than 20% [5, 6]. Therefore current guidelines recommend endoscopic surveillance of BE patients to detect HGD or EAC at an early and potentially curable stage [7, 8].

BE surveillance

Nowadays, histological diagnosis of LGD is used for risk assessment of neoplastic progression in BE. Guidelines recommend endoscopic surveillance every 3 to 5 years in patients without dysplasia, and surveillance every 6 to 12 months in patients with LGD [7, 8]. Endoscopic surveillance is standardized and at each endoscopy targeted biopsies from mucosal abnormalities as well as quadrant biopsies every 2 cm from the most distal to the most proximal part of the Barrett's epithelium are taken to obtain histological diagnosis and grading of dysplasia. However, diagnosis of LGD has a low predictive value, owing to sample error and overlapping features with reactive changes as well as considerable inter- and intraobserver variation [9, 10, 11]. Identification of other biomarkers for neoplastic progression could improve risk stratification and hence the effectiveness of BE surveillance.

Biomarkers for BE surveillance

SOX2

Application of biomarkers in addition to histology may contribute to the identification of BE patients at risk for neoplastic progression. Many biomarkers have been investigated and immunohistochemical staining of p53 appears to be the most promising so far [12]. However, only 40% of the BE patients with progression to HGD or EAC showed an aberrant p53 protein

expression using immunohistochemistry during surveillance, indicating that additional biomarkers are needed [12]. SOX2 is a high-mobility group box transcription factor, involved in the maintenance of pluripotency and self-renewal in embryonic stem cells and regulates an array of genes involved in normal and malignant processes [13, 14, 15]. Recent studies in gastric cancer cells revealed a role of SOX2 in growth inhibition through cell-cycle arrest and induction of apoptosis, indicating cancer-suppressive functions [16]. Only one study reported SOX2 in human BE and observed that SOX2 was present in multilayered esophageal epithelium, but was downregulated in biopsy samples with intestinal metaplasia [17]. However, the role of SOX2 in the development of BE and its expression during the metaplasia-dysplasia-adenocarcinoma sequence in BE epithelium has not yet been investigated.

Cyclin A

Another potential biomarker is cyclin A, a protein that plays an important role in the G1-S transition of the cell cycle. Overexpression of cell-cycle related proteins, including cyclin A, has been linked to the metaplasia-dysplasia-carcinoma sequence in BE and associated with an increased risk of neoplastic progression [18, 19, 20]. The results of previous studies evaluating the value of cyclin A expression for predicting neoplastic progression are conflicting. A small case-control study showed that cyclin A surface expression was significantly associated with an increased risk of neoplastic progression (OR 7.6; 95% CI 1.6 to 37.0), whereas a more recent larger population-based study could not confirm this correlation and only found a trend towards an increased risk of progression, which eventually lost significance in a multivariate analysis (OR 1.32; 95% CI 0.66 to 2.66) [18, 19, 20]. However, validation of cyclin A in a large prospective cohort of BE patients is still missing. In addition, there is a lack of studies testing performance of multiple biomarker simultaneously in the same cohort of BE patients.

Cost-effectiveness and survival

Although BE surveillance seems reasonable and is incorporated in international guidelines, there is little scientific evidence that BE surveillance is actually beneficial in daily clinical practice. To date no randomized controlled trails have been performed and the value of BE surveillance is still under discussion. The relevant key questions in this discussion are: (I) is BE surveillance cost-effective and (II) can BE surveillance reduce mortality due to EAC. Important

is that over the past decades there has been a major shift in treatment modalities for patients with neoplastic progression. Nowadays endoscopic treatment with endoscopic mucosal resection and radiofrequency ablation is frequently used, while in the previous century almost all patients underwent esophagectomy. Application of these new treatment modalities may improve the cost-effectiveness of BE surveillance and reduce mortality due to EAC.

Esophageal adenocarcinoma

Over the past decades, the incidence of EAC has increased in the western world by at least six-fold, making EAC the cancer with the most rapid rise in incidence [21]. Although neoadjuvant chemoradiotherapy (nCRT) followed by surgery has recently become standard of care for locally advanced esophageal cancers (EC) and achieves a 5-year overall survival benefit of 10-15% compared to surgery alone. The overall prognosis for most patients including those treated with curative intent is still dismal with a 5-year survival of 47% at most [6, 22]. However, response to nCRT and overall prognosis is highly variable in EAC patients. Surgical resection, immediately after nCRT is associated with significant morbidity and substantial impact on the quality of life [23, 24]. So if clinicians were able to accurately identify (near-) complete responders, these patients might be candidates to postpone or even omit surgical resection. In addition, patients without substantial pathological response do not seem to benefit from nCRT but experience unnecessary side-effects and curative surgery is delayed [25, 26]. Therefore, there is a need for informative biomarkers that determine the biological behavior of individual tumors and may contribute to predict response to nCRT and prognosis, to eventually improve individualized decision-making in these patients. The tumor suppressor gene p53 has been identified as an important molecular factor associated with tumor tolerance to chemotherapy and radiation in patients with EAC [27, 28, 29]. Other potential biomarkers are SOX2 and CD44, both linked to cancer stem cells (CSCs), a small population of cells, found to be more resistant to chemo- and radiotherapy in various malignant tumor types [30, 31]. Although the first studies have shown promising results, the protein expression of p53, SOX2 and CD44 and the possible relation with response to nCRT in EAC has barely been investigated. Additionally, in previous literature the pattern of SOX2 expression also has been associated with prognosis in various malignancies, including gastric adenocarcinoma [32, 33]. But little is known about the role of SOX2 in EAC in terms of prognostication.

Outline of this thesis

The overall aim of this thesis is to evaluate whether the use of biomarkers can improve risk stratification in BE patients, as well as treatment and prognostication of EAC patients. In addition, the cost-effectiveness of BE surveillance and impact on survival was assessed. To investigate this, the following studies were performed:

In **chapter 2** the existing literature is systematically reviewed regarding the value of immunohistochemical biomarkers for predicting neoplastic progression in BE patients and a meta-analysis is performed for the biomarkers investigated multiple times in independent studies. In **chapter 3 and 4** we assessed the predictive value of the biomarkers SOX2 and cyclin A for cancer risk stratification in BE patients. In **chapter 5** the cost-effectiveness of different surveillance intervals and treatment strategies is evaluated. In **Chapter 6** the survival of BE patients diagnosed with HGD or EAC in a surveillance program is explored and compared to the survival of patients diagnosed with EAC in the general population. In **chapter 7 and chapter 8** the predictive value of the biomarkers p53, SOX2 and CD44 for response to nCRT, as well as the value of SOX2 as prognostic marker in EAC patients is investigated. In **chapter 9 and 10** the results of this thesis are discussed and summarized.

Reference

1 Falk GW. Barrett's esophagus. Gastroenterology 2002;122:1569-91.

2 Desai TK, Krishnan K, Samala N, Singh J, Cluley J, Perla S, et al. The incidence of oesophageal adenocarcinoma in non-dysplastic Barrett's oesophagus: a meta-analysis. Gut 2012;61:970-6.

3 Hvid-Jensen F, Pedersen L, Drewes AM, Sorensen HT, Funch-Jensen P. Incidence of Adenocarcinoma among Patients with Barrett's Esophagus. New Engl J Med 2011;365:1375-83.

4 Sikkema M, de Jonge PJ, Steyerberg EW, Kuipers EJ. Risk of esophageal adenocarcinoma and mortality in patients with Barrett's esophagus: a systematic review and meta-analysis. Clin Gastroenterol Hepatol 2010;8:235-44; quiz e32.

5 Buttar NS, Wang KK. Mechanisms of disease: Carcinogenesis in Barrett's esophagus. Nat Clin Pract Gastroenterol Hepatol 2004;1:106-12.

6 van Hagen P, Hulshof MC, van Lanschot JJ, Steyerberg EW, van Berge Henegouwen MI, Wijnhoven BP, et al. Preoperative chemoradiotherapy for esophageal or junctional cancer. The New England journal of medicine 2012;366:2074-84.

7 Fitzgerald RC, di Pietro M, Ragunath K, Ang Y, Kang JY, Watson P, et al. British Society of Gastroenterology guidelines on the diagnosis and management of Barrett's oesophagus. Gut 2014;63:7-42.

8 Wang KK, Sampliner RE, Practice Parameters Committee of the American College of G. Updated guidelines 2008 for the diagnosis, surveillance and therapy of Barrett's esophagus. Am J Gastroenterol 2008;103:788-97.

9 Curvers WL, ten Kate FJ, Krishnadath KK, Visser M, Elzer B, Baak LC, et al. Low-grade dysplasia in Barrett's esophagus: overdiagnosed and underestimated. The American journal of gastroenterology 2010;105:1523-30.

10 Kerkhof M, van Dekken H, Steyerberg EW, Meijer GA, Mulder AH, de Bruine A, et al. Grading of dysplasia in Barrett's oesophagus: substantial interobserver variation between general and gastrointestinal pathologists. Histopathology 2007;50:920-7.

11 Skacel M, Petras RE, Gramlich TL, Sigel JE, Richter JE, Goldblum JR. The diagnosis of low-grade dysplasia in Barrett's esophagus and its implications for disease progression. Am J Gastroenterol 2000;95:3383-7.

12 Kastelein F, Biermann K, Steyerberg EW, Verheij J, Kalisvaart M, Looijenga LH, et al. Aberrant p53 protein expression is associated with an increased risk of neoplastic progression in patients with Barrett's oesophagus. Gut 2012.

13 Wang JL, Rao S, Chu JL, Shen XH, Levasseur DN, Theunissen TW, et al. A protein interaction network for pluripotency of embryonic stem cells. Nature 2006;444:364-8.

14 Liu K, Lin B, Zhao M, Yang X, Chen M, Gao A, et al. The multiple roles for Sox2 in stem cell maintenance and tumorigenesis. Cell Signal 2013;25:1264-71.

15 Boiani M, Scholer HR. Regulatory networks in embryo-derived pluripotent stem cells. Nat Rev Mol Cell Bio 2005;6:872-84.

16 Otsubo T, Akiyama Y, Yanagihara K, Yuasa Y. SOX2 is frequently downregulated in gastric cancers and inhibits cell growth through cell-cycle arrest and apoptosis. Br J Cancer 2008;98:824-31.

17 Chen XX, Qin R, Liu B, Ma Y, Su YH, Yang CS, et al. Multilayered epithelium in a rat model and human Barrett's esophagus: Similar expression patterns of transcription factors and differentiation markers. Bmc Gastroenterol 2008;8.

18 Bird-Lieberman EL, Dunn JM, Coleman HG, Lao-Sirieix P, Oukrif D, Moore CE, et al. Population-based study reveals new risk-stratification biomarker panel for Barrett's esophagus. Gastroenterology 2012;143:927-35 e3.

19 di Pietro M, Boerwinkel DF, Shariff MK, Liu X, Telakis E, Lao-Sirieix P, et al. The combination of autofluorescence endoscopy and molecular biomarkers is a novel diagnostic tool for dysplasia in Barrett's oesophagus. Gut 2015;64:49-56.

20 Lao-Sirieix P, Lovat L, Fitzgerald RC. Cyclin A immunocytology as a risk stratification tool for Barrett's esophagus surveillance. Clin Cancer Res 2007;13:659-65.

21 Rubenstein JH, Shaheen NJ. Epidemiology, Diagnosis, and Management of Esophageal Adenocarcinoma. Gastroenterology 2015;149:302-17 e1.

22 Gebski V, Burmeister B, Smithers BM, Foo K, Zalcberg J, Simes J, et al. Survival benefits from neoadjuvant chemoradiotherapy or chemotherapy in oesophageal carcinoma: a meta-analysis. Lancet Oncol 2007;8:226-34.

23 Donohoe CL, McGillycuddy E, Reynolds JV. Long-term health-related quality of life for disease-free esophageal cancer patients. World J Surg 2011;35:1853-60.

24 Djarv T, Lagergren J, Blazeby JM, Lagergren P. Long-term health-related quality of life following surgery for oesophageal cancer. Br J Surg 2008;95:1121-6.

25 Mariette C, Piessen G, Triboulet JP. Therapeutic strategies in oesophageal carcinoma: role of surgery and other modalities. Lancet Oncol 2007;8:545-53.

26 Noordman BJ, Shapiro J, Spaander MC, Krishnadath KK, van Laarhoven HW, van Berge Henegouwen MI, et al. Accuracy of Detecting Residual Disease After Cross Neoadjuvant Chemoradiotherapy for Esophageal Cancer (preSANO Trial): Rationale and Protocol. JMIR Res Protoc 2015;4:e79.

27 Mendonca JA, Francischone CE, Senna PM, Matos de Oliveira AE, Sotto-Maior BS. A retrospective evaluation of the survival rates of splinted and non-splinted short dental implants in posterior partially edentulous jaws. J Periodontol 2014;85:787-94.

28 Vallbohmer D, Holscher AH, Schneider PM, Schmidt M, Dietlein M, Bollschweiler E, et al. [18F]-fluorodeoxyglucose-positron emission tomography for the assessment of histopathologic response and prognosis after completion of neoadjuvant chemotherapy in

gastric cancer. J Surg Oncol 2010;102:135-40.

29 Clara A, Fonseca I, Francisca A, Bettencourt A, Vaz F. Unexpected long-term survival in a BRCA2 patient with metastatic carcinosarcoma associated with tamoxifen. Gynecol Oncol Case Rep 2013;4:44-6.

30 Phillips TM, McBride WH, Pajonk F. The response of CD24(-/low)/CD44+ breast cancer-initiating cells to radiation. J Natl Cancer Inst 2006;98:1777-85.

31 Baumann M, Krause M, Hill R. Exploring the role of cancer stem cells in radioresistance. Nat Rev Cancer 2008;8:545-54.

32 Wang Q, He W, Lu C, Wang Z, Wang J, Giercksky KE, et al. Oct3/4 and Sox2 are significantly associated with an unfavorable clinical outcome in human esophageal squamous cell carcinoma. Anticancer Res 2009;29:1233-41.

33 Zhang X, Yu H, Yang Y, Zhu R, Bai J, Peng Z, et al. SOX2 in gastric carcinoma, but not Hath1, is related to patients' clinicopathological features and prognosis. J Gastrointest Surg 2010;14:1220-6.

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Immunohistochemical biomarkers for risk stratification of neoplastic progression in Barrett Esophagus: A systematic review and meta-analysis

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* contributed equally to the work.

Submitted

Abstract

Introduction: The low incidence of esophageal adenocarcinoma (EAC) in Barrett's esophagus (BE) patients reinforces the need for risk stratification tools to make BE surveillance more effective. Currently, none of the clinical and endoscopic criteria are able to accurately predict which BE patient will progress to EAC. Immunohistochemical (IHC) biomarkers are relatively easy applicable and may prove of additional value to predict neoplastic progression in BE. Therefore, this study aims to provide a systematic review and meta-analyses of published studies on IHC biomarkers in Barrett's esophagus.
Methods: We searched MEDLINE, EMBASE, Web of Science, CENTRAL, Pubmed publisher, and Google scholar up to October 1st of 2015. All studies on IHC biomarkers in BE surveillance were included.
Results: A total of 16 different IHC biomarkers have been studied in 31 studies. These studies included 414 cases and 1695 controls from a screening population of more than 7.000 BE patients. A meta- analysis was performed for P53, aspergillus oryzae lectin (AOL), Cyclin A, and Cyclin D. Aberrant p53 expression was significantly associated with an increased risk of neoplastic progression with an OR of 3.18 (95% CI 1.68 to 6.03). This association was confirmed for both non-dysplastic BE and BE with low grade dysplasia (LGD). Another promising biomarker to predict neoplastic progression was AOL, with an OR of 3.04 (95% CI 2.05 to 4.49).
Conclusions: Use of p53 IHC staining will improve risk stratification in BE surveillance. Aberrant P53 expression in BE patients appeared to be associated with a significantly increased risk of neoplastic progression for both non-dysplastic and dysplastic BE patients.

Introduction

Development of EAC is related to Barrett's esophagus (BE), a premalignant condition of the distal esophagus. In BE, the pre-existent squamous epithelium is replaced by columnar epithelium which develops under the influence of chronic acid reflux and frequently contains goblet cells [1, 2, 3]. The progression from BE to EAC is a gradual process, in which intestinal metaplasia (IM) evolves to low-grade dysplasia (LGD), high-grade dysplasia (HGD) and eventually EAC [4]. Therefore, current guidelines recommend endoscopic surveillance in BE patients to detect HGD or EAC at an early stage, with the aim to improve survival rates [5, 6]. Several studies have shown that patients diagnosed with EAC during BE surveillance have earlier staged tumors and better survival compared to those diagnosed after the onset of symptoms [7, 8, 9, 10].

The estimated incidence of EAC in patients with BE is reported to be between 0.5 and 1% per year [11, 12, 13, 14]. However, more recent population-based studies and two meta-analyses have set this risk around 0.12% to 0.38% per year [15, 16, 17, 18]. This relatively low annual risk reinforces the need for risk stratification tools to make BE surveillance more effective. BE length, male gender, smoking, and LGD are known risk factors for progression to HGD and EAC [13, 15, 18, 19, 20]. Two large population studies confirmed that patients with LGD have an approximately five times higher risk of progression compared to patients with non-dysplastic BE [15, 18]. Thus, more intensive surveillance is recommended in BE patients with LGD [5, 6]. However, the histological diagnosis of LGD is subject to a considerable inter- and intra-observer variation, because of sample error and overlap with features of non-neoplastic regenerative changes [21, 22, 23, 24].

Because none of the current clinical and histologic criteria are able to accurately predict which patients are likely to progress to HGD or EAC, there is an increasing interest in (molecular) biomarkers. Many immunohistochemical (IHC) biomarkers have been studied in BE progression, mainly because they can be applied to standard histological samples. In clinical practice, IHC biomarkers are relatively easily applicable compared to other techniques. Currently, only p53 IHC is recommended in the guideline of the British Society of Gastroenterology to improve the diagnostic reproducibility of the histological diagnosis of LGD [5]. The use of IHC biomarkers as independent predictor of neoplastic progression is not yet performed in routine clinical care, neither for p53, nor for other IHC biomarkers. Therefore, this study aims

to provide a systematic review and meta-analyses of all IHC biomarkers as predictor of neoplastic progression in patients with BE.

Methods

Definitions

BE was defined as columnar lined esophagus (CLE). Neoplastic progression was defined as the development of HGD or EAC during follow up. Patients with neoplastic progression were classified as cases and patients without neoplastic progression as controls.

Data sources and Searches

Records were identified by searching the following electronic databases: 1. EMBASE 1980 - 01-10-2015, 2. MEDLINE 1950 - 01-10-2015, 3. Web of Science until 01-10-2015, 4. CENTRAL 1994 - 01-10-2015, 5. Pubmed Publisher until 01-10-2015, 6. Google scholar until 01-10-2015. The search strategy was constructed by applying a sensitivity maximizing approach. A combination of MESH subject headings and text words were used related to IHC markers for progression in patients with BE. The search was confined to English language publications. Conference abstracts indexed in Embase from the years 2014 and 2015 were included.

Study selection

Search results were combined and duplicates removed. Every article was screened on title avvnd abstract level for relevance by a single author (SvO or VJ). Articles were reviewed full text by the same two independent authors and included if they met the following criteria: (1) association between IHC biomarker expression on formalin fixed paraffin embedded material and risk of neoplastic progression was assessed; (2) a cohort or case-control study design; (3) patients with known or newly diagnosed BE with or without LGD at baseline; (4) patients defined as cases must have progressed to either HGD or EAC during follow-up; (5) mean follow-up of at least one year from the time of initial BE diagnosis. Studies were excluded if: (1) BE cohorts included patients with HGD at baseline; (2) endoscopic therapies affecting neoplastic progression were performed during follow-up (see figure 1).

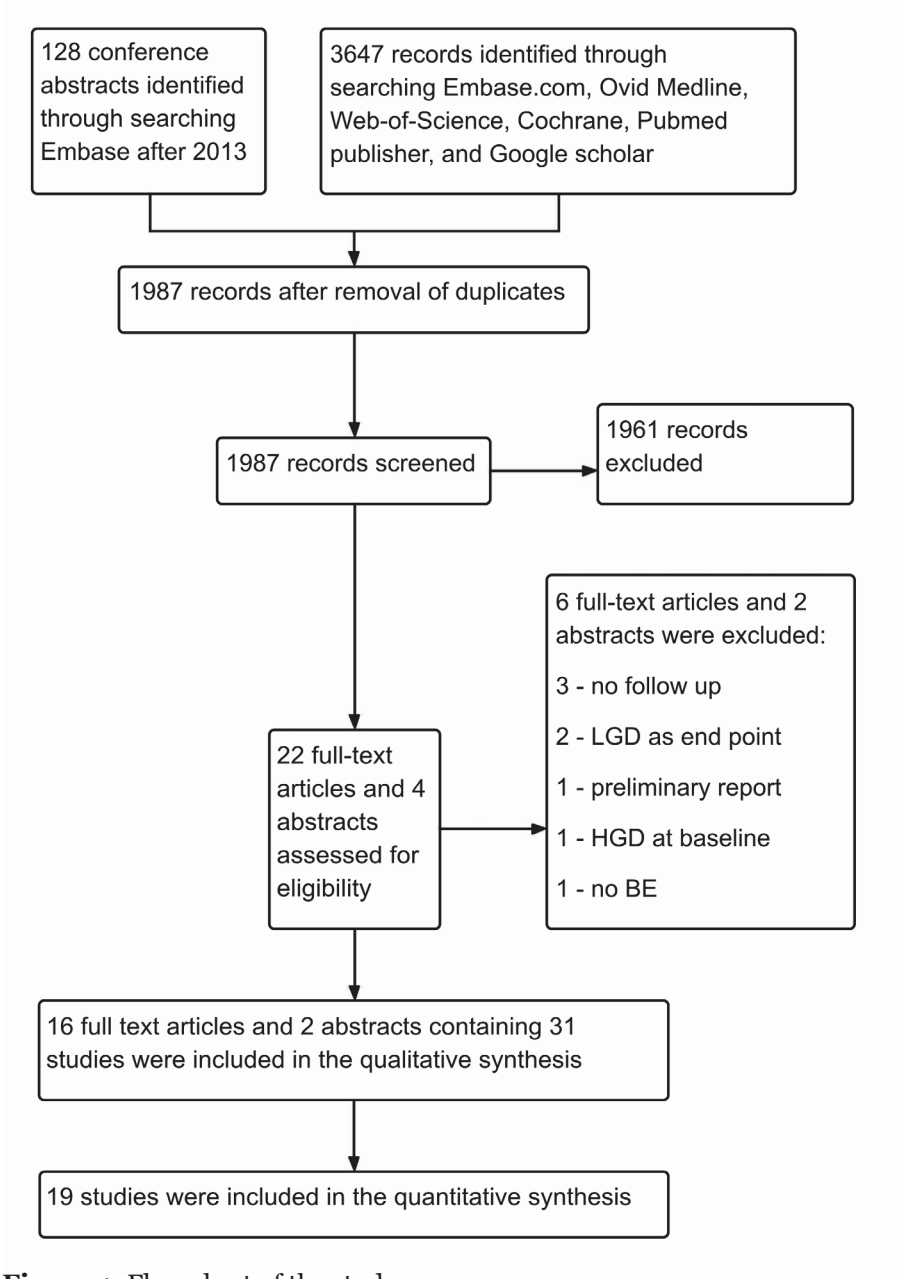

Figure 1. Flow chart of the study.

Data extraction and quality assessment

For each included study two independent authors extracted data according to a standardized data extraction form and assessed the quality of the eligible studies. In case of disagreement consensus was reached by consulting a third author (MS). Concerning quality assessment, the items baseline histology, age, duration of follow-up, sex, length of BE segment, control staining(s), number of pathologists, their agreement, and blinding were extracted.

Data synthesis and analyses

Odds ratio's (OR)s and 95% confidence intervals (CI)s were extracted or estimated from the data. If ORs could not be extracted directly from the text or the tables, ORs were calculated indirectly by using the numbers of cases and controls with a positive and negative IHC biomarker expression from text, tables, or figures. In the meta-analyses different studies using the same IHC biomarker were combined. An inverse variance random-effect model was used. If data on multiple definitions of aberrant staining were available, definitions were chosen to resemble those from other included studies for that IHC biomarker. Pooled estimates of effect were calculated and results investigated for statistical heterogeneity by visual inspection and the I-squared test (I2) = [(Q-df)/Q]*100% was used, where Q was the chi squared statistic and df was its degree of freedom). Small study effects such as publication bias were assessed using a funnel plot.

Sensitivity analyses

Sensitivity analyses were performed in case of small sample size (if small study effects were present as observed in the funnel plot), if no adjustments were made for known predictors of progression, and if only an abstract was available. Additional analyses were performed to assess if an IHC biomarker can be used as a predictor of neoplastic progression, independent of the presence of dysplasia. Thereto, all studies including non-dysplastic BE, BE with LGD, or studies adjusted for histology type were summarized.

Stringency of the definition for aberrant staining used and its interpretation

The stringency level of the definition for aberrant staining and its interpretation could lead to variation in the predictive ability of the IHC biomarker investigated. To investigate whether this effect might be present, the proportion of controls deemed positive was plotted against the OR of each

study.

Results

Included studies

1987 records were retrieved, after removal of duplicates. After excluding 1966 records based on title and abstract, a total of 22 full text articles and four abstracts were assessed in detail (figure 1). Of these, 16 full text articles and two abstracts were included in this review [25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43]. These articles contained a total of 31 studies.

Characteristics

A total of 31 studies were included, containing 2118 patients of which 417 cases, selected from a populations of more than 7.000 BE patients. The proportion of male patients ranged from 66% to 100%. Mean duration of follow-up varied from 11.3 months to 94.8 months. Most studies were retrospective case-control studies (n=28), and three prospective cohort studies One study defined BE as CLE without IM, other studies defined BE as CLE with IM (n=22), or gave no definition (n=8). Endpoint was EAC in five studies and either HGD or EAC in 26 studies.

Dilutions and definitions for aberrant staining used

For p53, the antibody DO-7 (Dako, Glostrup, Denmark) was used most frequently with a dilution ranging from 1:25 to 1:1000. The definition for aberrant IHC staining was heterogeneous. Very intense staining was considered aberrant by all studies (being independent of the concentration used). However, intensity of staining was not a prerequisite for considering a staining pattern aberrant in six studies. The three more recent studies also considered a total absence of staining as aberrant [32, 33, 36]. For aspergillus oryzae lectin (AOL), one study calculated the OR for aberrant AOL in 2 or 3 epithelial compartments versus 0 or 1 compartment [31]. Another study reported multiple ORs for aberrant AOL in 1, 2, or 3 versus 0 epithelial compartments [36]. The OR of aberrant AOL IHC in 2 or 3 versus 0 or 1 compartment was extracted from this second study and analyzed together with the data from the first study for the meta-analysis.

Quality of studies

In 13 of the 31 studies there was at least a 10% difference in baseline histology between cases and controls. In these studies, around 31% of the cases had IND or LGD at baseline, versus 8% in the controls. In five studies an age difference at baseline of at least 5 years was found between cases and controls, in four of these studies the case group was older. In three studies 10% more males were included in the case groups, 93% males on average in the cases, versus 70% in controls. Information on length of the BE segment for both cases and controls was provided in nine studies. In cases a longer BE segment was present, on average 6 cm versus 5.2 cm in the controls. In 17 studies the total follow-up time differed by at least 10% between case and control groups. On average, follow up time was 48 months versus 58 months for cases versus controls, respectively. Five studies did provide adjusted outcome measures and for four studies adjusted ORs could be extracted and were included meta-analyses.
IHC staining was scored by one observer in 12 studies, by two observers in 12 studies, and by three observers in two studies. Kappa values were mentioned in only seven studies, and 15 studies did not mention the use of any positive or negative controls in order to validate the staining process. Whether slides were assessed in a blinded manner was not mentioned in five studies.

Meta-analyses

Meta-analyses were performed for biomarkers studied more than once. This was possible for P53, AOL, Cyclin A, and Cyclin D, which were studied 12, 2, 3, and 2 times respectively. The most frequent reasons for excluding articles were the absence of follow-up data and LGD being defined as neoplastic progression and end-point of the study (see figure 1). Biomarkers studied only once were MCM2, CD1a, β-catenin, hERG1, COX2, Ki67, HER2, Sialyl Lewis, Wheat germ, Lewis, AMACR, and SOX2.

P53

A total of 12 studies, containing 2023 patients, of which 372 cases, were included in the meta-analysis. One study gave multiple ORs for various expression levels of p53, but only intense overexpression of p53 staining was considered positive [31]. Individual patient data of one study were converted in order to extract an adjusted OR [32]. The overall OR for neoplastic progression was 6.67 (95% CI 3.64 to 12.22) for patients with aberrant p53 expression (see table 1 and figure 2). Aberrant p53 expression detected in both non-dysplastic

BE and LGD patients was significantly associated with the development of HGD or EAC. Significant heterogeneity (I2=55%, P<0.01) was observed between the included studies. The 12 studies were plotted in a funnel plot showing small study effects. In order to reduce the influence of such effects a sensitivity analysis was performed, which excluded all studies with a standard error above 1. Based on this criterion, six studies were selected, containing 1610 patients and 323 cases. The overall OR for neoplastic progression was 4.40 (95% CI 2.30 to 8.40) in patients with an aberrant p53 expression (see table 1 and figure 3).

Table 1. summary of meta-analyses of studies investigating p53 IHC as an independent predictor of neoplastic progression.

Analysis	Studies	Cases	Controls	OR	95% CI	I^2
P53 (main)	12	372	1651	6.67	3.64-12.22	64%
P53 (excluded SE > 1)	6	323	1287	4.40	2.30-8.40	63%
P53 (also excluded unadjusted ORs)	4	278	1044	3.18	1.68-6.03	55%
P53 (also excluded abstracts)	3	138	905	3.78	1.65-8.68	52%
P53 (only ORs stratified for histology)	7	316	1221	4.13	2.36-7.21	41%
P53 (only non-dysplastic BE)	2	61	659	6.12	2.99-12.52	0%
P53 (only LGD)	4	37	145	8.64	3.62-20.62	0%

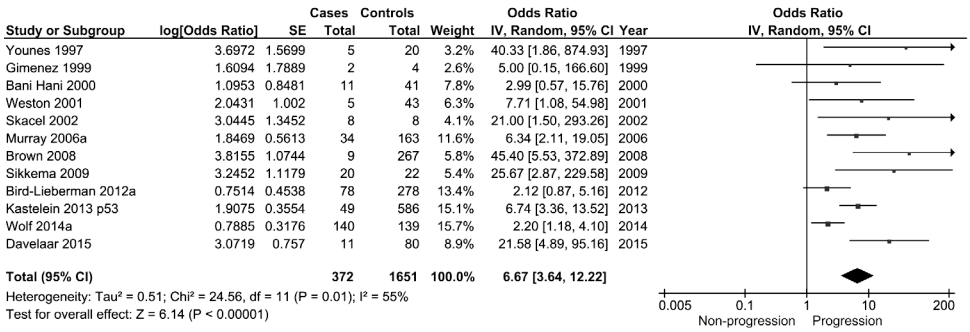

Figure 2. Forest plot of 12 studies investigating p53 as a predictor of progression.

The use of a more stringent definition of aberrant staining may lead to loss of aberrant expression in cases, in controls, or in both. In order to investigate this, the proportion of controls deemed aberrant was plotted against the OR of each study. Further sensitivity analyses were performed excluding studies for which unadjusted ORs were used in the meta-analyses and studies presented as abstracts. These sensitivity analyses showed similar results with slightly lower point estimates (see table 1). For three studies both unadjusted and adjusted ORs were available, and all three adjusted ORs had a lower point estimate compared to the unadjusted ones, in line with the outcome of our meta-analysis.

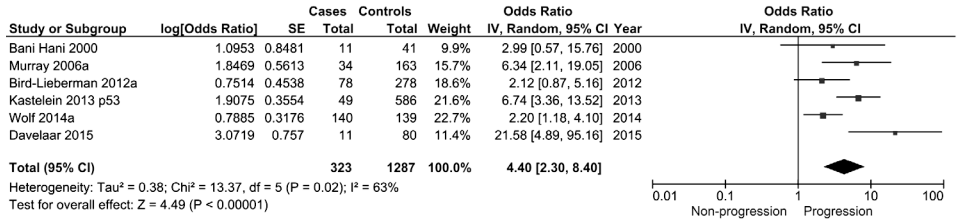

Figure 3. Forest plot of the first sensitivity analysis of studies investigating p53 IHC as a predictor of progression. All studies with a SE above one were excluded in order to reduce the effect of small study effects.

For the analysis whether P53 can serve as a predictor of progression independently of histology, studies that did not adjust for histology at baseline were excluded. The overall OR for aberrant p53 IHC on neoplastic progression, after stratification for histology, was 4.13 (95% CI 2.36 to 7.21), (see table 1).

P53 in non-dysplastic Barrett

Two studies, with a total of 720 BE patients, of which 61 cases, were included for this analysis [29]. Individual patient data of one study [32] was re-analyzed to provide an OR for non-dysplastic BE patients only. The overall OR for neoplastic progression to HGD or EAC in non-dysplastic BE patients was 6.12 (95% CI 2.99 to 12.52; I2=0%, P=0.93), (see table 1).

P53 in low grade dysplasia Barrett

For BE with LGD, four studies, with a total of 182 BE patients, of which 37 cases, were included. One study [32] was re-analyzed to provide an OR for the LGD subgroup only. The overall OR for neoplastic progression to HGD or EAC was 8.64 (95% CI 3.62 to 20.62; I2=0%, P=0.91), see table 1).

AOL

Two studies, containing 573 BE patients, of which 204 cases, were included in this meta-analysis. The overall OR for neoplastic progression in BE patients with an aberrant AOL staining in 2 or 3 compartments, versus 0 or 1 compartments of the tissue was 3.04 (95% CI 2.05 to 4.49) (see table 2). Results of the two studies were consistent in their findings (I2=0%, P=0.85).

Table 2. Summary of meta-analyses of studies investigating IHC biomarkers other than p53 as a predictor of neoplastic progression.

Analysis	Studies	Cases	Controls	OR	95% CI	I^2
AOL	2	204	369	3.04	2.04-4.49	0%
Cyclin A	3	235	415	1.54	0.62-3.79	71%
Cyclin B	2	46	212	1.87	0.17-20.63	87%

Cyclin A

Three studies, containing 650 patients, of which 235 cases, were included in this meta-analysis. The overall OR for neoplastic progression in BE patients with cyclin A positivity was 1.54 (95% CI 0.62 to 3.79). Results of the three studies were inconsistent in their findings (I2=71%, P=0.03).

Cyclin D

Two studies containing 258 patients, of which 46 cases, were included in this meta-analysis. The overall OR for neoplastic progression in BE patients with cyclin D positivity was 1.87 (95% CI 0.17 to 20.63) (see table 2). Results of the two studies were inconsistent in their findings (I2=87%, P=0.006).

Studies pertaining other IHC biomarkers

The following IHC biomarkers were investigated only once: AMACR, β-catenin, CD1a, COX2, hERG1, HER2, Ki67, Lewis, Mcm2, Sialyl Lewis, SOX2, and WGA. In the CD1a study CLE without IM was used as baseline histology [42]. When considering study size and point estimate, CD1a, SOX2, and AMACR appeared most promising (see table 2).

Discussion

This is the first systematic review and meta-analysis to assess if IHC biomarkers can be used as a risk stratification tool to predict neoplastic

progression in BE surveillance. Sixteen biomarkers have been investigated in this setting, of which four biomarkers have been investigated more than once. The meta-analysis showed that aberrant p53 expression was associated with a significantly increased risk of neoplastic progression. Moreover, aberrant p53 expression predicted neoplastic progression in both non-dysplastic BE patients and BE patients with LGD. Of the other three IHC biomarkers, AOL appeared to be most promising in predicting neoplastic progression, whereas Cyclin A and Cyclin D appeared to have limited value.

Current use of p53 IHC in BE patients differs in international guidelines. The guideline of the British Society of Gastroenterology recommends the addition of p53 IHC staining for the pathological assessment of BE to improve the diagnostic reproducibility of dysplasia [5]. While the American Gastroenterological Association guideline states that: "data supporting the use of biomarkers to confirm the histologic diagnosis of dysplasia must be considered preliminary [44]. No guideline has yet adopted the use of IHC biomarkers to predict neoplastic progression. Two large population based studies confirmed that patients with LGD have an approximately 5 times higher risk of neoplastic progression compared to patients without LGD [15, 18]. Our meta-analysis is the first to show that BE patients with aberrant p53 IHC have approximately the same increased cancer risk as patients with LGD, independent of presence of dysplasia.

Although routine p53 IHC will incur higher cost than histological assessment alone, application of this marker has the potential to reduce the overall costs related to BE surveillance by improved risk stratification. The disparity in ORs of neoplastic progression found in the various studies may be explained by differences in staining methods, including antibodies used, antigen retrieval methods, definitions, and interpretations of aberrant staining used. Therefore, special consideration should be given to the protocol of staining and the definition and interpretation used for aberrant expression. Some studies did not consider loss of p53 staining aberrant, which might have contributed to the protocol being less predictive compared to other studies. By using a more stringent definition of aberrant expression, cases appeared to remain p53 aberrant, while controls were not considered as aberrant. Thus, the use of more stringent definitions and interpretations for aberrant staining will likely lead to an even higher predictive ability of p53 IHC.

The strength of this paper is the focus on IHC biomarkers as a relatively easy applicable tool to improve risk stratification in BE surveillance. No previous

publications with this approach were identified. Additionally, we performed a broad search, and the extraction of ORs from text, tables and figures resulted in the inclusion of quite a large number of trials. The inclusion of abstracts results in an up to date overview of this field. Several limitations were present in the data set, such as the apparent presence of publication bias, differences in baseline comparability within studies, and various adjustments made for these baseline differences. Thereto, we performed sensitivity analyses of the p53 meta-analyses, these show that the point estimate of the OR decreased from 6.67 to 3.18 when we accounted for these limitations. Because aberrant p53 IHC co-occurs with LGD, separate analyses were performed in which we stratified for dysplastic and non-dysplastic patients. These analyses show that aberrant p53 expression is an independent prognostic factor for neoplastic progression.

In conclusion, we show that sixteen IHC biomarkers in BE surveillance have been studied. Aberrant P53 expression is the most studied IHC biomarker and associated with a significantly increased risk to develop HGD or EAC, this association was independent of the presence of LGD. Consensus amongst pathologists concerning the appropriate staining method, definition, and interpretation of aberrant p53 expression is currently low, and more consensus is required. Other promising biomarkers such as AOL need further investigation.

References

1 American Gastroenterological A, Spechler SJ, Sharma P, Souza RF, Inadomi JM, Shaheen NJ. American Gastroenterological Association medical position statement on the management of Barrett's esophagus. Gastroenterology 2011;140:1084-91.

2 Kastelein F, Spaander MC, Biermann K, Vucelic B, Kuipers EJ, Bruno MJ. Role of acid suppression in the development and progression of dysplasia in patients with Barrett's esophagus. Dig Dis 2011;29:499-506.

3 Winters C, Jr., Spurling TJ, Chobanian SJ, Curtis DJ, Esposito RL, Hacker JF, 3rd, et al. Barrett's esophagus. A prevalent, occult complication of gastroesophageal reflux disease. Gastroenterology 1987;92:118-24.

4 Buttar NS, Wang KK. Mechanisms of disease: Carcinogenesis in Barrett's esophagus. Nat Clin Pract Gastroenterol Hepatol 2004;1:106-12.

5 Fitzgerald RC, di Pietro M, Ragunath K, Ang Y, Kang JY, Watson P, et al. British Society of Gastroenterology guidelines on the diagnosis and management of Barrett's oesophagus. Gut 2014;63:7-42.

6 Wang KK, Sampliner RE, Practice Parameters Committee of the American College of G. Updated guidelines 2008 for the diagnosis, surveillance and therapy of Barrett's esophagus. Am J Gastroenterol 2008;103:788-97.

7 Cooper GS, Kou TD, Chak A. Receipt of previous diagnoses and endoscopy and outcome from esophageal adenocarcinoma: a population-based study with temporal trends. Am J Gastroenterol 2009;104:1356-62.

8 Fountoulakis A, Zafirellis KD, Dolan K, Dexter SP, Martin IG, Sue-Ling HM. Effect of surveillance of Barrett's oesophagus on the clinical outcome of oesophageal cancer. Br J Surg 2004;91:997-1003.

9 Kastelein F, van Olphen SH, Steyerberg EW, Spaander MC, Bruno MJ, ProBar-study g. Impact of surveillance for Barrett's oesophagus on tumour stage and survival of patients with neoplastic progression. Gut 2015.

10 Rubenstein JH, Sonnenberg A, Davis J, McMahon L, Inadomi JM. Effect of a prior endoscopy on outcomes of esophageal adenocarcinoma among United States veterans. Gastrointest Endosc 2008;68:849-55.

11 Reid BJ, Levine DS, Longton G, Blount PL, Rabinovitch PS. Predictors of progression to cancer in Barrett's esophagus: baseline histology and flow cytometry identify low- and high-risk patient subsets. Am J Gastroenterol 2000;95:1669-76.

12 Sikkema M, de Jonge PJ, Steyerberg EW, Kuipers EJ. Risk of esophageal adenocarcinoma and mortality in patients with Barrett's esophagus: a systematic review and meta-analysis. Clin Gastroenterol Hepatol 2010;8:235-44; quiz e32.

13 Yousef F, Cardwell C, Cantwell MM, Galway K, Johnston BT, Murray L. The incidence

of esophageal cancer and high-grade dysplasia in Barrett's esophagus: a systematic review and meta-analysis. Am J Epidemiol 2008;168:237-49.

14 Hage M, Siersema PD, van Dekken H, Steyerberg EW, Dees J, Kuipers EJ. Oesophageal cancer incidence and mortality in patients with long-segment Barrett's oesophagus after a mean follow-up of 12.7 years. Scand J Gastroenterol 2004;39:1175-9.

15 Bhat S, Coleman HG, Yousef F, Johnston BT, McManus DT, Gavin AT, et al. Risk of malignant progression in Barrett's esophagus patients: results from a large population-based study. J Natl Cancer Inst 2011;103:1049-57.

16 Desai TK, Krishnan K, Samala N, Singh J, Cluley J, Perla S, et al. The incidence of oesophageal adenocarcinoma in non-dysplastic Barrett's oesophagus: a meta-analysis. Gut 2012;61:970-6.

17 Desai TK, Singh J, Samala N, Subbiah P. The incidence of esophageal adenocarcinoma in Barrett's esophagus has been overestimated. Am J Gastroenterol 2011;106:1364-5; author reply 5-6.

18 Hvid-Jensen F, Pedersen L, Drewes AM, Sorensen HT, Funch-Jensen P. Incidence of adenocarcinoma among patients with Barrett's esophagus. N Engl J Med 2011;365:1375-83.

19 Wani S, Falk G, Hall M, Gaddam S, Wang A, Gupta N, et al. Patients with nondysplastic Barrett's esophagus have low risks for developing dysplasia or esophageal adenocarcinoma. Clin Gastroenterol Hepatol 2011;9:220-7; quiz e6.

20 Coleman HG, Bhat S, Johnston BT, McManus D, Gavin AT, Murray LJ. Tobacco smoking increases the risk of high-grade dysplasia and cancer among patients with Barrett's esophagus. Gastroenterology 2012;142:233-40.

21 Curvers WL, ten Kate FJ, Krishnadath KK, Visser M, Elzer B, Baak LC, et al. Low-grade dysplasia in Barrett's esophagus: overdiagnosed and underestimated. Am J Gastroenterol 2010;105:1523-30.

22 Kerkhof M, van Dekken H, Steyerberg EW, Meijer GA, Mulder AH, de Bruine A, et al. Grading of dysplasia in Barrett's oesophagus: substantial interobserver variation between general and gastrointestinal pathologists. Histopathology 2007;50:920-7.

23 Cameron AJ, Carpenter HA. Barrett's esophagus, high-grade dysplasia, and early adenocarcinoma: a pathological study. Am J Gastroenterol 1997;92:586-91.

24 Levine DS, Haggitt RC, Blount PL, Rabinovitch PS, Rusch VW, Reid BJ. An endoscopic biopsy protocol can differentiate high-grade dysplasia from early adenocarcinoma in Barrett's esophagus. Gastroenterology 1993;105:40-50.

25 Younes M, Ertan A, Lechago LV, Somoano JR, Lechago J. p53 Protein accumulation is a specific marker of malignant potential in Barrett's metaplasia. Dig Dis Sci 1997;42:697-701.

26 Bani-Hani K, Martin IG, Hardie LJ, Mapstone N, Briggs JA, Forman D, et al. Prospective study of cyclin D1 overexpression in Barrett's esophagus: association with increased

risk of adenocarcinoma. J Natl Cancer Inst 2000;92:1316-21.

27 Weston AP, Banerjee SK, Sharma P, Tran TM, Richards R, Cherian R. p53 protein overexpression in low grade dysplasia (LGD) in Barrett's esophagus: immunohistochemical marker predictive of progression. Am J Gastroenterol 2001;96:1355-62.

28 Skacel M, Petras RE, Rybicki LA, Gramlich TL, Richter JE, Falk GW, et al. p53 expression in low grade dysplasia in Barrett's esophagus: correlation with interobserver agreement and disease progression. Am J Gastroenterol 2002;97:2508-13.

29 Murray L, Sedo A, Scott M, McManus D, Sloan JM, Hardie LJ, et al. TP53 and progression from Barrett's metaplasia to oesophageal adenocarcinoma in a UK population cohort. Gut 2006;55:1390-7.

30 Sikkema M, Kerkhof M, Steyerberg EW, Kusters JG, van Strien PM, Looman CW, et al. Aneuploidy and overexpression of Ki67 and p53 as markers for neoplastic progression in Barrett's esophagus: a case-control study. Am J Gastroenterol 2009;104:2673-80.

31 Bird-Lieberman EL, Dunn JM, Coleman HG, Lao-Sirieix P, Oukrif D, Moore CE, et al. Population-based study reveals new risk-stratification biomarker panel for Barrett's esophagus. Gastroenterology 2012;143:927-35 e3.

32 Kastelein F, Biermann K, Steyerberg EW, Verheij J, Kalisvaart M, Looijenga LH, et al. Aberrant p53 protein expression is associated with an increased risk of neoplastic progression in patients with Barrett's oesophagus. Gut 2013;62:1676-83.

33 Davelaar AL, Calpe S Fau - Lau L, Lau L Fau - Timmer MR, Timmer Mr Fau - Visser M, Visser M Fau - Ten Kate FJ, Ten Kate Fj Fau - Parikh KB, et al. Aberrant TP53 detected by combining immunohistochemistry and DNA-FISH improves Barrett's esophagus progression prediction: a prospective follow-up study.

34 Gimenez A, de Haro LM, Parrilla P, Bermejo J, Perez-Guillermo M, Ortiz MA. Immunohistochemical detection of p53 protein could improve the management of some patients with Barrett esophagus and mild histologic alterations. Arch Pathol Lab Med 1999;123:1260-3.

35 Brown K, Younes M, Ertan A, Verm R, Meriano FV. M1928 P53 Immunostaining Predicts Malignant Progression in Barrett's Metaplasia: WB Saunders, 2008.

36 Wolf WA, Lao-Sirieix P, Duits LC, Chak A, Shaheen NJ, Fitzgerald R, et al. Utility of a biomarker panel to predict progression to esophageal adenocarcinoma in barrett's esophagus. Gastroenterology 2014;146:S-330.

37 Lao-Sirieix P, Lovat L, Fitzgerald RC. Cyclin A immunocytology as a risk stratification tool for Barrett's esophagus surveillance. Clin Cancer Res 2007;13:659-65.

38 Sirieix PS, O'Donovan M, Brown J, Save V, Coleman N, Fitzgerald RC. Surface expression of minichromosome maintenance proteins provides a novel method for detecting patients at risk for developing adenocarcinoma in Barrett's esophagus. Clin Cancer Res 2003;9:2560-6.

39 Kastelein F, Biermann K, Steyerberg EW, Verheij J, Kalisvaart M, Looijenga LH, et al. Value of alpha-methylacyl-CoA racemase immunochemistry for predicting neoplastic progression in Barrett's oesophagus. Histopathology 2013;63:630-9.

40 van Olphen S, Biermann K, Spaander MC, Kastelein F, Steyerberg EW, Stoop HA, et al. SOX2 as a Novel Marker to Predict Neoplastic Progression in Barrett's Esophagus. Am J Gastroenterol 2015.

41 Rossi E, Grisanti S Fau - Villanacci V, Villanacci V Fau - Della Casa D, Della Casa D Fau - Cengia P, Cengia P Fau - Missale G, Missale G Fau - Minelli L, et al. HER-2 overexpression/amplification in Barrett's oesophagus predicts early transition from dysplasia to adenocarcinoma: a clinico-pathologic study.

42 Cappello F, Rappa F Fau - Anzalone R, Anzalone R Fau - La Rocca G, La Rocca G Fau - Zummo G, Zummo G. CD1a expression by Barrett's metaplasia of gastric type may help to predict its evolution towards cancer.

43 Lastraioli E, Taddei A Fau - Messerini L, Messerini L Fau - Comin CE, Comin Ce Fau - Festini M, Festini M Fau - Giannelli M, Giannelli M Fau - Tomezzoli A, et al. hERG1 channels in human esophagus: evidence for their aberrant expression in the malignant progression of Barrett's esophagus.

44 Spechler SJ, Sharma P, Souza RF, Inadomi JM, Shaheen NJ. American gastroenterological association technical review on the management of Barrett's esophagus. Gastroenterology 2011;140:e18-e52.

3

SOX2 as a novel marker to predict neoplastic progression in Barrett esophagus

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Abstract

Introduction: The value of Barrett's esophagus (BE) surveillance based on histological diagnosis of low-grade dysplasia (LGD) remains debated given the lack of adequate risk stratification. The aim of this study was to evaluate the predictive value of SOX2 expression for neoplastic progression in BE patients.
Methods: We conducted a case-control study within a prospective cohort of 720 BE patients. Patients with neoplastic progression, defined as development of high-grade dysplasia (HGD) or esophageal adenocarcinoma (EAC), were classified as cases and patients without neoplastic progression as controls. SOX2 expression was determined by immunohistochemistry in more than 12,000 biopsies from 635 patients; these results were combined with our previous p53 immunohistochemical data.
Results: Non-dysplastic BE showed homogeneous nuclear staining for SOX2, while SOX2 was progressively lost in dysplastic BE. Loss of SOX2 was seen in only 2% of biopsy series without dysplasia, in contrast to 28% in LGD and 67% in HGD/EAC. Loss of SOX2 expression was associated with an increased risk of neoplastic progression in BE patients after adjusting for gender, age, BE length and esophagitis (adjusted relative risk 4.8; 95% CI 3.2 to 7.0). The positive predictive value (PV) for neoplastic progression increased from 16% with LGD alone to 56% with concurrent loss of SOX2 and aberrant p53 expression.
Conclusions: SOX2 expression is lost during transition from non-dysplastic BE to HGD/EAC and associated with an increased risk of neoplastic progression. The highest PV is achieved by concurrent loss of SOX2 and aberrant p53 expression in BE patients with LGD. The use of these markers has the potential to significantly improve risk stratification of Barrett surveillance.

Introduction

Over the past decades, the incidence of esophageal adenocarcinoma (EAC) has increased rapidly in the Western world [1, 2]. In most cases development of EAC is related to Barrett's esophagus (BE). BE is a premalignant condition in which the squamous epithelium of the distal esophagus is replaced by columnar epithelium containing goblet cells, under the influence of chronic esophageal acid exposure [3, 4, 5]. Patients with BE have an increased risk to develop EAC with an estimated incidence of 0.1% to 0.5% per year [6, 7, 8]. The transition from BE to EAC is a gradual process, in which intestinal metaplasia evolves to low-grade dysplasia (LGD), high-grade dysplasia (HGD) and finally EAC [5, 9]. Current guidelines recommend endoscopic surveillance of BE patients to detect HGD or EAC at an early and potentially curable stage [10, 11]. However, the current endoscopic surveillance strategy based on histological diagnosis alone remains debated given the lack of discriminative power to stratify BE patients at high risk for neoplastic progression from those at low risk [12, 13].

Histological diagnosis of LGD is the only accepted predictor for neoplastic progression to date and more intensive surveillance is recommended in BE patients with LGD (yearly instead of every 3 years) [10, 14]. However, histological diagnosis of LGD has a low predictive value, owing to sample error and considerable interobserver variation [15, 16, 17]. The use of biomarkers in addition to histological assessment may improve risk stratification in BE patients and has the potential to improve cost-effectiveness of BE surveillance. Many biomarkers are under investigation and our group previously reported that aberrant p53 protein expression appears to be a highly informative predictor for neoplastic progression [18, 19, 20]. The British Society of Gastroenterology guidelines already recommend the addition of p53 immunochemistry to the histopathological assessment of BE to improve the diagnostic reproducibility of histological diagnosis of dysplasia [11]. Recent study by Weaver et al. showed that only TP53 mutations differentiated between never-dysplastic BE and malignant stages HGD and EAC, which underlines the role of p53 as potential biomarker [21]. However, only 40% of the BE patients with progression to HGD or EAC showed an aberrant P53 protein expression during surveillance, suggesting that additional biomarkers are needed [18].

Another potential biomarker is SOX2, a transcription factor involved in the maintenance of pluripotency and self-renewal in embryonic stem cells [22]. During embryonal development, SOX2 plays a pivotal role in the formation and

differentiation of esophageal and gastric epithelium [23]. In adult organism, SOX2 is expressed in many tissues including esophageal squamous epithelium as well as foveolar gastric epithelium. In the past, SOX2 was introduced mainly as a relevant oncogene; gene amplification and overexpression of SOX2 have been detected in squamous cancers of the lung and esophagus [24, 25, 26]. However, the role of SOX2 might be cell-dependent. Immunohistochemically, SOX2 protein was shown to be progressively downregulated in intestinal metaplasia and adjacent gastric cancer [27, 28]. Recent studies in gastric cancer cells revealed a role of SOX2 in growth inhibition through cell-cycle arrest and induction of apoptosis, indicating cancer-suppressive functions [29]. Furthermore, loss of SOX2 expression was detected in EAC in earlier studies and associated with poor survival [30, 31, 32]. However, the role of SOX2 in the development of BE and its expression during the metaplasia-dysplasia-adenocarcinoma sequence in BE epithelium has not yet been investigated.

Therefore, the aim of the present study was to evaluate SOX2 expression in a prospective BE cohort within different grades of dysplasia and to assess the value of SOX2 to predict neoplastic progression in patients with BE. In addition, the results obtained from this analysis were combined with our previously reported p53 immunohistochemical data.

Methods

Study design

We conducted a case-control study within a large ongoing prospective cohort of 720 consecutive BE patients. Patients were included between November 2003 and December 2004 in three university medical centers and 12 regional hospitals throughout the Netherlands and received endoscopic surveillance according to the guidelines of the American College of Gastroenterology [10]. Inclusion criterion was known or newly diagnosed BE of at least 2 cm, histologically confirmed by the presence of intestinal metaplasia on initial biopsy. Patients with a history of HGD or esophageal cancer were excluded. All endoscopic procedures were performed by an experienced gastroenterologist, according to a standard protocol. An experienced gastroenterologist was defined as a gastroenterologist with at least several years of experience in endoscopic procedures and with an interest for BE. Prior to taking biopsies, endoscopic landmarks such as the diaphragm impression, gastroesophageal

junction and squamocolumnar junction were noted. The presence of esophagitis was graded according to the Los Angeles Classification, and abnormalities were reported [33]. At each endoscopy quadrant biopsies were taken every 2 cm from the most distal to the most proximal part of the Barrett's epithelium, according to the Seattle protocol and targeted biopsies were taken from mucosal abnormalities [34]. Patients without dysplasia, based on a histological consensus diagnosis, underwent upper endoscopy with biopsy sampling every three years and patients with LGD every year.

Histology

Biopsy samples were fixed in 10% buffered formalin and embedded in paraffin, according to standard procedure. From each biopsy set, four-micrometer thick sections were cut and stained with haematoxylin-eosin. After examination of all biopsy samples, the highest degree of abnormality was reported for each endoscopy. Slides were assessed for the presence of BE and grade of dysplasia, first by a local pathologist and second by an expert pathologist. When the local pathologist and expert pathologist disagreed on the grade of dysplasia, a second expert pathologist evaluated the slides. Pathologists were blinded to each other's diagnosis and a final diagnosis was made only if at least two pathologists agreed on the grade of dysplasia. If there was still disagreement, a panel of expert pathologists reviewed the slides and a final diagnosis was made based on consensus agreement. Given the equal surveillance strategy according the ACG guidelines, the biopsies (n=7) with the diagnosis of indefinite for dysplasia were included in the group of biopsies with LGD.

Patient selection

We collected paraffin material suitable for immunohistochemistry from all 720 patients in our BE cohort. Paraffin material was not available in 85 patients, leaving 635 patients to be included in this study. Patients who developed HGD or EAC during follow-up were classified as cases and patients without neoplastic progression were classified as controls (Figure 1). In accordance with our previous p53 paper, the minimum time interval between the index biopsies and a diagnosis of HGD or EAC was nine months to prevent inclusion of prevalent cases. A sensitivity analysis with a minimum time interval of 1 year was performed and had no impact on the results. Immunohistochemistry was performed on the complete series of biopsies of all surveillance endoscopies of patients who developed any form of dysplasia i.e. LGD, HGD or EAC during follow-up. This included the total number of biopsies taken during

endoscopic surveillance at different levels of the Barrett segment, according to the Seattle protocol. In patients without any dysplasia during follow-up, immunohistochemistry was performed on biopsies of a random surveillance endoscopy.

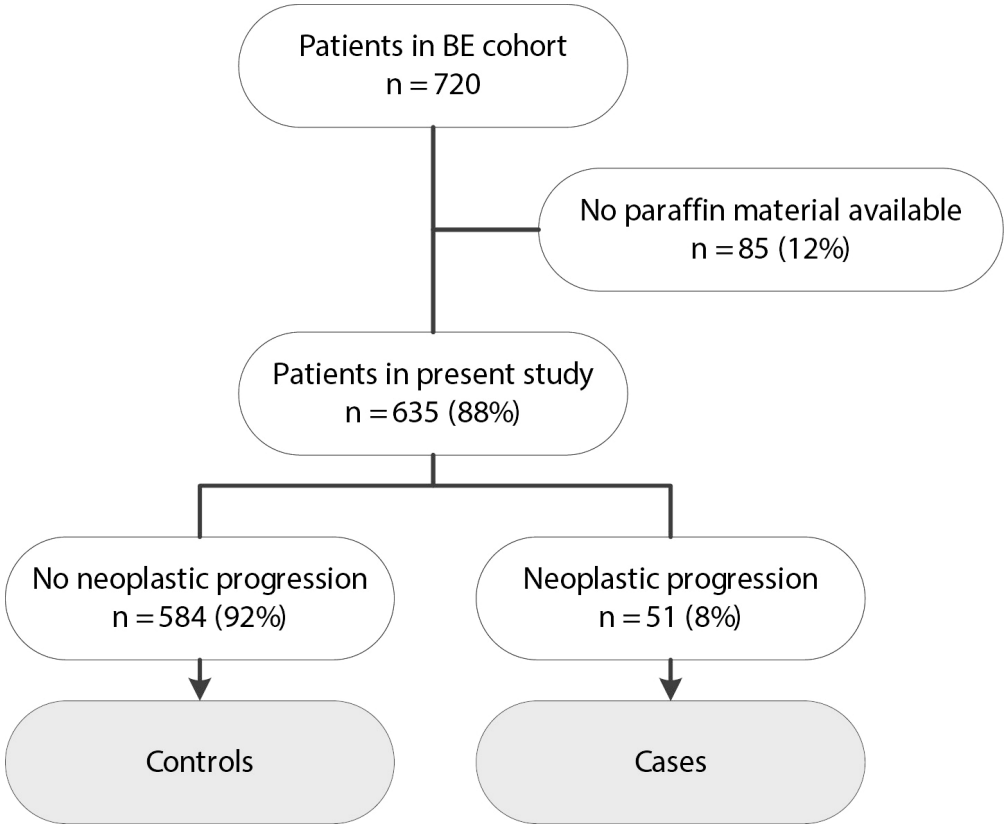

Figure 1. Flowchart of patients included in the study. Patients with neoplastic progression were classified as cases and patients without neoplastic progression were classified as controls.

Immunohistochemistry

For SOX2 immunohistochemistry, paraffin-embedded tissue sections were deparaffinized in xylene and rehydrated in graded alcohols. Antigen retrieval was enhanced by heating in Tris buffer and endogenous peroxidase activity was blocked by incubating the slides in a solution of 0.3% hydrogen peroxide in phosphate-buffered saline. Primary antibody (AF2018; R&D systems, Abingdon, United Kingdom: goat, polyclonal) with a dilution of 1:400 was incubated overnight at 4 degrees Celsius. As secondary antibody, a biotinylated horse anti-goat IgG antibody was used (1:150; BA-950, Vector, United Kingdom). Visualization was achieved by using the horseradish peroxidase avidin-biotin complex (HRP-ABC) method and diaminobenzidine (DAB). Finally, the slides were counterstained with haematoxylin. A sample of embryonal carcinoma was used as positive control for each section [35]. Immunohistochemical staining for p53 was performed by using an automatic immunohistochemical staining machine, as previously described [18]. Briefly, sections were deparaffinized and heat-induced epitope retrieval was performed at 97 °C for 15 min. Endogenous peroxidase activity was blocked by incubating the slides in a solution of 3.0% hydrogen peroxide in phosphate-buffered saline. The primary antibody (Clone DO-7, Dako, Glostrup, Denmark: mouse monoclonal) with a dilution of 1:25 was applied for 30 min. Visualization was achieved by using the Dako REAL EnVision system (peroxidase/DAB, Rabbit/Mouse, Dako, Glostrup, Denmark).

Immunohistochemically stained slides were examined in tandem with the haematoxylin-eosin stained slides to determine SOX2 and p53 expression in areas with dysplasia. Nuclear SOX2 expression was scored on a binary two-point scale; positive or loss of expression. Positive expression included strong as well as weak nuclear SOX2 positivity and was interpreted as normal expression. As previously described, P53 expression was scored on a three-point scale; normal expression, overexpression or loss of expression. Only intense nuclear staining for p53 was scored as overexpression. All stained slides were scored by two independent experienced investigators (KB and SO) who were blinded for long-term outcome. Loss of SOX2 expression in a cluster of glands, excluding BE glands containing many goblet cells (i.e., colon-like BE) was defined as aberrant SOX2 expression. Aberrant p53 expression was defined as either overexpression or complete loss of expression in at least one gland. In biopsy series with dysplasia, SOX2 and p53 expression was scored in the dysplastic areas. After scoring all biopsies, the highest degree of

abnormality was reported for each endoscopy. When there was disagreement between the two investigators, slides were reviewed by both investigators simultaneously to reach a consensus diagnosis.

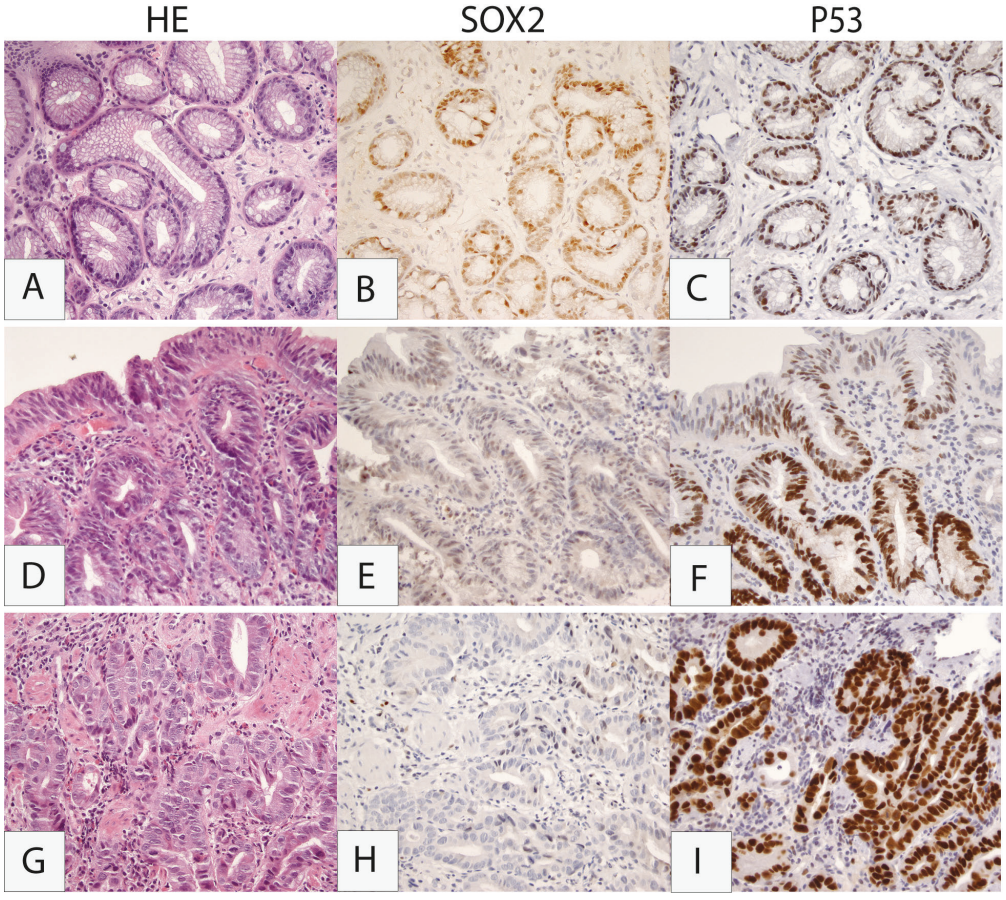

Figure 3. Representative examples of SOX2 and p53 immunohistochemistry in non-dysplastic Barrett's epithelium (BE), LGD and HGD (A-I). (A-C) Non-dysplastic BE with normal nuclear SOX2 (B) and normal p53 expression(C); (D-F) BE with LGD, loss of SOX2 expression (E) and overexpression of p53 (F) in dysplastic epithelial cells; (G-I) BE with HGD, loss of SOX2 expression (H) and p53 overexpression (I) in corresponding glands. Magnification 1:200 (A-I).

Ethics

The study protocol was approved by the institutional review board of the Erasmus Medical Center, including those of all participating hospitals. Before the first endoscopy, written informed consent was obtained from all patients.

Statistical analysis

Characteristics of cases and controls were compared using Mann-Whitney-Wilcoxon tests for continuous variables and $\chi 2$ tests for categorical variables. To compare SOX2 expression in biopsy series of cases and controls with different grade of dysplasia, the Mann-Whitney-Wilcoxon test and Kruskal-Wallis test were used, thereby ignoring that multiple biopsy series could be from the same patient. Neoplastic progression was defined as the development of HGD or EAC after inclusion and follow-up time was defined as the time between two consecutive surveillance endoscopies. The value of SOX2 and p53 immunohistochemistry to predict neoplastic progression was estimated in loglinear regression models. The fact that immunohistochemistry was not performed on all biopsy series, data were split up by endoscopy. Loglinear models were used to calculate relative risks (RRs) and 95% Confidence Intervals (CIs) with the logarithm of follow-up time as offset variable. In multivariable models we adjusted for gender, age, BE length and esophagitis to estimate adjusted RRs. Interobserver agreement for SOX2 expression was determined by using Cohen kappa statistics. Kappa value of <0.21 reflects 'poor', 0.21 to 0.40 'fair', 0.41 to 0.60 'moderate', 0.61 to 0.8 'substantial', and above 0.81 'very good' [36]. Two sided p values <0.05 were considered statistical significant. Data were analyzed using SPSS software (V.22.0).

Results

Patient characteristics

We followed 635 patients with BE (73% men, median age of 60 years (interquartile range (IQR) 53-69)) for a median duration of 6.9 years (IQR 5.1-7.5). Thirty-seven (6%) patients developed HGD and 14 (2%) patients developed EAC during follow-up. These 51 (8%) patients with neoplastic progression were classified as cases. The incidence rate of HGD and EAC together was 1.3 per 100 patients-years. The remaining 584 (92%) patients without neoplastic progression were classified as controls (Figure 1). Biopsy

series were defined as a total number of biopsies from one endoscopy. Histology, SOX2 and p53 protein expression were assessed in biopsy series of 1,486 endoscopies, 194 endoscopies were performed in cases and 1,292 in controls. The highest degree of abnormality was reported for each endoscopy after evaluation of all biopsies. In total more than 12,000 biopsies were reviewed. Except for a smaller number of follow-up endoscopies, a higher number of biopsies per endoscopy, longer Barrett length and more often diagnosed with LGD at baseline there were no significant differences between cases and controls (Table 1).

Table 1. Characteristics of cases and controls

		Controls n = 584	Cases n = 51	p Value
Follow-up	Median, years (IQR)	6.5 (5.2-7.2)	3.3 (1.9-5.3)	<0.001
Endoscopies	Median number (IQR)	4 (4-5)	3 (2-4)	<0.001
Biopsies	Median per endoscopy (IQR)	6 (4-9)	8 (6-12)	0.001
	Total Number	10.560	1837	
Age	Median, years (IQR)	60 (53-69)	65 (54-71)	0.145
Sex	Male	424 (73%)	42 (82%)	0.131
Alcohol use	Never	68 (12%)	6 (12%)	0.992
	Former	53 (9%)	5 (10%)	
	Current	451 (79%)	40 (78%)	
Smoking	Never	190 (33%)	12 (24%)	0.312
	Former	261 (46%)	25 (49%)	
	Current	121 (21%)	14 (27%)	
Reflux symptoms	Yes	176 (31%)	19 (37%)	0.326
Barrett diagnosis	≤ 1999	237 (41%)	16 (33%)	0.401
	2000-2002	199 (34%)	21 (39%)	
	2003-2004	143 (25%)	14 (30%)	
Barrett length	Median, cm (IQR)	4 (3-6)	5 (3-7)	<0.001
Baseline histology	Low-grade dysplasia	89 (15%)	24 (47%)	<0.001
Esophagitis	Yes	110 (19%)	14 (28%)	0.117

Patients with neoplastic progression were classified as cases and patients without neoplastic progression were classified as controls.
Mann-Whitney U-test and chi-squares test were used to compare the characteristics of cases and controls
IQR, interquartile range

Histology

Histology was assessed in biopsies series of 1,486 endoscopies of both cases and controls. This included 1,094 (74%) biopsy series without dysplasia, 341 (23%) with LGD, 37 (2%) with HGD and 14 (1%) with EAC. Presence of LGD was more common in biopsy series of cases (47%) than in biopsy series of controls (21%) and was associated with an increased risk of neoplastic progression with a RR of 4.3 (95% CI 3.0 to 6.0) and this association remained after adjusting for gender, age, BE length and esophagitis (adjusted RR 4.0; 95% CI 2.8 to 5.7) (Table 2). In total, 227 (36%) patients were diagnosed with LGD during follow-up, of these patients only 37 (16%) eventually developed HGD or EAC. The sensitivity for predicting neoplastic progression was 47% with a specificity of 79%.

Table 2. Histology, p53 and SOX2 expression in biopsy series of cases and controls

	Controls n = 1292	Cases n = 143	RR (95% CI)	RR[a] (95% CI)
Histology				
ND	1018 (79%)	76 (53%)	Reference	Reference
LGD	274 (21%)	67 (47%)	4.3 (3.0-6.0)	4.0 (2.8-5.7)
SOX2 expression				
Normal SOX2 expression	1207 (93%)	107 (75%)	Reference	Reference
Aberrant SOX2 expression	85 (7%)	36 (25%)	5.2 (3.5-7.6)	4.8 (3.2-7.0)
P53 expression				
Normal p53 expression	1119 (86%)	70 (49%)	Reference	Reference
Aberrant p53 expression	173 (14%)	73 (51%)	6.1 (4.3-8.4)	5.7 (4.1-8.0)
Histology and SOX2 expression				
ND & normal SOX2	1000 (78%)	69 (48%)	Reference	Reference
LGD & normal SOX2	207 (16%)	38 (27%)	3.5 (2.4-5.2)	3.2 (2.1-4.8)
ND & aberrant SOX2	18 (1%)	7 (5%)	4.6 (1.8-10.2)	4.0 (1.8-8.8)
LGD & aberrant SOX2	67 (5%)	29 (20%)	8.0 (5.2-12.4)	7.6 (4.9-11.8)
Histology and p53 expression				
ND & normal p53	930 (72%)	50 (35%)	Reference	Reference
LGD & normal p53	189 (15%)	20 (14%)	2.4 (0.9-5.8)	2.2 (0.8-5.0)
ND & aberrant p53	88 (7%)	26 (18%)	4.7 (2.9-7.7)	4.4 (2.7-7.1)
LGD & aberrant p53	85 (7%)	47 (33%)	11.0 (7.3-16.4)	10.4 (6.9-15.7)
Histology, p53 and SOX2 expression				
ND, normal SOX2 & normal p53	918 (71%)	44 (31%)	Reference	Reference
LGD, normal SOX2 & normal p53	141 (11%)	13 (9%)	2.1 (0.6-4.9)	2.0 (0.5-4.5)

	Controls n = 1292	Cases n = 143	RR (95% CI)	RR[a] (95% CI)
ND, aberrant SOX2 or aberrant p53	94 (7%)	31 (22%)	5.9 (3.8-9.4)	5.3 (3.3-8.3)
LGD, aberrant SOX2 or aberrant p53	114 (9%)	32 (22%)	8.0 (5.1-12.7)	7.3 (4.6-11.5)
ND, aberrant SOX2 & aberrant p53	6 (1%)	1 (1%)	2.9 (0.5-24.8)	2.8 (0.4-27.5)
LGD, aberrant SOX2 & aberrant p53	19 (1%)	22 (15%)	18.2 (10.7-30.5)	18.5 (11.1-31.2)
Number of aberrant markers[b]				
0	918 (71%)	44 (31%)	Reference	Reference
1	235 (18%)	44 (31%)	4.3 (2.9-6.6)	3.9 (2.6-6.0)
2	120 (9%)	33 (23%)	7.7 (4.9-12.1)	7.0 (4.4-11.0)
3	19 (2%)	22 (15%)	18.2 (10.7-30.5)	18.5 (10.9-31.1)

The highest degree of abnormality was reported for each endoscopy after examining all biopsies.
RR, relative risks; CI, confidence interval; ND, no dysplasia; LGD, low-grade dysplasia.
[a] RR adjusted for gender, age, BE length and esophagitis.
[b] Included aberrant markers were LGD, aberrant p53 expression and aberrant SOX2 expression.

SOX2 immunohistochemistry

Homogeneous nuclear SOX2 protein expression, classified as normal expression, was seen in all biopsies where squamous epithelium was present, as well as in most biopsies with non-dysplastic BE. In cases and controls together, normal SOX2 expression was seen in 1,333 (90%) biopsy series whereas loss of SOX2 expression, classified as aberrant SOX2 expression, was seen in 153 (10%) biopsy series. The interobserver agreement for SOX2 expression was good with a kappa-value of 0.70 (95% CI 0.67 to 0.73). The observers disagreed on SOX2 expression in 106 (7%) biopsy series (Table 3). Interestingly, while loss of SOX2 expression was seen in 25 (2%) biopsy series without dysplasia, it was more frequent in dysplastic BE, including 96 (28%) biopsy series with LGD, 22 (63%) biopsy series with HGD and 10 (71%) biopsies with EAC ($p<0.001$) (Figure 2). In addition, loss of SOX2 expression was more common in biopsy series of cases (25%) than in biopsy series of controls (7%) and was associated with an increased risk of neoplastic progression with a RR of 5.2 (95% CI 3.5 to 7.6). This association remained after adjusting for gender, age, BE length and esophagitis with an adjusted RR of 4.8 (95% CI 3.2 to 7.0) and was seen in biopsy series without dysplasia and biopsy series with LGD (Table 2). The sensitivity of aberrant SOX2 expression for predicting neoplastic progression was 25% with a specificity of 94%. In total, 73 (12%) patients were diagnosed with LGD and concurrent aberrant SOX2 expression during follow-up. Of these patients, 22 (30%) eventually developed HGD or

EAC.

Table 3. Interobserver agreement for SOX2 expression

SOX2 expression	Positive expression	Loss of expression	K value
Positive expression	1253 (84%)	45 (3%)	0.70
Loss of expression	61 (4%)	127 (9%)	

The highest degree of abnormality was reported for each endoscopy after examination of all biopsies. Cohen K statistics were used to determine interobserver agreement.
The observers disagreed on SOX2 expression in 106 (7%) of the biopsy series with balanced results between the two observers in final SOX2 score (55% observer 1 versus 45% observer 2).

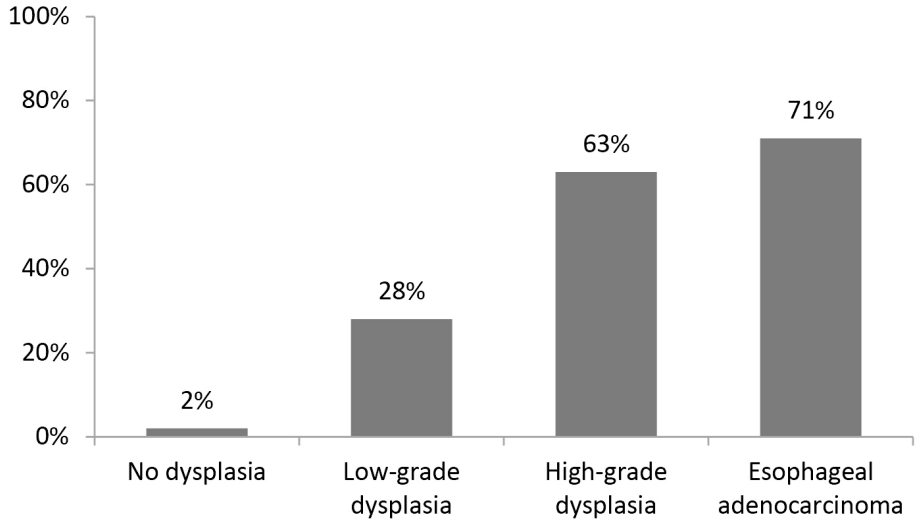

Figure 2. Percentage of biopsy series with loss of SOX2 expression stratified by grade of dysplasia. ■ Loss of SOX2 expression

Histology, SOX2 and p53 immunohistochemistry

Previous p53 stained slides were re-evaluated in this study.[18] Briefly, aberrant p53 expression was more common in biopsy series of cases (51%) than in biopsy series of controls (14%) and was associated with an increased risk of neoplastic progression with an adjusted RR of 5.7 (95% CI 4.1 to 8.0) (Table 2). A total of 72 (11%) patients were diagnosed with LGD and concurrent aberrant p53 expression during follow-up. Of these patients, 26 (36%) eventually developed HGD or EAC.

Histology and SOX2 expression were correlated with our previously published p53 immunohistochemical data (Figure 3 and Table 2). Non-dysplastic BE with both normal SOX2 and p53 expression was seen in 962 (67%) biopsy series. In contrast, non-dysplastic BE with aberrant SOX2 or p53 expression was detected in only 125 (9%) of the biopsy series of cases and controls. LGD with normal SOX2 and p53 expression was more common (11%) than LGD with presence of aberrant SOX2 or p53 expression (10%), as also more common than LGD with the presence of both aberrant SOX2 and p53 expression (3%). Aberrant expression of either SOX2 or p53 was more common in biopsy series of cases than in biopsy series of controls, in both, non-dysplastic BE (22% vs 7%) and in LGD (22% vs 9%). Also, concurrent aberrant SOX2 and p53 expression was more common in biopsy series of cases than in biopsy series of controls in LGD (15% vs 1%) but not in non-dysplastic BE (1% vs 1%). Aberrant SOX2 or p53 expression in non-dysplastic BE was associated with an increased risk of neoplastic progression with an adjusted RR of 5.3 (95% CI 3.3 to 8.3), the risk was even higher with concurrent LGD (adjusted RR 7.3; 95% CI 4.6 to 11.5), but aberrant expression of both SOX2 and p53 in BE with LGD was associated with the highest risk of neoplastic progression (adjusted RR 18.5; 95% CI 11.1 to 31.2). The risk to develop HGD or EAC in the individual BE patient increases with the number of aberrant markers, including LGD, aberrant SOX2 and p53 expression (Table 2).

During follow-up, 32 (5%) patients were diagnosed with aberrant SOX2 and concurrent aberrant p53 expression in BE with LGD. Of these patients, 18 (56%) eventually developed HGD or EAC (positive predictive value of 56%).

Calculated RR to predict neoplastic progression subdivided into cases with progression to HGD and cases with progression to EAC are presented in the supplementary data.

Discussion

In this case-control study, we confirmed the independent value of SOX2 immunohistochemistry to predict neoplastic progression in patients with BE when combined with our previously reported p53 immunohistochemical data. SOX2 expression was progressively lost in dysplastic BE and associated with an increased risk of neoplastic progression. The risk of neoplastic progression was the highest in patients with LGD and concurrent aberrant SOX2 and p53 expression.

Surveillance of BE patients, especially of those with LGD is under significant debate. Several previous studies demonstrated repeatedly the value of LGD as a risk factor for neoplastic progression, although with a low predictive value [14, 16, 33, 34, 37]. In our study up to 36% of patients were diagnosed with LGD during surveillance, while only 16% of these patients eventually developed HGD or EAC. Ultimately, the predictive value was low, despite using a consensus diagnosis of dysplasia. The diagnosis of LGD in BE is challenging due to sampling error and considerable interobserver variation, mainly because features of dysplasia may overlap with features of non-neoplastic regenerative changes [15, 17]. Even though the predictive value of LGD increases with consensus of multiple pathologists, still one-third of the BE patients are diagnosed with LGD during surveillance, while the 10-year cumulative incidence of neoplastic progression is only around 15% in this group [16, 37, 38]. This obviously has important implications for the cost-effectiveness of a surveillance program [39].

SOX2 is a high-mobility group box transcription factor, involved in the maintenance of pluripotency and self-renewal in embryonic stem cells and regulates an array of genes involved in normal and malignant processes [22, 40, 41]. Until now, only one publication reported on SOX2 in BE. Chen and coworkers studied SOX2 in rat and human BE and observed that SOX2 was present in multilayered esophageal epithelium, but was downregulated in biopsy samples with intestinal metaplasia in both species [42]. Our study is the first study to explore SOX2 expression in different grades of dysplasia in BE and to test its value to predict neoplastic progression. Because it was shown by us and others, that p53 is one of the most promising predictive experimental molecular markers so far, we also tested the combined value of SOX2 and p53 within the same cohort [37, 43, 44, 45].

In the present study strong nuclear SOX2 expression was seen in all biopsies were squamous epithelium was present, as well as in most non-dysplastic BE.

Interestingly, expression of SOX2 was progressively lost in BE with neoplastic changes. The percentage of biopsy series with loss of SOX2 expression increased from 2% in BE samples without dysplasia to 71% in samples with EAC. Our results suggest that SOX2 may play a role along the metaplasia-dysplasia-adenocarcinoma sequence in BE. Loss of SOX2 expression might reflect the loss of differentiation potential of metaplastic columnar epithelial cells. Previous studies reported loss of SOX2 expression in 65-87% of human samples with EAC, which corresponds to the results of our study [30, 31]. Loss of SOX2 expression was identified more frequently in cases than in controls and was associated with an increased risk of developing HGD or EAC. The positive predictive value for neoplastic progression increased from 16% with histological diagnosis of LGD alone to 30% with concurrent aberrant SOX2. P53 as a single biomarker has more power to predict neoplastic progression than SOX2 alone, but the highest predictive value was achieved by the combination of histological diagnosis of LGD with concurrent loss of SOX2 and aberrant p53 expression amounting to a positive predictive value of 56%. This finding might have important and clinically relevant implications. A recent study from Phoa et al. showed that in patients with BE and a consensus diagnosis of LGD, treatment with radiofrequency ablation resulted in a reduced risk of neoplastic progression [46]. To date, we have limited ability to predict which patients with LGD and no visible mucosal irregularities are truly at risk to develop an esophageal malignancy. Assessment of SOX2 and p53 immunohistochemistry offers an added tool to select these high risk patients for either intensified surveillance or ablation of BE epithelium and may contribute to a more cost-effective management.

In this study we have also shown good interobserver agreement for the assessment of SOX2 expression similar to the results on p53 expression, which indicates that both markers are clinically suitable markers to predict progression in BE [18]. The evaluation of SOX2 immunohistochemistry was simple and straightforward; surrounding squamous epithelium as well as BE without dysplasia with positive SOX2 expression, contrasts well to the areas of SOX2 loss. Although routine p53 and SOX2 immunohistochemistry incur higher costs than histology alone, application of this panel of biomarkers has the potential to reduce overall costs of BE surveillance. Patients at low-risk of neoplastic progression, i.e. the majority of LGD patients, might be followed up less intensively. However, cost-effectiveness analysis should be performed to examine the economic value of both, p53 and SOX2 immunohistochemistry in

BE surveillance, which is beyond the scope of this study.
Our study has several strengths. Patients were prospectively followed according to a stringent scheme during a long follow-up time and both clinical and pathological data were collected. Additionally, a standardized endoscopy and biopsy protocol was used. All slides were reviewed by at least two experienced observers to obtain a final diagnosis based on consensus. A limitation of the study is that we cannot exclude that patients presently classified as controls may develop HGD or EAC in the future. This may lead to underestimation of the value of SOX2 and p53 to predict neoplastic progression. Further validation of these markers in large, prospective studies is required to confirm our findings.
In conclusion, loss of SOX2 expression is associated with an increased risk of neoplastic progression in patients with BE. The combination of histological diagnosis of LGD, aberrant p53 and loss of SOX2 expression has the highest predictive value to identify BE patients at high risk to develop HGD or EAC (56%). Clinical application of these biomarkers in routine practice has the potential to improve cost-effectiveness of BE surveillance.

References

1 Brown LM, Devesa SS, Chow WH. Incidence of adenocarcinoma of the esophagus among white Americans by sex, stage, and age. J Natl Cancer Inst 2008;100:1184-7.

2 Thrift AP, Whiteman DC. The incidence of esophageal adenocarcinoma continues to rise: analysis of period and birth cohort effects on recent trends. Ann Oncol 2012;23:3155-62.

3 Kastelein F, Spaander MC, Biermann K, Vucelic B, Kuipers EJ, Bruno MJ. Role of acid suppression in the development and progression of dysplasia in patients with Barrett's esophagus. Dig Dis 2011;29:499-506.

4 Winters C, Jr., Spurling TJ, Chobanian SJ, Curtis DJ, Esposito RL, Hacker JF, 3rd, et al. Barrett's esophagus. A prevalent, occult complication of gastroesophageal reflux disease. Gastroenterology 1987;92:118-24.

5 American Gastroenterological A, Spechler SJ, Sharma P, Souza RF, Inadomi JM, Shaheen NJ. American Gastroenterological Association medical position statement on the management of Barrett's esophagus. Gastroenterology 2011;140:1084-91.

6 de Jonge PJ, van Blankenstein M, Looman CW, Casparie MK, Meijer GA, Kuipers EJ. Risk of malignant progression in patients with Barrett's oesophagus: a Dutch nationwide cohort study. Gut 2010;59:1030-6.

7 Sikkema M, de Jonge PJ, Steyerberg EW, Kuipers EJ. Risk of esophageal adenocarcinoma and mortality in patients with Barrett's esophagus: a systematic review and meta-analysis. Clin Gastroenterol Hepatol 2010;8:235-44; quiz e32.

8 Hvid-Jensen F, Pedersen L, Drewes AM, Sorensen HT, Funch-Jensen P. Incidence of Adenocarcinoma among Patients with Barrett's Esophagus. New Engl J Med 2011;365:1375-83.

9 Buttar NS, Wang KK. Mechanisms of disease: Carcinogenesis in Barrett's esophagus. Nat Clin Pract Gastroenterol Hepatol 2004;1:106-12.

10 Wang KK, Sampliner RE, Practice Parameters Committee of the American College of G. Updated guidelines 2008 for the diagnosis, surveillance and therapy of Barrett's esophagus. Am J Gastroenterol 2008;103:788-97.

11 Fitzgerald RC, di Pietro M, Ragunath K, Ang Y, Kang JY, Watson P, et al. British Society of Gastroenterology guidelines on the diagnosis and management of Barrett's oesophagus. Gut 2014;63:7-42.

12 Ronkainen J, Aro P, Storskrubb T, Johansson SE, Lind T, Bolling-Sternevald E, et al. Prevalence of Barrett's esophagus in the general population: an endoscopic study. Gastroenterology 2005;129:1825-31.

13 Wang JS, Canto MI. Predicting Neoplastic Progression in Barrett's Esophagus. Ann Gastroentol Hepatol 2010;1:1-10.

14 Sikkema M, Looman CW, Steyerberg EW, Kerkhof M, Kastelein F, van Dekken H, et al. Predictors for neoplastic progression in patients with Barrett's Esophagus: a prospective cohort

study. Am J Gastroenterol 2011;106:1231-8.

15 Kerkhof M, van Dekken H, Steyerberg EW, Meijer GA, Mulder AH, de Bruine A, et al. Grading of dysplasia in Barrett's oesophagus: substantial interobserver variation between general and gastrointestinal pathologists. Histopathology 2007;50:920-7.

16 Skacel M, Petras RE, Gramlich TL, Sigel JE, Richter JE, Goldblum JR. The diagnosis of low-grade dysplasia in Barrett's esophagus and its implications for disease progression. Am J Gastroenterol 2000;95:3383-7.

17 Curvers WL, ten Kate FJ, Krishnadath KK, Visser M, Elzer B, Baak LC, et al. Low-grade dysplasia in Barrett's esophagus: overdiagnosed and underestimated. Am J Gastroenterol 2010;105:1523-30.

18 Kastelein F, Biermann K, Steyerberg EW, Verheij J, Kalisvaart M, Looijenga LH, et al. Aberrant p53 protein expression is associated with an increased risk of neoplastic progression in patients with Barrett's oesophagus. Gut 2012.

19 Kastelein F, Biermann K, Steyerberg EW, Verheij J, Kalisvaart M, Looijenga LH, et al. Value of alpha-methylacyl-CoA racemase immunochemistry for predicting neoplastic progression in Barrett's oesophagus. Histopathology 2013;63:630-9.

20 di Pietro M, Boerwinkel DF, Shariff MK, Liu X, Telakis E, Lao-Sirieix P, et al. The combination of autofluorescence endoscopy and molecular biomarkers is a novel diagnostic tool for dysplasia in Barrett's oesophagus. Gut 2015;64:49-56.

21 Weaver JM, Ross-Innes CS, Shannon N, Lynch AG, Forshew T, Barbera M, et al. Ordering of mutations in preinvasive disease stages of esophageal carcinogenesis. Nat Genet 2014;46:837-43.

22 Liu K, Lin B, Zhao M, Yang X, Chen M, Gao A, et al. The multiple roles for Sox2 in stem cell maintenance and tumorigenesis. Cell Signal 2013;25:1264-71.

23 Que J, Okubo T, Goldenring JR, Nam KT, Kurotani R, Morrisey EE, et al. Multiple dose-dependent roles for Sox2 in the patterning and differentiation of anterior foregut endoderm. Development 2007;134:2521-31.

24 Wilbertz T, Wagner P, Petersen K, Stiedl AC, Scheble VJ, Maier S, et al. SOX2 gene amplification and protein overexpression are associated with better outcome in squamous cell lung cancer. Mod Pathol 2011;24:944-53.

25 Wang Q, He W, Lu C, Wang Z, Wang J, Giercksky KE, et al. Oct3/4 and Sox2 are significantly associated with an unfavorable clinical outcome in human esophageal squamous cell carcinoma. Anticancer Res 2009;29:1233-41.

26 Yang F, Gao Y, Geng J, Qu D, Han Q, Qi J, et al. Elevated expression of SOX2 and FGFR1 in correlation with poor prognosis in patients with small cell lung cancer. Int J Clin Exp Pathol 2013;6:2846-54.

27 Tsukamoto T, Inada K, Tanaka H, Mizoshita T, Mihara M, Ushijima T, et al. Down-

regulation of a gastric transcription factor, Sox2, and ectopic expression of intestinal homeobox genes, Cdx1 and Cdx2: inverse correlation during progression from gastric/intestinal-mixed to complete intestinal metaplasia. J Cancer Res Clin Oncol 2004;130:135-45.

28 Tsukamoto T, Mizoshita T, Mihara M, Tanaka H, Takenaka Y, Yamamura Y, et al. Sox2 expression in human stomach adenocarcinomas with gastric and gastric-and-intestinal-mixed phenotypes. Histopathology 2005;46:649-58.

29 Otsubo T, Akiyama Y, Yanagihara K, Yuasa Y. SOX2 is frequently downregulated in gastric cancers and inhibits cell growth through cell-cycle arrest and apoptosis. Br J Cancer 2008;98:824-31.

30 DiMaio MA, Kwok S, Montgomery KD, Lowe AW, Pai RK. Immunohistochemical panel for distinguishing esophageal adenocarcinoma from squamous cell carcinoma: a combination of p63, cytokeratin 5/6, MUC5AC, and anterior gradient homolog 2 allows optimal subtyping. Hum Pathol 2012;43:1799-807.

31 Long KB, Hornick JL. SOX2 is highly expressed in squamous cell carcinomas of the gastrointestinal tract. Hum Pathol 2009;40:1768-73.

32 Honing J, Pavlov KV, Meijer C, Smit JK, Boersma-van Ek W, Karrenbeld A, et al. Loss of CD44 and SOX2 expression is correlated with a poor prognosis in esophageal adenocarcinoma patients. Ann Surg Oncol 2014;21 Suppl 4:S657-64.

33 DiBaise JK. The LA classification for esophagitis: a call for standardization. Am J Gastroenterol 1999;94:3403-4.

34 Levine DS, Haggitt RC, Blount PL, Rabinovitch PS, Rusch VW, Reid BJ. An endoscopic biopsy protocol can differentiate high-grade dysplasia from early adenocarcinoma in Barrett's esophagus. Gastroenterology 1993;105:40-50.

35 de Jong J, Stoop H, Gillis AJ, van Gurp RJ, van de Geijn GJ, Boer M, et al. Differential expression of SOX17 and SOX2 in germ cells and stem cells has biological and clinical implications. J Pathol 2008;215:21-30.

36 Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics 1977;33:159-74.

37 Kaye PV, Haider SA, Ilyas M, James PD, Soomro I, Faisal W, et al. Barrett's dysplasia and the Vienna classification: reproducibility, prediction of progression and impact of consensus reporting and p53 immunohistochemistry. Histopathology 2009;54:699-712.

38 Duits LC, Phoa KN, Curvers WL, Ten Kate FJ, Meijer GA, Seldenrijk CA, et al. Barrett's oesophagus patients with low-grade dysplasia can be accurately risk-stratified after histological review by an expert pathology panel. Gut 2014.

39 Kastelein F, van Olphen S, Steyerberg EW, Sikkema M, Spaander MC, Looman CW, et al. Surveillance in patients with long-segment Barrett's oesophagus: a cost-effectiveness analysis. Gut 2014.

40 Wang JL, Rao S, Chu JL, Shen XH, Levasseur DN, Theunissen TW, et al. A protein interaction network for pluripotency of embryonic stem cells. Nature 2006;444:364-8.

41 Boiani M, Scholer HR. Regulatory networks in embryo-derived pluripotent stem cells. Nat Rev Mol Cell Bio 2005;6:872-84.

42 Chen XX, Qin R, Liu B, Ma Y, Su YH, Yang CS, et al. Multilayered epithelium in a rat model and human Barrett's esophagus: Similar expression patterns of transcription factors and differentiation markers. Bmc Gastroenterol 2008;8.

43 Skacel M, Petras RE, Rybicki LA, Gramlich TL, Richter JE, Falk GW, et al. p53 expression in low grade dysplasia in Barrett's esophagus: Correlation with interobserver agreement and disease progression. American Journal of Gastroenterology 2002;97:2508-13.

44 Sikkema M, Kerkhof M, Steyerberg EW, Kusters JG, van Strien PMH, Looman CWN, et al. Aneuploidy and Overexpression of Ki67 and p53 as Markers for Neoplastic Progression in Barrett's Esophagus: A Case-Control Study. American Journal of Gastroenterology 2009;104:2673-80.

45 Murray L, Sedo A, Scott M, McManus D, Sloan JM, Hardie LJ, et al. TP53 and progression from Barrett's metaplasia to oesophageal adenocarcinoma in a UK population cohort. Gut 2006;55:1390-7.

46 Phoa KN, van Vilsteren FG, Weusten BL, Bisschops R, Schoon EJ, Ragunath K, et al. Radiofrequency ablation vs endoscopic surveillance for patients with Barrett esophagus and low-grade dysplasia: a randomized clinical trial. JAMA 2014;311:1209-17.

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Value of cyclin A immunohistochemistry for cancer risk-stratification in Barrett esophagus surveillance

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Abstract

Introduction: The value of endoscopic Barrett's esophagus (BE) surveillance based on histological diagnosis of low-grade dysplasia (LGD) remains debated given the lack of adequate risk-stratification. The aim of this study was (I) to evaluate the predictive value of cyclin A expression and (II) to combine these results with our previously reported immunohistochemical p53, AMACR and SOX2 data, to identify a panel of biomarkers predicting neoplastic progression in BE.

Methods: We conducted a case-control study within a prospective cohort of 720 BE patients. BE patients who progressed to high-grade dysplasia (HGD, n=37) or esophageal adenocarcinoma (EAC, n=13), defined as neoplastic progression, were classified as cases and patients without neoplastic progression were classified as controls (n=575). Cyclin A expression was determined by immunohistochemistry in all 625 patients; these results were combined with the histological diagnosis and our previous p53, AMACR and SOX2 data in loglinear regression models. Differences in discriminatory ability were quantified as changes in area under the ROC curve (AUC) for predicting neoplastic progression.

Results: Cyclin A surface positivity significantly increased throughout the metaplasia-dysplasia-carcinoma sequences and was seen in 10% (107/1050) of biopsy series without dysplasia, 33% (109/335) in LGD and 69% (34/50) in HGD/EAC. Positive cyclin A expression was associated with an increased risk of neoplastic progression (adjusted relative risk (RRa) 2.4; 95% CI 1.7 to 3.4). Increases in AUC were substantial for P53 (+0.05), smaller for SOX2 (+0.014), minor for Cyclin A (+0.003) and none for AMARC (0.00).

Conclusions: Cyclin A immunopositivity was associated with an increased progression risk in BE patients. However, compared to p53 and SOX2, the incremental value of cyclin A was limited. The use of biomarkers has the potential to significantly improve risk-stratification in BE.

Introduction

Barrett's esophagus (BE) is a premalignant condition of the distal esophagus in which the normal squamous epithelium is replaced by columnar epithelium containing goblet cells, as a result of chronic acid exposure [1, 2, 3]. Patients with BE have an increased risk to develop esophageal adenocarcinoma (EAC) with an estimated incidence of 0.2 to 0.5% per year [4, 5, 6, 7]. The transition from BE to EAC is a gradual process, in which intestinal metaplasia evolves via low-grade dysplasia (LGD), to high-grade dysplasia (HGD) and finally to EAC, a cancer with an overall 5-year survival of less than 20% [8, 9]. Current guidelines recommend endoscopic surveillance of BE patients to detect HGD or EAC at an early and potentially curable stage when endoscopic treatment is still feasible [10, 11]. However, the applied endoscopic surveillance strategy to date based on histological diagnosis alone remains debated given the overall low incidence of neoplastic progression, and the lack of discriminative power to stratify BE patients at high risk for neoplastic progression from those at low risk.

Histological diagnosis of LGD is nowadays used for the risk assessment of neoplastic progression in BE surveillance and more intensive follow-up is recommended in LGD patients (yearly instead of every 3 years) [10, 11, 12]. However, diagnosis of LGD has a low predictive value, owing to sample error and a considerable inter- and intraobserver variation [13, 14, 15]. The use of (a panel of) biomarkers in addition to histology may improve risk stratification in BE patients, and several immunohistochemical biomarkers are under investigation. Our group previously reported on the predictive value for neoplastic progression of p53, AMACR and SOX2 in a large prospective cohort of patients with BE [16, 17, 18].

Another potential biomarker is cyclin A, a protein that plays an important role in the G1-S transition of the cell cycle. Overexpression of cell-cycle related proteins, including cyclin A, has been linked to the metaplasia-dysplasia-carcinoma sequence in BE and associated with an increased risk of neoplastic progression [19, 20, 21]. However, clinical validation of cyclin A in a large prospective cohort of BE patients is still missing. In addition, there is a lack of studies testing performance of multiple biomarker simultaneously in the same cohort of BE patient.

The aim of the present study was (I) to assess the value of cyclin A immunohistochemistry to predict neoplastic progression in a large cohort of BE patients and (II) to combine the results obtained with our previously

reported p53, AMACR and SOX2 immunohistochemical data in the same prospective cohort, to identify a panel of biomarkers for predicting neoplastic progression in BE.

Methods

Study design

We conducted a case-control study nested within a large multi-center prospective cohort of 720 BE patients. All patients were included between November 2003 and December 2004 from three university medical centers and 12 regional hospitals throughout the Netherlands and received endoscopic surveillance according to the guidelines of the American College of Gastroenterology (ACG) [11]. Inclusion criterion was known or newly diagnosed BE of at least 2 cm according to the Prague C&M criteria, histologically confirmed by the presence of intestinal metaplasia on initial biopsies [22]. Patients with a history of HGD or esophageal malignancy were excluded. All endoscopic procedures were performed according to a standardized protocol, by an experienced gastroenterologist with at least several years of experience in endoscopic procedures and with interest for BE. Prior to taking biopsies, endoscopic landmarks such as the diaphragm impression, gastroesophageal junction and squamocolumnar junction were reported. The presence of esophagitis was graded according to the Los Angeles Classification, and abnormalities were noted, including nodules, ulcers and erosions [23]. At each endoscopic procedure targeted biopsies were taken from mucosal abnormalities and quadrant biopsies were taken every 2 cm from the most distal to the most proximal part of the Barrett segment, according to the Seattle protocol [24]. Patients without dysplasia in the biopsy samples, based on histological consensus diagnosis, underwent endoscopy surveillance with biopsy sampling every three year and patients with LGD every year.

Histology

According to standard procedure, all biopsy samples were fixed with buffered formalin and embedded in paraffin. From each biopsy set, 4-micrometer thick sections were cut and stained with haematoxylin-eosin to assess the presence of BE and grade of dysplasia. After assessment of all the biopsies, the highest degree of abnormality was reported for each endoscopy. Slides were graded first by a local pathologist and second by an expert academic

pathologist. In case of disagreement on the grade of dysplasia between the local pathologist and expert academic pathologist, the slides were reviewed by a second expert academic pathologist. Pathologists were blinded for each other's diagnosis and a final diagnosis was made if at least two pathologists agreed on the grade of dysplasia. When there was still disagreement, a panel of expert pathologists reviewed the slides and a final diagnosis was made based on consensus agreement. Given the equal surveillance strategy according to the ACG guidelines, the biopsies (n=7) with the final diagnosis of indefinite for dysplasia were included in the group of biopsies with the diagnosis of LGD.

Patient selection

We collected formalin-fixed paraffin-embedded (FFPE) material suitable for immunohistochemistry from all 720 BE patients in our cohort. However, no material or not enough material was available in 95 patients, leaving 625 patients to be included in this analysis. Patients with progression to HGD or EAC during follow-up were classified as cases and patients without neoplastic progression were classified as controls. In accordance with our previous analyses, the minimal time interval between the index endoscopy and diagnosis of HGD or EAC was nine months to prevent inclusion of prevalent cases. Immunohistochemistry was performed on the complete series of FFPE material of all surveillance endoscopies of patients who developed any form of dysplasia i.e. LGD, HGD or EAC during follow-up. This included the total number of biopsies taken during surveillance at different levels of the Barrett segment. In patients without any form of dysplasia during follow-up, immunohistochemistry was performed on biopsies of a random surveillance endoscopy.

Immunohistochemistry

For cyclin A immunohistochemistry, FFPE tissue sections were deparaffinized in xylene and rehydrated in graded alcohols. Antigen retrieval was done by heating in Tris buffer and endogenous peroxidase activity was blocked by incubating the slides in a solution of 0.3% hydrogen peroxide in phosphate-buffered saline. Primary antibody (Leica, Novocastra, Newcastle upon Tyns, United Kingdom: monoclonal, mouse) with a dilution of 1:200 was incubated overnight at 4 degrees Celsius. Rabbit anti-mouse (1:150; E0413, Dako, Heverlee, Belgium) was used as secondary antibody. Visualization was achieved by using the horseradish peroxidase avidin-biotin complex (HRP-ABC) method and diaminobenzidine (DAB) substrate. Finally, slides

were counterstained with haematoxylin. A negative control was obtained by omission of the primary antibody. Positive nuclei in the proliferation zone of the BE epithelium were used as internal positive control. Immunohistochemical staining for p53, AMACR and SOX2 was performed as previously described [16, 17, 18].

Scoring of immunohistochemistry

Immunohistochemically stained slides were examined in tandem with the haematoxylin-eosin stained slides to determine cyclin A, and previously p53, AMACR and SOX2 expression in areas with dysplasia [16, 17, 18]. Nuclear cyclin A expression was scored on a two-point scale; negative or positive expression. The surface cells were counted up to a maximum of 600 cells to determine the percentage of cyclin A positive cells. Only surface cells with strong nuclear staining were considered as positive. The epithelial surface was defined as the columnar cells at the luminal side of the biopsy, as described previously [25]. Based on published data, a cut-off value of 1% or more was used for cyclin A positivity [21]. Cyclin A expression was scored in BE epithelium with the highest percentage of positive cyclin A cells and in biopsy series with dysplasia, cyclin A expression was scored in the dysplastic area. After scoring all biopsies, the highest degree of abnormality was reported for each surveillance endoscopy. All stained slides were scored by two independent expert investigators who were blinded for long-term outcome as well as each other's results. When there was disagreement between the two investigators, slides were reviewed by an experienced academic pathologist (KB or MD) and final diagnosis was made if two investigators agreed on the extend of cyclin A expression.

P53, AMACR and SOX2 expression was scored as previously described [16, 17, 18]. Briefly, nuclear p53 and cytoplasmatic AMACR expression were scored on a three-point scale (p53; normal expression, overexpression or loss of expression and for AMACR; no expression, mild expression or strong expression). Only intense nuclear staining for p53 was scored as overexpression and aberrant p53 expression was defined as either overexpression or complete loss of expression in at least one gland. Nuclear SOX2 expression was scored on a two-point scale; positive or loss of expression. Positive expression included strong as well as weak nuclear SOX2 positivity and was interpreted as normal expression. Loss of SOX2 expression in a cluster of glands, excluding BE glands containing many goblet cells was defined as aberrant SOX2 expression.

Ethics

The study protocol was approved by the institutional review board of the Erasmus University Medical Center, including those of all participating hospitals. Before the first endoscopy, written informed consent was obtained from all 720 BE patients.

Statistical analysis

Patient characteristics of cases and controls were compared using Mann-Whitney U-tests for continuous variables and $\chi 2$ tests for categorical variables. To compare cyclin A expression in biopsy series of cases and controls with different grade of dysplasia, the Mann-Whitney U-tests test and Kruskal-Wallis test were used, thereby ignoring that multiple biopsy series could be from the same patient. Neoplastic progression was defined as the development of HGD or EAC at least 9 months after inclusion in the study, and follow-up time was defined as the time between two consecutive surveillance endoscopies. The value of cyclin A immunohistochemistry to predict neoplastic progression was estimated in loglinear regression models. Previous stained slides for p53, AMACR and SOX2 expression in the same cohort of BE patients were re-evaluated in this study to explore the classification performance of different combinations of biomarkers for predicting neoplastic progression in BE. Because immunohistochemical staining was not performed on all biopsy series, data were split up by endoscopy (1,243 in 575 controls, 142 in 50 cases). Loglinear models were used to calculate relative risks (RRs) and 95% Confidence Intervals (CIs) with the logarithm of follow-up time (time between two consecutive endoscopies) as offset variable. In multivariable analysis we adjusted for gender, age, BE length and esophagitis to estimate adjusted RRs and 95% CIs. For each of the biomarkers the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) was calculated. The areas under the Receiver Operating Characteristic (ROC) curves for neoplastic progression were calculated for the individual markers as well as for the comparison between a selection of models, in which the studied biomarkers were included or excluded. These included pathological diagnosis of grade of dysplasia alone, pathological diagnosis in combination with p53 and SOX2 immunohistochemistry and pathological diagnosis in combination with p53, SOX2 and cyclin A immunohistochemistry. The incremental value of each biomarker was calculated by the change in AUC after exclusion of the concerning biomarker in the 'fully adjusted model'

(model including histological diagnosis, cyclin A, p53, AMACR and SOX2 immunohistochemistry) as described earlier 26. Interobserver agreement for cyclin A expression was determined by Cohen kappa statistics. Kappa value of below 0.21 were considered 'poor', 0.21 to 0.40 'fair', 0.41 to 0.60 'moderate', 0.61 to 0.8 'substantial', and above 0.81 'very good'27. Two sided p values of <0.05 were considered statistically significant. Data were analyzed using SPSS statistical software (V.21.0; IBM SPSS, Chicago, IL, USA).

Results

Patient characteristics

Six hundred and twenty-five patients with BE were included in this study (74% men, median age of 60 years (interquartile range (IQR) 53-69)) and followed for a median duration of 6.7 years (IQR 5.0-7.4). Thirty-seven (6%) patients developed HGD and 13 (2%) patients developed EAC during surveillance after a median follow-up of 3.2 years (IQR 1.9-5.3). These 50 (8%) BE patients with neoplastic progression were classified as cases and the remaining 575 (92%) patients without neoplastic progression were classified as controls. Cyclin A expression was scored separately and subsequently correlated with histological diagnosis and expression of p53, AMACR and SOX2 in biopsy series of 1,432 endoscopies: 189 endoscopies were performed in 50 cases and 1,243 endoscopies in 575 controls. Biopsy series were defined as the total number of biopsies from one endoscopy and the highest degree of abnormality was reported for each surveillance endoscopy after evaluation of all biopsies taken at that respective endoscopy procedure. Except for a smaller number of endoscopies, a higher number of biopsies per endoscopy, longer BE length and more frequent diagnosis of LGD at baseline there were no significant differences between the cases and controls (Table 1).

Table 1. Baseline characteristics of cases and controls

		Controls n = 575	Cases n = 50	p Value
Follow-up	Median, years (IQR)	6.5 (5.2-7.2)	3.2 (1.9-5.3)	<0.001
Endoscopies	Median number (IQR)	4 (4-5)	3 (2-4)	<0.001
Biopsies available	Median number per endoscopy (IQR)	6 (4-9)	9 (6-12)	<0.001
Age	Median, years (IQR)	60 (53-69)	65 (56-71	0.103
Sex	Male	419 (73%)	41 (82%)	0.160
Alcohol use	Never	66 (12%)	6 (12%)	0.981
	Former	52 (9%)	5 (10%)	
	Current	445 (79%)	39 (78%)	
Smoking	Never	189 (34%)	12 (24%)	0.362
	Former	256 (45%)	25 (50%)	
	Current	118 (21%)	13 (26%)	
Reflux symptoms	Yes	172 (30%)	19 (38%)	0.265
Barrett diagnosis	≤ 1999	231 (41%)	16 (32%)	0.473
	2000-2002	197 (34%)	19 (38%)	
	2003-2004	141 (25%)	15 (30%)	
Barrett length	Median, cm (IQR)	4 (3-6)	5 (4-7)	0.010
Baseline	Low-grade dysplasia	88 (15%)	24 (48%)	<0.001
Esophagitis	Yes	109 (19%)	14 (30%)	0.104

IQR, Interquartile range.
Patients with neoplastic progression were classified as cases and patients without neoplastic progression were classified as controls.
Mann-Whitney U-test and chi-squares test were used to compare the characteristics of cases and controls.

Histology

Consensus histology assessments included, 1,050 (73%) biopsy series with non-dysplastic BE (NDBE), 335 (23%) with LGD, 34 (3%) with HGD and 13 (1%) with EAC. The local pathologist and expert academic pathologist disagreed on grade of dysplasia in 421 (29%) biopsy series and these samples were reviewed by a second expert pathologist (kappa-value of 0.34; 95% CI 0.32 to 0.36). In 22 (19%) biopsy series there was still disagreement and a second expert pathologist or a panel of expert pathologists reviewed the slides for a final diagnosis. The presence of LGD was more frequent in biopsy series of cases (47%) than in biopsy series of controls (22%) and was associated with an increased risk of neoplastic progression after adjusting for gender, age,

BE length and esophagitis (adjusted RR of 3.9; 95% CI 2.8 to 5.4), with an AUC of 0.62 (95% CI 0.58 to 0.68) (Table 2 and Figure 1). The sensitivity of histological diagnosis of LGD for predicting neoplastic progression was 47%, with a specificity of 78%. The PPV and NPV were respectively 20% and 93% (Table 3).

Table 2. Histology and cyclin A immunohistochemistry in biopsy series of cases and controls

	Controls n = 1,243	Cases n = 142	RR (95% CI)	RR[a] (95% CI)
Histology				
ND	975 (78%)	75 (53%)	Reference	Reference
LGD	268 (22%)	67 (47%)	4.2 (3.0 to 5.8)	3.9 (2.8 to 5.4)
Cyclin A expression				
< 1% cyclin A positivity	1073 (86%)	96 (68%)	Reference	Reference
≥ 1% cyclin A positivity	170 (14%)	46 (32%)	2.7 (1.9 to 3.8)	2.4 (1.7 to 3.4)
Histology and cyclin A expression				
ND and < 1% cyclin A positivity	883 (71%)	60 (42%)	Reference	Reference
LGD and < 1% cyclin A positivity	190 (15%)	36 (25%)	3.8 (2.5 to 5.8)	3.5 (2.3 to 5.3)
ND and ≥ 1% cyclin A positivity	92 (8%)	15 (11%)	2.0 (1.2 to 3.6)	1.7 (0.9 to 3.0)
LGD and ≥ 1% cyclin A positivity	78 (6%)	31 (22%)	6.4 (4.1 to 9.9)	5.8 (3.7 to 9.0)

The highest degree of abnormality was reported for each endoscopy after examining all biopsies.
RR, relative risk as calculated from a log-linear regression model; CI, confidence interval; ND, no dysplasia; LGD, low-grade dysplasia.
[a] RR adjusted for gender, age, BE length and esophagitis.

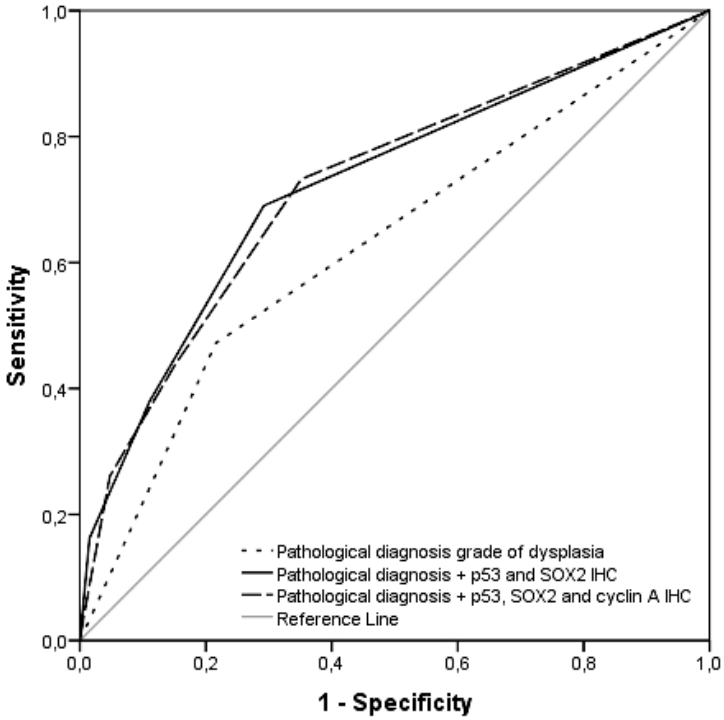

Figure 1. Receiver operating characteristic (ROC) comparing different biomarker models with the basic pathological diagnosis of grade of dysplasia. Area under the curve (AUC) for predicting neoplastic progression was calculated (pathological diagnosis grade of dysplasia AUC of 0.62 (95% CI 0.58 to 0.68), pathological diagnosis + p53 and SOX2 immunohistochemistry AUC of 0.72 (95% CI 0.67 to 0.77) and pathological diagnosis + p53, SOX2 and cyclin A immunohistochemistry AUC of 0.72 (95% CI 0.67 to 0.77).

Table 3. Performance of each individual marker for predicting neoplastic progression

Biomarker	Sensitivity	Specificity	PPV	NPV	AUC (95% CI)
Low-grade dysplasia	47%	78%	20%	93%	0.62 (0.58 to 0.68)
Cyclin A positivity	32%	86%	21%	92%	0.59 (0.54 to 0.64)
Aberrant p53	51%	87%	30%	94%	0.69 (0.64 to 0.74)
Strong AMACR	11%	96%	25%	90%	0.53 (0.48 to 0.59)
Loss of SOX2	25%	93%	29%	92%	0.60 (0.55 to 0.65)

PPV, positive predictive value; NPV, negative predictive value; AUC, area under the ROC curve; CI, confidence interval
The highest degree of abnormality was reported for each endoscopy after examining all biopsies.

Cyclin A immunohistochemistry

A positive cyclin A expression was seen in 250/1,432 (17%) of the biopsy series. The interobserver agreement for cyclin A expression was moderate with a kappa-value of 0.46 (95% CI 0.43 to 0.49). The observers disagreed on cyclin A surface expression in 278 (19%) biopsy series (Table 4). Cyclin A surface positivity was seen in 107 (10%) biopsy series without dysplasia, and was more common in dysplastic BE, including 109 (33%) biopsy series with LGD, 26 (76%) biopsy series with HGD and eight (62%) with EAC ($p<0.001$). Positive cyclin A surface expression was more common in biopsy series of cases (32%) than in biopsy series of controls (14%), and it was associated with an increased risk of neoplastic progression with a RR of 2.7 (95% CI 1.9 to 3.8). This association remained after adjusting for gender, age, BE length and esophagitis (adjusted RR 2.4; 95% CI 1.7 to 3.4) and was particularly seen in biopsy series with LGD (adjusted RR of 5.8; 95% CI 3.7 to 9.0) (Table 2). In per-biopsy analysis, cyclin A had an AUC of 0.59 (95% CI 0.54 to 0.64) for predicting neoplastic progression with a sensitivity of 32%, a specificity of 86%, a PPV of 21% and a NPV of 92% (Table 3).

Table 4. Interobserver agreement for cyclin A expression

Cyclin A surface expression	Cyclin A positivity < 1%	Cyclin A positivity ≥ 1%	K value
Cyclin A positivity < 1%	47%	93%	0.62 (0.58 to 0.68)
Cyclin A positivity≥ 1%	32%	92%	0.59 (0.54 to 0.64)

The highest degree of abnormality was reported for each endoscopy after examination of all biopsies.
Cohen K statistics were used to determine interobserver agreement.

P53, AMACR and SOX2 immunohistochemistry and incremental value of cyclin A

The pattern of p53, AMACR and SOX2 expression were studied previously and the data discussed elsewhere [16, 17, 18]. Aberrant p53 expression, as well as strong AMACR expression and aberrant SOX2 expression were more common in biopsy series of cases than in biopsy series of controls (p53; 51% vs. 13%, AMACR; 11% vs. 4%, SOX2; 25% vs. 7%) and were associated with an increased risk of neoplastic progression with adjusted RR of 5.6 (95% CI; 4.0 to 7.8) for aberrant p53 expression, 2.8 (95% CI; 1.6 to 4.8) for strong AMACR expression and 4.4 (95% CI; 3.0 to 6.5) for aberrant SOX2 expression, respectively. The highest risk of neoplastic progression was detected in patients with LGD and concurrent aberrant p53 expression (adjusted RR of 9.9; 95% CI 6.6 to 14.9). The addition of p53 immunohistochemistry improved the AUC compared to the histological diagnosis alone (from AUC 0.62 to AUC 0.70; 95% CI 0.66 to 0.76).

Next, we combined the information on histology, cyclin A, p53, AMACR and SOX2 immunohistochemistry in a fully adjusted model for predicting neoplastic progression in BE (Table 5). Aberrant p53 expression showed the highest change in AUC (0.05), to a lesser extent aberrant SOX2 expression (0.014) and histological diagnosis of LGD (0.005). The biomarkers cyclin A and AMACR only showed a minimal drop or no drop in AUC after exclusion (cyclin A: 0.003 and AMACR: 0.0) (Table 5). Importantly, the addition of SOX2 slightly improved the AUC compared with the model including only histological diagnosis and p53 immunohistochemistry (from AUC 0.70 to AUC 0.72; 95% CI 0.67 to 0.77)(Figure 1).

Table 5. Fully adjusted model with histology, cyclin A, p53, AMACR and SOX2 immunohistochemistry in biopsy series of cases and controls.

Variable	RR[a] (95% CI)	Change in AUC[b]
Low-grade dysplasia	1.8 (1.2 to 2.6)	0.005
Cyclin A positivity	1.4 (1.0 to 2.1)	0.003
Aberrant p53 expression	3.7 (2.6 to 5.4)	0.050
Strong AMACR expression	1.3 (0.8 to 2.3)	0.000
Loss of SOX2 expression	2.2 (1.4 to 3.4)	0.014

[a] RR adjusted for gender, age, BE length and esophagitis and all the other biomarkers
[b] Calculated drop of AUC after exclusion of the concerning biomarker compared to AUC of the total model (AUC of 0.734; 95% CI 0.687 to 0.780)

Discussion

In this large case-control study we evaluated the value of cyclin A expression for predicting neoplastic progression in patients with BE. These results were combined with our previously reported p53, AMACR and SOX2 immunohistochemical data within the same cohort using AUC in ROC analysis, to explore the classification performance of different combinations of biomarkers. This modeling is a valuable tool for the overall judgment of the incremental value of the biomarkers studied but not intended as an exact analytic method [26]. Cyclin A surface positivity significantly increased throughout the metaplasia-dysplasia-carcinoma progression steps and was associated with an increased risk of neoplastic progression. However, the incremental value of cyclin A expression was limited compared to histological diagnosis of LGD, p53 and SOX2.

Surveillance of BE patients is under significant debate given the lack of discriminative tools for adequate risk stratification. Additionally, with the introduction of minimally invasive endoscopic therapy and the evidence of cancer prevention by radiofrequency ablation in patients with LGD, there is an increasing need for accurate dysplasia detection during BE surveillance [28, 29]. Previous studies demonstrated repeatedly the value of LGD as a risk factor for neoplastic progression, albeit with a low predictive value due to sampling error and considerable interobserver variation [4, 6, 12, 13, 14, 15]. Even though the predictive value of LGD increases with consensus of multiple pathologists, approximately one-third of the patients with BE are diagnosed with LGD during surveillance, whereas the 5-year cumulative incidence of neoplastic progression is only between 5%-30% in this group [15, 30, 31]. Although the result of our study support the use of LGD diagnosed by expert GE pathologists, as indicator for increased risk of neoplastic progression, its sensitivity is only 47% and specificity 78%, despite using a consensus diagnosis of dysplasia. These results exemplify the interest in identifying molecular biomarkers to improve risk stratification and eventually cost-effectiveness of BE surveillance.

In the present study, cyclin A expression was confined to the base of the crypts in normal columnar gastrointestinal epithelium, as well as in most non-dysplastic BE. With increasing grades of dysplasia the expression of cyclin A progressively shifted towards the surface epithelium. The percentage of biopsy series with a positive cyclin A surface expression increased from 10% in non-dysplastic BE to 62% in biopsy series with EAC, which corresponds to previous

studies [20, 21]. A recent study identified cyclin A expression as one of a three-biomarker panel which provides a more accurate and objective diagnosis of dysplasia in BE [20]. Our results confirmed the correlation between dysplasia and cyclin A expression and hence potential as diagnostic tool for dysplasia detection.

Positive cyclin A surface expression was detected more frequently in cases than in controls, and was significantly associated with an increased risk of developing HGD or EAC (adjusted RR 2.4; 95% CI 1.7 to 3.4), particularly in dysplastic BE. The results of previous studies evaluating the value of cyclin A expression for predicting neoplastic progression are conflicting. A small case-control study showed that cyclin A surface expression was significantly associated with an increased risk of neoplastic progression (OR 7.6; 95% CI 1.6 to 37.0), whereas a more recent larger population-based study could not confirm this correlation and only found a trend towards an increased risk of progression, which eventually lost significance in a multivariate analysis (OR 1.32; 95% CI 0.66 to 2.66)[19, 21]. These conflicting results might be explained by a rather challenging interpretation of cyclin A immunohistochemistry. We found a moderate interobserver agreement with a kappa value of 0.46. This is low compared to the interobserver agreement of the other biomarkers p53 and SOX2 (kappa values between 0.70 and 0.86) [17, 18, 32].

The biomarker with the greatest body of evidence remains aberrant p53 expression (adjusted RR in fully adjusted model of 3.7 (95% CI 2.6 to 5.4), change in AUC 0.05) and to a lesser extent aberrant SOX2 expression (change in AUC 0.014). Cyclin A positivity showed only a minimal drop in AUC after exclusion (0.003). These findings might have important and clinically relevant implications. Assessment of p53 and SOX2 are promising to select high-risk patients for either intensified surveillance or ablation therapy and may eventually contribute to a more cost-effective management. Although routine p53 and SOX2 staining and assessment incur higher costs than histology alone, application of this panel of biomarkers has the potential to reduce the overall costs related of Barrett surveillance. Patients at low-risk of neoplastic progression, i.e. the majority of the patients with LGD, might be followed-up less intensively with the potential to eventually discharge them. However, a more detailed cost-effectiveness analysis should be performed to evaluate the economic value of p53 and SOX2 immunohistochemistry, which is beyond the scope of this study.

Our study has several strengths. The large cohort of BE patients was

prospectively followed-up according to a stringent scheme during a long follow-up time, clinical, endoscopic and pathological data were collected. Additionally, a standardized endoscopy and biopsy protocol was used. All stained slides were assessed by at least two experienced observers blinded for clinical outcome and in case of disagreement an expert pathologist reviewed the slides for final diagnosis. Another major strength of this study was that we tested multiple biomarkers in the same cohort of BE patients so we could identify the smallest panel of biomarkers with the highest predictive value for neoplastic progression, and which can be performed on routine clinical collected FFPE tissue.

Our study also has some limitations. Although immunohistochemistry is an established clinical examination method and easily applicable to standard clinical pathological laboratories, the scoring of the expression is a subjective assessment. It will require standardization of processing and scoring for reliable routine clinical application. In spite of this, our previous studies have shown good interobserver agreement for both p53 and SOX2 and they were relatively simple and straightforward to interpret [17, 18]. Further validation of this panel of biomarkers in large prospective studies is required to confirm our findings. Secondly, as all patients with BE, the patients considered as controls in this study still have the potential to progress to HGD or EAC during the future follow-up. However, since their median follow-up time was 6.5 years (which is more the twice the follow-up time of the cases), and the incidence of progression in only 2,6/1000 patients per year, the chance of progression in the controls is slim [6].

In conclusion, cyclin A surface expression was associated with an increased risk of neoplastic progression in BE patients, but its ability to predict neoplastic progression is limited compared to the biomarkers p53 and SOX2. The use of biomarkers has the potential to significantly improve risk-stratification in Barrett surveillance and hence the cost-effectiveness of Barrett surveillance programs.

Reference

1 American Gastroenterological A, Spechler SJ, Sharma P, Souza RF, Inadomi JM, Shaheen NJ. American Gastroenterological Association medical position statement on the management of Barrett's esophagus. Gastroenterology 2011;140:1084-91.

2 Kastelein F, Spaander MC, Biermann K, Vucelic B, Kuipers EJ, Bruno MJ. Role of acid suppression in the development and progression of dysplasia in patients with Barrett's esophagus. Dig Dis 2011;29:499-506.

3 Winters C, Jr., Spurling TJ, Chobanian SJ, Curtis DJ, Esposito RL, Hacker JF, 3rd, et al. Barrett's esophagus. A prevalent, occult complication of gastroesophageal reflux disease. Gastroenterology 1987;92:118-24.

4 Bhat S, Coleman HG, Yousef F, Johnston BT, McManus DT, Gavin AT, et al. Risk of malignant progression in Barrett's esophagus patients: results from a large population-based study. J Natl Cancer Inst 2011;103:1049-57.

5 Desai TK, Krishnan K, Samala N, Singh J, Cluley J, Perla S, et al. The incidence of oesophageal adenocarcinoma in non-dysplastic Barrett's oesophagus: a meta-analysis. Gut 2012;61:970-6.

6 Hvid-Jensen F, Pedersen L, Drewes AM, Sorensen HT, Funch-Jensen P. Incidence of adenocarcinoma among patients with Barrett's esophagus. N Engl J Med 2011;365:1375-83.

7 Sikkema M, de Jonge PJ, Steyerberg EW, Kuipers EJ. Risk of esophageal adenocarcinoma and mortality in patients with Barrett's esophagus: a systematic review and meta-analysis. Clin Gastroenterol Hepatol 2010;8:235-44; quiz e32.

8 Buttar NS, Wang KK. Mechanisms of disease: Carcinogenesis in Barrett's esophagus. Nat Clin Pract Gastroenterol Hepatol 2004;1:106-12.

9 van Hagen P, Hulshof MC, van Lanschot JJ, Steyerberg EW, van Berge Henegouwen MI, Wijnhoven BP, et al. Preoperative chemoradiotherapy for esophageal or junctional cancer. N Engl J Med 2012;366:2074-84.

10 Fitzgerald RC, di Pietro M, Ragunath K, Ang Y, Kang JY, Watson P, et al. British Society of Gastroenterology guidelines on the diagnosis and management of Barrett's oesophagus. Gut 2014;63:7-42.

11 Wang KK, Sampliner RE, Practice Parameters Committee of the American College of G. Updated guidelines 2008 for the diagnosis, surveillance and therapy of Barrett's esophagus. Am J Gastroenterol 2008;103:788-97.

12 Sikkema M, Looman CW, Steyerberg EW, Kerkhof M, Kastelein F, van Dekken H, et al. Predictors for neoplastic progression in patients with Barrett's Esophagus: a prospective cohort study. Am J Gastroenterol 2011;106:1231-8.

13 Curvers WL, ten Kate FJ, Krishnadath KK, Visser M, Elzer B, Baak LC, et al. Low-grade dysplasia in Barrett's esophagus: overdiagnosed and underestimated. Am J Gastroenterol

2010;105:1523-30.
14 Kerkhof M, van Dekken H, Steyerberg EW, Meijer GA, Mulder AH, de Bruine A, et al. Grading of dysplasia in Barrett's oesophagus: substantial interobserver variation between general and gastrointestinal pathologists. Histopathology 2007;50:920-7.
15 Skacel M, Petras RE, Gramlich TL, Sigel JE, Richter JE, Goldblum JR. The diagnosis of low-grade dysplasia in Barrett's esophagus and its implications for disease progression. Am J Gastroenterol 2000;95:3383-7.
16 Kastelein F, Biermann K, Steyerberg EW, Verheij J, Kalisvaart M, Looijenga LH, et al. Value of alpha-methylacyl-CoA racemase immunochemistry for predicting neoplastic progression in Barrett's oesophagus. Histopathology 2013;63:630-9.
17 Kastelein F, Biermann K, Steyerberg EW, Verheij J, Kalisvaart M, Looijenga LH, et al. Aberrant p53 protein expression is associated with an increased risk of neoplastic progression in patients with Barrett's oesophagus. Gut 2013;62:1676-83.
18 van Olphen S, Biermann K, Spaander MC, Kastelein F, Steyerberg EW, Stoop HA, et al. SOX2 as a Novel Marker to Predict Neoplastic Progression in Barrett's Esophagus. Am J Gastroenterol 2015.
19 Bird-Lieberman EL, Dunn JM, Coleman HG, Lao-Sirieix P, Oukrif D, Moore CE, et al. Population-based study reveals new risk-stratification biomarker panel for Barrett's esophagus. Gastroenterology 2012;143:927-35 e3.
20 di Pietro M, Boerwinkel DF, Shariff MK, Liu X, Telakis E, Lao-Sirieix P, et al. The combination of autofluorescence endoscopy and molecular biomarkers is a novel diagnostic tool for dysplasia in Barrett's oesophagus. Gut 2015;64:49-56.
21 Lao-Sirieix P, Lovat L, Fitzgerald RC. Cyclin A immunocytology as a risk stratification tool for Barrett's esophagus surveillance. Clin Cancer Res 2007;13:659-65.
22 Sharma P, Dent J, Armstrong D, Bergman JJ, Gossner L, Hoshihara Y, et al. The development and validation of an endoscopic grading system for Barrett's esophagus: the Prague C & M criteria. Gastroenterology 2006;131:1392-9.
23 DiBaise JK. The LA classification for esophagitis: a call for standardization. Am J Gastroenterol 1999;94:3403-4.
24 Levine DS, Haggitt RC, Blount PL, Rabinovitch PS, Rusch VW, Reid BJ. An endoscopic biopsy protocol can differentiate high-grade dysplasia from early adenocarcinoma in Barrett's esophagus. Gastroenterology 1993;105:40-50.
25 Sirieix PS, O'Donovan M, Brown J, Save V, Coleman N, Fitzgerald RC. Surface expression of minichromosome maintenance proteins provides a novel method for detecting patients at risk for developing adenocarcinoma in Barrett's esophagus. Clin Cancer Res 2003;9:2560-6.
26 Steyerberg EW, Pencina MJ, Lingsma HF, Kattan MW, Vickers AJ, Van Calster B.

Assessing the incremental value of diagnostic and prognostic markers: a review and illustration. Eur J Clin Invest 2012;42:216-28.

27 Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics 1977;33:159-74.

28 Shaheen NJ, Sharma P, Overholt BF, Wolfsen HC, Sampliner RE, Wang KK, et al. Radiofrequency ablation in Barrett's esophagus with dysplasia. N Engl J Med 2009;360:2277-88.

29 Phoa KN, van Vilsteren FG, Weusten BL, Bisschops R, Schoon EJ, Ragunath K, et al. Radiofrequency ablation vs endoscopic surveillance for patients with Barrett esophagus and low-grade dysplasia: a randomized clinical trial. JAMA 2014;311:1209-17.

30 Duits LC, Phoa KN, Curvers WL, Ten Kate FJ, Meijer GA, Seldenrijk CA, et al. Barrett's oesophagus patients with low-grade dysplasia can be accurately risk-stratified after histological review by an expert pathology panel. Gut 2015;64:700-6.

31 Kaye PV, Haider SA, Ilyas M, James PD, Soomro I, Faisal W, et al. Barrett's dysplasia and the Vienna classification: reproducibility, prediction of progression and impact of consensus reporting and p53 immunohistochemistry. Histopathology 2009;54:699-712.

32 Murray L, Sedo A, Scott M, McManus D, Sloan JM, Hardie LJ, et al. TP53 and progression from Barrett's metaplasia to oesophageal adenocarcinoma in a UK population cohort. Gut 2006;55:1390-7.

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

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Abstract

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

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

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

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

Introduction

Barrett's esophagus (BE) is a premalignant condition in which patients have an increased risk of developing esophageal adenocarcinoma (EAC) with an estimated incidence of 0.1 to 0.5% per year [1, 2, 3, 4]. The development of EAC in BE is a gradual process, in which metaplastic epithelium with no dysplasia (ND) evolves to low-grade dysplasia (LGD), high-grade dysplasia (HGD) and eventually EAC under the influence of chronic esophageal acid exposure [5]. Once a patient has developed EAC the prognosis is poor with a 5-year survival of less than 20% [6, 7]. Endoscopic surveillance is therefore recommended for BE to detect EAC at an early stage, when curative treatment is still feasible [8, 9]. Histological diagnosis of dysplasia is the golden standard for predicting neoplastic progression in BE and is therefore used for defining surveillance intervals. Current guidelines recommend surveillance every three to five years in patients with ND, every six to twelve months in patients with LGD and every three months in patients with HGD (in absence of endoscopic therapy). Most patients with BE belong to the group with ND and have an overall low risk of neoplastic progression. The majority of patients with non-dysplastic BE will never develop HGD or EAC and die of causes not related to BE, which makes surveillance controversial in this patient group [10]. In patients with LGD the risk of neoplastic progression is increased, which makes surveillance more effective. However, histological diagnosis of LGD is subject to considerable intra- en interobserver variation which limits its predictive value [11, 12].

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

the palliative treatment of EAC [16].
One of the key questions in the discussion about BE surveillance is whether surveillance and (endoscopic) treatment is cost-effective. The cost-effectiveness of BE surveillance has been investigated in previous studies, where transition rates to HGD and EAC were mostly based on pooled literature data [17, 18, 19, 20, 21, 22, 23, 24, 25, 26]. For a more accurate representation of the natural history of BE and its progression to EAC, true transition and misclassification rates can be calculated in a multi-state Markov (MSM) model using prospectively collected follow-up data from a large cohort of BE patients [27]. The aim of this study was to evaluate the cost-effectiveness of different surveillance intervals and treatment strategies for patients without dysplasia and LGD in long-segment BE, within a large multicenter prospective cohort study.

Methods

Study Design

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

are treated similarly. Carcinomas invading the submucosa (T1b), muscularis propria (T2), adventitia (T3) or adjacent structures (T4) were considered as another category (advanced EAC).

Incidence, misclassification and transition rates

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

Surveillance strategies

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

strategies consisted of surveillance with different intervals (one to five years) for patients with ND or LGD and endoscopic or surgical intervention for patients with HGD or EAC. Treatment strategies for patients with HGD or early EAC consisted of RFA alone, EMR followed by RFA, or esophagectomy with neoadjuvant chemoradiotherapy. We assumed that complications occurred in 2.2% after EMR, 6.5% after RFA and 22.9% after esophagectomy and considered costs associated with additional treatment [29, 30, 31]. After endoscopic treatment with EMR or RFA we assumed that patients returned to ND and surveillance was resumed. We assumed that 5 to 10% of patients had early recurrence for which they received endoscopic treatment. After endoscopic treatment, patients remained at risk for neoplastic progression. Treatment of patients with advanced EAC consisted of esophagectomy with neoadjuvant chemoradiotherapy. Palliative treatment of EAC consisted of chemotherapy, esophageal stenting or brachytherapy and terminal care.

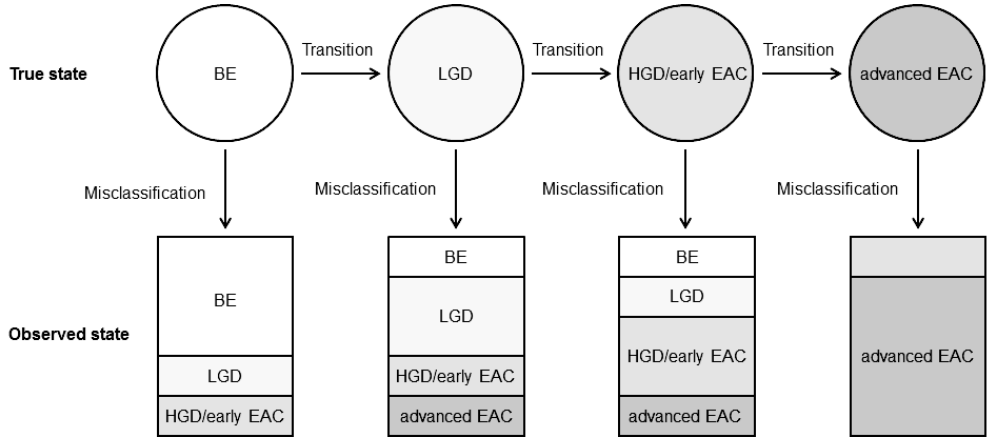

Figure 1. Multi-state Markov model
BE, Barrett's esophagus; LGD, low-grade dysplasia; HGD, high-grade dysplasia; EAC, esophageal adenocarcinoma

Costs and quality of life

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

costs of medical procedures, equipment, overhead, personnel and honoraria of medical specialists. Hospitals get these costs reimbursed by the health insurance. Data on quality of life (utilities) associated with different health states were derived from the published literature and were used to convert absolute life-years of survival into quality-adjusted-life-years (QALYs) [33, 34, 35]. Costs and utilities were discounted at an annual rate of 5%, which allows to compare our results to those of previous studies (Table 1).

Table 1. Variables included in cost-effectiveness analysis

Variables		Base value	Reference
Transition rates (per year)			
ND to LGD		0.023	Own data
LGD to HGD/early EAC		0.043	Own data
HGD/early EAC to advanced EAC		0.250	(28)
Misclassification rates			
True state	*Observed state*		
ND	LGD	0.086	Own data
ND	HGD/early EAC	0.004	Own data
LGD	ND	0.247	Own data
LGD	HGD/early EAC	0.123	Own data
LGD	Advanced EAC	0.008	Own data
HGD/early EAC	LGD	0.016	Own data
HGD/early EAC	Advanced EAC	0.287	Own data
Advanced EAC	HGD/early EAC	0.036	Own data
Probabilities			
Probability of surgery		0.600	Cancer register
Probability of curative treatment		0.500	Cancer register
Probability of dying from surgery		0.018	(49)
Probability of complications from surgery		0.229	(29)
Probability of complications from endoscopy		0.001	(50)
Probability of complications from EMR		0.022	(31)
Probability of complications from RFA		0.065	(30)
Costs			
Cost of endoscopy		€ 629	NZa
Cost of endoscopy with complication		€ 1677	NZa
Cost of EMR		€ 1925	Expert opinion
Cost of EMR with complication		€ 3425	Expert opinion

Variables	Base value	Reference
Cost of RFA	€ 6210	Expert opinion
Cost of RFA with complication	€ 8710	Expert opinion
Cost of staging adenocarcinoma	€ 2499	NZa
Cost of esophagectomy	€ 17.887	NZa
Cost of esophagectomy with complication	€ 38.930	NZa
Cost of postoperative follow-up, per year	€ 948	NZa
Cost of neoadjuvant chemoradiation	€ 8792	NZa
Cost of palliative chemotherapy	€ 3867	NZa
Cost of palliative stenting	€ 1215	NZa
Cost of brachytherapy	€ 3004	NZa
Cost of terminal care, per year	€ 32565	(22)
Quality of life		
Quality of life after HGD diagnosis	0.84	(33, 35)
Quality of life after EAC diagnosis	0.66	(33, 35)
Quality of life after endoscopic treatment (short term)	0.93	(33, 35)
Quality of life after esophagectomy (short term)	0.86	(34)
Quality of life after esophagectomy (long term)	0.90	(34)
Duration of short term morbidity		
After endoscopic treatment	3 days	(30)
After esophagectomy	4 weeks	(34)
Discount rate	0.05	(22)

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

Ethics

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

Cost-effectiveness analysis

For the analysis we used a modification of a previously published decision-analytic Markov model, which was constructed in Windows Decision Maker (Beta test version 2010) [22]. In this computer model a BE cohort was simulated with as base case a 55-years old male BE patient with ND or LGD. The natural history of the BE cohort was modelled to examine the costs of no surveillance

and its effects on quality of life. Subsequently, the effect of multiple surveillance strategies was evaluated with various surveillance intervals for patients with ND or LGD and endoscopic or surgical interventions for patients with HGD or EAC. Simulation of the BE cohort started with baseline endoscopy and was continued with cycles of 3 months until death. True progression rates from ND to LGD, HGD, and advanced EAC were estimated in a MSM model based on the progression and misclassification rates observed in our cohort. Death from other causes than EAC was possible in any state and was modelled as a time-dependent variable with the risk increasing with age.

Statistical analysis

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

Results

Patient characteristics

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

Table 2. Characteristics of patients included in the Barrett's esophagus cohort

		Cohort n = 714
Follow-up	Median, years (IQR)	6.1 (4.4-7.0)
	Total, person-years	3992
Age	Median, years (IQR)	61 (53-69)
Gender	Male	520 (73%)
	Female	194 (27%)
BE diagnosis	< Inclusion	529 (74%)
	≥ Inclusion	185 (26%)
BE length	Median, cm (IQR)	4 (2-6)
Baseline esophagitis	No	642 (90%)
	Yes	72 (10%)
Baseline histology	No dysplasia	606 (85%)
	Low-grade dysplasia	108 (15%)
Mucosal abnormalities	No	694 (97%)
	Yes	20 (3%)

IQR, interquartile range; BE, Barrett's esophagus

Incidence and transition rates

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

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

Transition	Observed	Cases Interpolated	Total	Follow-up in years	Incidence rate with 95% CI
ND to LGD	180	27	207	3640	5.7% (4.9-6.5)
LGD to HGD/early EAC	18	28	46	350	13.1% (9.6-17.5)
LGD to ND	198	-	198	350	56.6% (49.0-65.0)
ND/LGD to HGD/early EAC	42	4	46	3990	1.2% (0.9-1.6)
ND/LGD to advanced EAC	4	-	4	3992	0.1% (0.0-0.3)

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

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

Surveillance in patients with no dysplasia

In patients with ND, the costs of no surveillance were € 5.695 for 12.62 discounted QALYs. Surveillance every five years with RFA for HGD or early EAC and esophagectomy for advanced EAC resulted in an increase in life expectancy by 0.25 QALYs and an increase in costs by €1.324, representing an ICER of €5.283 per QALY. Surveillance every four years resulted in an additional increase in life expectancy by 0.02 QALYs and an additional increase in costs by €802, representing an ICER of €62.619 per QALY. Strategies with surveillance intervals shorter than four years provided substantial higher costs with similar or less QALYs gained (Table 4).

Strategies using EMR prior to RFA had similar effects on QALYs compared to strategies using RFA alone, but were slightly more expensive. Strategies using esophagectomy were much more expensive with less QALYs gained. However, use of RFA alone is still controversial and EMR contributed significantly to tumor staging, which may justify the slightly higher costs. In summary, when assuming a willingness-to-pay threshold of €35.000 per QALY, surveillance every five years with EMR followed by RFA or RFA alone for HGD or early EAC and esophagectomy with neoadjuvant chemoradiotherapy for advanced EAC is a cost-effective strategy for long-segment BE with ND. When assuming a willingness-to-pay threshold of €80.000 per QALY, surveillance every four years is cost-effective (Figure 2).

Surveillance in patients with low-grade dysplasia

In patients with LGD, the costs of no surveillance were € 21.806 for 10.95 discounted QALYs. Surveillance every five years with RFA for HGD or early EAC and esophagectomy for advanced EAC resulted in an increase in life expectancy by 0.96 QALYs and an increase in costs by €4.756, representing an ICER of €4.922 per QALY. Surveillance every one to four years resulted in an additional increase in life expectancy, but at increasing costs (Table 4). EMR followed by RFA for patients with HGD or early EAC had similar effects on QALYs compared to strategies using RFA alone, but costs were slightly higher. Esophagectomy was much more expensive with less QALYs gained. When assuming a willingness-to-pay threshold of €35.000 per QALY, surveillance every three years with EMR followed by RFA or RFA alone for HGD or early EAC and esophagectomy with neoadjuvant chemoradiotherapy for advanced EAC is a cost-effective strategy for long-segment BE with LGD. When assuming a willingness-to-pay threshold of €80.000 per QALY, surveillance every year is cost-effective.

Table 4. Cost-effectiveness of different surveillance intervals and treatment strategies in patients with Barrett's esophagus.

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

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

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

Sensitivity analysis

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

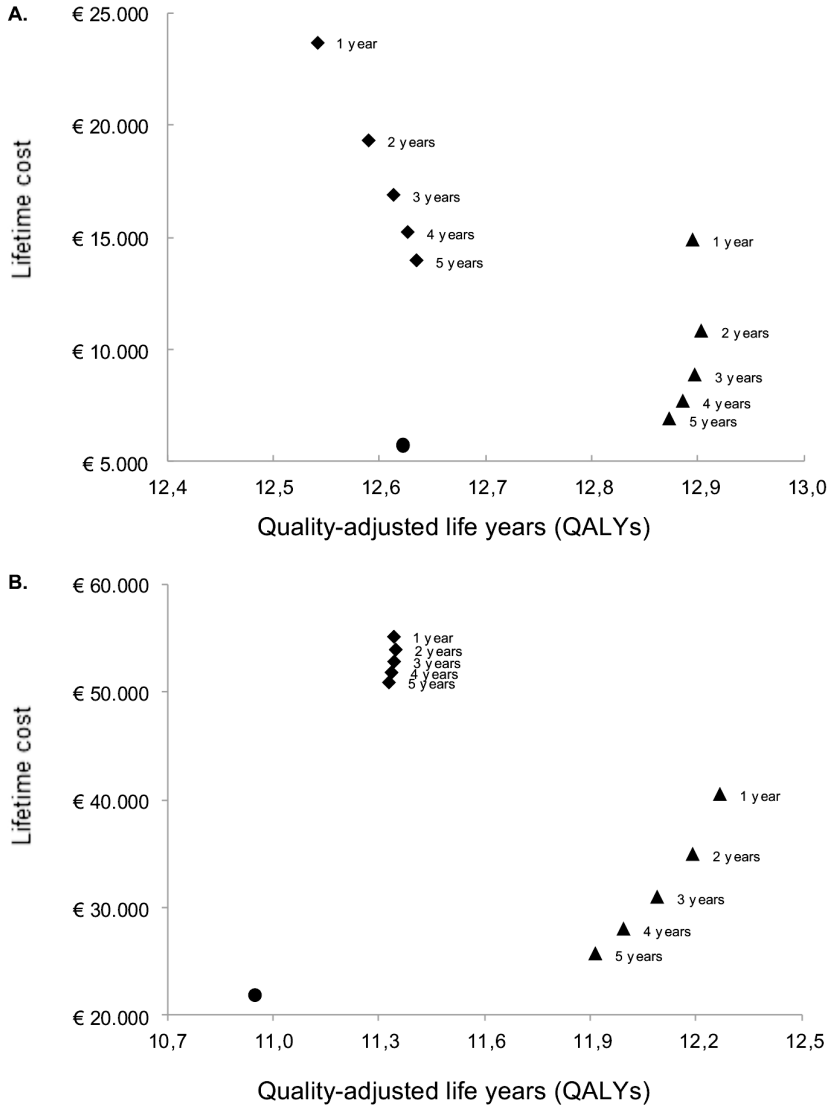

Figure 2. Costs and quality-adjusted life years (QALYs) associated with different surveillance strategies in patients with no dysplasia (A) or low-grade dysplasia (B). ● No surveillance, ▲ Surveillance with radiofrequency ablation for high-grade dysplasia (HGD) or early esophageal adenocarcinoma (EAC)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

Table 5. Cost-effectiveness of different surveillance intervals in case of higher or lower transition rates (sensitivity analysis).

No dysplasia	Transition rates 200%			Transition rates 50%			Transition rates 25%		
	Costs	QALYs	ICER	Costs	QALYs	ICER	Costs	QALYs	ICER
No surveillance	€ 9.886	11.89		€ 3.501	12.87		€ 2.443	12.95	
every 5 years with RFA	€ 9.731	12.54	x	€ 5.864	12.95	€ 29.802	€ 5.357	12.97	€ 126.139
every 4 years with RFA	€ 10.510	12.60	€ 12.560	€ 6.667	12.95	x	€ 6.152	12.97	x
every 3 years with RFA	€ 11.624	12.67	€ 16.152	€ 7.868	12.95	x	€ 7.352	12.96	x
every 2 years with RFA	€ 13.473	12.74	€ 27.073	€ 9.883	12.94	x	€ 9.376	12.95	x
every year with RFA	€ 17.403	12.78	€ 87.727	€ 10.411	12.93	x	€ 13.510	12.94	x

Low-grade dysplasia	Transition rates 200%			Transition rates 50%			Transition rates 25%		
	Costs	QALYs	ICER	Costs	QALYs	ICER	Costs	QALYs	ICER
No surveillance	€ 24.747	9.44		€ 19.772	11.84		€ 18.636	12.26	
every 5 years with RFA	€ 29.778	10.76	€ 3.817	€ 24.548	12.46	€ 7.631	€ 23.503	12.68	€ 11.753
every 4 years with RFA	€ 32.095	10.90	€ 16.398	€ 27.034	12.48	€ 135.848	€ 26.027	12.67	x
every 3 years with RFA	€ 35.053	11.11	€ 14.100	€ 30.249	12.50	€ 206.087	€ 29.287	12.65	x
every 2 years with RFA	€ 39.024	11.39	€ 14.080	€ 34.540	12.50	€ 670.480	€ 33.626	12.62	x
every year with RFA	€ 44.671	11.70	€ 17.973	€ 40.499	12.49	x	€ 39.634	12.58	x

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

Discussion

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

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

Over the past years there has been a major shift in the treatment BE patients with the introduction of endoscopic treatment strategies. We therefore included EMR and RFA in this cost-effectiveness analysis [8, 9]. An advantage of EMR is that it not only removes mucosal abnormalities suspect for dysplasia, but also allows for evaluation of tissue invasion [39, 40]. RFA is used in addition to EMR for complete eradication of BE, but may also be used as a single treatment modality [30, 41]. Previous studies have shown that RFA is effective in eradicating HGD, early EAC and complete segments of BE with low complication rates [30, 41, 42, 43]. The current study shows that RFA is also cost-effective, which corresponds to the results of previous studies [17, 18, 19, 20, 21, 22, 23, 24, 25, 26]. Some recent studies suggested that RFA might also be cost-effective in patients with confirmed LGD [43, 44]. However, it is

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

The cost-effectiveness of a surveillance strategy not only depends on the costs and effects on quality of life, but also on the willingness-to-pay threshold [22]. We considered a willingness-to-pay threshold between €20.000 to €80.000 per QALY with special emphasis on the threshold of €35.000 per QALY, which is used in the United Kingdom and the United States of America [36, 37, 38]. The most critical variables in the cost-effectiveness analysis were the true progression rates. We used advanced statistical techniques to estimate these rates from prospectively collected follow-up data. The incidence rate of EAC was estimated at 0.1% per year which corresponds to the results of recent population-based studies, which confirms that our model is a good reflection of the natural history of neoplastic progression in BE [2]. For patients with LGD, the incidence rate of EAC was estimated at 4.9% per year. Previous studies have shown highly variable results for LGD with incidence rates of 0-26% and 1.7% in a recent meta-analysis [45]. The estimated progression rate in the current study was higher than in the meta-analysis which can be explained by the fact that we only included patients with long-segment BE, that LGD diagnosis was made only when at least two pathologists agreed on the diagnosis and that patients were under strict surveillance. When progression rates were halved, surveillance every five years was cost-effective for both ND and LGD. When progression rates were 25% of the calculated values, surveillance was only cost-effective for LGD. Changes in other variables such as costs and quality of life data had less impact on outcome.

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

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

pump inhibitors, nonsteroidal anti-inflammatory drugs and statins and use of biomarkers such as p53 [46, 47, 48]. When new risk factors become available our model needs to be updated for a more personalized surveillance strategy.
In conclusion this study shows that surveillance every five years with EMR followed by RFA for HGD or early EAC and esophagectomy with neoadjuvant chemoradiotherapy for advanced EAC is a cost-effective strategy in patients with long-segment BE without dysplasia, assuming a willingness-to-pay threshold of € 35.000 per QALY. In patients with LGD surveillance every three years with EMR followed by RFA for HGD or early EAC and esophagectomy with neoadjuvant chemoradiotherapy for advanced EAC is cost-effective. In the future new risk factors or biomarkers may identify patients at high risk for neoplastic progression and thereby improve the cost-effectiveness of BE surveillance.

Reference

1 de Jonge PJ, van Blankenstein M, Looman CW, Casparie MK, Meijer GA, Kuipers EJ. Risk of malignant progression in patients with Barrett's oesophagus: a Dutch nationwide cohort study. Gut 2010;59:1030-6.

2 Hvid-Jensen F, Pedersen L, Drewes AM, Sorensen HT, Funch-Jensen P. Incidence of adenocarcinoma among patients with Barrett's esophagus. N Engl J Med 2011;365:1375-83.

3 Sikkema M, de Jonge PJ, Steyerberg EW, Kuipers EJ. Risk of esophageal adenocarcinoma and mortality in patients with Barrett's esophagus: a systematic review and meta-analysis. Clinical gastroenterology and hepatology 2010;8:235-44; quiz e32.

4 Bhat S, Coleman HG, Yousef F, Johnston BT, McManus DT, Gavin AT, et al. Risk of malignant progression in Barrett's esophagus patients: results from a large population-based study. J Natl Cancer Inst 2011;103:1049-57.

5 Kastelein F, Spaander MC, Biermann K, Vucelic B, Kuipers EJ, Bruno MJ. Role of acid suppression in the development and progression of dysplasia in patients with Barrett's esophagus. Dig Dis 2011;29:499-506.

6 Lund O, Kimose HH, Aagaard MT, Hasenkam JM, Erlandsen M. Risk stratification and long-term results after surgical treatment of carcinomas of the thoracic esophagus and cardia. A 25-year retrospective study. The Journal of thoracic and cardiovascular surgery 1990;99:200-9.

7 http://www.cijfersoverkanker.nl/.

8 Fitzgerald RC, di Pietro M, Ragunath K, Ang Y, Kang JY, Watson P, et al. British Society of Gastroenterology guidelines on the diagnosis and management of Barrett's oesophagus. Gut 2013.

9 Spechler SJ, Sharma P, Souza RF, Inadomi JM, Shaheen NJ. American Gastroenterological Association medical position statement on the management of Barrett's esophagus. Gastroenterology 2011;140:1084-91.

10 Sikkema M, de Jonge PJ, Steyerberg EW, Kuipers EJ. Risk of esophageal adenocarcinoma and mortality in patients with Barrett's esophagus: a systematic review and meta-analysis. Clin Gastroenterol Hepatol 2010;8:235-44; quiz e32.

11 Kerkhof M, van Dekken H, Steyerberg EW, Meijer GA, Mulder AH, de Bruine A, et al. Grading of dysplasia in Barrett's oesophagus: substantial interobserver variation between general and gastrointestinal pathologists. Histopathology 2007;50:920-7.

12 Sikkema M, Looman CW, Steyerberg EW, Kerkhof M, Kastelein F, van Dekken H, et al. Predictors for neoplastic progression in patients with Barrett's Esophagus: a prospective cohort study. Am J Gastroenterol 2011;106:1231-8.

13 Haidry RJ, Dunn JM, Butt MA, Burnell MG, Gupta A, Green S, et al. Radiofrequency ablation and endoscopic mucosal resection for dysplastic barrett's esophagus and early

esophageal adenocarcinoma: outcomes of the UK National Halo RFA Registry. Gastroenterology 2013;145:87-95.

14 Kim HP, Bulsiewicz WJ, Cotton CC, Dellon ES, Spacek MB, Chen X, et al. Focal endoscopic mucosal resection before radiofrequency ablation is equally effective and safe compared with radiofrequency ablation alone for the eradication of Barrett's esophagus with advanced neoplasia. Gastrointestinal endoscopy 2012;76:733-9.

15 van Hagen P, Hulshof MC, van Lanschot JJ, Steyerberg EW, van Berge Henegouwen MI, Wijnhoven BP, et al. Preoperative chemoradiotherapy for esophageal or junctional cancer. N Engl J Med 2012;366:2074-84.

16 Homs MY, van Oijen MG, Wijnhoven BP, van Hillegersberg R, de Boer-Dennert M, Siersema PD. Changes in diagnostic and treatment strategies of oesophageal cancer in the period from 2001 to 2009: a survey in the Netherlands. European journal of gastroenterology & hepatology 2012;24:126-33.

17 Garside R, Pitt M, Somerville M, Stein K, Price A, Gilbert N. Surveillance of Barrett's oesophagus: exploring the uncertainty through systematic review, expert workshop and economic modelling. Health technology assessment (Winchester, England) 2006;10:1-142, iii-iv.

18 Gordon LG, Mayne GC. Cost-effectiveness of Barrett's oesophagus screening and surveillance. Best practice & research Clinical gastroenterology 2013;27:893-903.

19 Gordon LG, Mayne GC, Hirst NG, Bright T, Whiteman DC, Watson DI. Cost-effectiveness of endoscopic surveillance of non-dysplastic Barrett's esophagus. Gastrointestinal endoscopy 2013.

20 Inadomi JM, Sampliner R, Lagergren J, Lieberman D, Fendrick AM, Vakil N. Screening and surveillance for Barrett esophagus in high-risk groups: a cost-utility analysis. Annals of internal medicine 2003;138:176-86.

21 Inadomi JM, Somsouk M, Madanick RD, Thomas JP, Shaheen NJ. A cost-utility analysis of ablative therapy for Barrett's esophagus. Gastroenterology 2009;136:2101-14 e1-6.

22 Provenzale D, Schmitt C, Wong JB. Barrett's esophagus: a new look at surveillance based on emerging estimates of cancer risk. The American journal of gastroenterology 1999;94:2043-53.

23 Sonnenberg A, Fennerty MB. Medical decision analysis of chemoprevention against esophageal adenocarcinoma. Gastroenterology 2003;124:1758-66.

24 Sonnenberg A, Soni A, Sampliner RE. Medical decision analysis of endoscopic surveillance of Barrett's oesophagus to prevent oesophageal adenocarcinoma. Aliment Pharmacol Ther 2002;16:41-50.

25 Inadomi JM. Surveillance in Barrett's esophagus: a failed premise. The Keio journal of medicine 2009;58:12-8.

26 Das A, Wells C, Kim HJ, Fleischer DE, Crowell MD, Sharma VK. An economic analysis of endoscopic ablative therapy for management of nondysplastic Barrett's esophagus. Endoscopy 2009;41:400-8.

27 Jackson CH, Sharples LD, Thompson SG, Duffy SW, Couto E. Multistate Markov models for disease progression with classification error.

28 Verbeek RE, van Oijen MG, ten Kate FJ, Vleggaar FP, Schipper ME, Casparie MK, et al. Surveillance and follow-up strategies in patients with high-grade dysplasia in Barrett's esophagus: a Dutch population-based study. The American journal of gastroenterology 2012;107:534-42.

29 Rutegard M, Lagergren P, Rouvelas I, Mason R, Lagergren J. Surgical complications and long-term survival after esophagectomy for cancer in a nationwide Swedish cohort study. European journal of surgical oncology : the journal of the European Society of Surgical Oncology and the British Association of Surgical Oncology 2012;38:555-61.

30 Shaheen NJ, Sharma P, Overholt BF, Wolfsen HC, Sampliner RE, Wang KK, et al. Radiofrequency ablation in Barrett's esophagus with dysplasia. N Engl J Med 2009;360:2277-88.

31 Tomizawa Y, Iyer PG, Wong Kee Song LM, Buttar NS, Lutzke LS, Wang KK. Safety of endoscopic mucosal resection for Barrett's esophagus. The American journal of gastroenterology 2013;108:1440-7; quiz 8.

32 http://www.nza.nl/zorgonderwerpen/dossiers/dbc-dossier/.

33 Crockett SD, Lippmann QK, Dellon ES, Shaheen NJ. Health-related quality of life in patients with Barrett's esophagus: a systematic review. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association 2009;7:613-23.

34 de Boer AG, van Lanschot JJ, van Sandick JW, Hulscher JB, Stalmeier PF, de Haes JC, et al. Quality of life after transhiatal compared with extended transthoracic resection for adenocarcinoma of the esophagus. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2004;22:4202-8.

35 Hur C, Wittenberg E, Nishioka NS, Gazelle GS. Quality of life in patients with various Barrett's esophagus associated health states. Health and quality of life outcomes 2006;4:45.

36 CVZ. Het pakketprincipe kosteneffectiviteit achtergrondstudie ten behoeve van de 'appraisal' fase in pakketbeheer.

37 NICE. http://www.nice.org.uk/newsroom/features/measuringeffectivenessandcosteffectivenesstheqaly.jsp.

38 Grosse SD. Assessing cost-effectiveness in healthcare: history of the $50,000 per QALY threshold. Expert review of pharmacoeconomics & outcomes research 2008;8:165-78.

39 Moss A, Bourke MJ, Hourigan LF, Gupta S, Williams SJ, Tran K, et al. Endoscopic

resection for Barrett's high-grade dysplasia and early esophageal adenocarcinoma: an essential staging procedure with long-term therapeutic benefit. The American journal of gastroenterology 2010;105:1276-83.

40 Pech O, Behrens A, May A, Nachbar L, Gossner L, Rabenstein T, et al. Long-term results and risk factor analysis for recurrence after curative endoscopic therapy in 349 patients with high-grade intraepithelial neoplasia and mucosal adenocarcinoma in Barrett's oesophagus. Gut 2008;57:1200-6.

41 Fleischer DE, Overholt BF, Sharma VK, Reymunde A, Kimmey MB, Chuttani R, et al. Endoscopic radiofrequency ablation for Barrett's esophagus: 5-year outcomes from a prospective multicenter trial. Endoscopy 2010;42:781-9.

42 Pech O, May A, Manner H, Behrens A, Pohl J, Weferling M, et al. Long-term Efficacy and Safety of Endoscopic Resection for Patients With Mucosal Adenocarcinoma of the Esophagus. Gastroenterology 2013.

43 Hur C, Choi SE, Rubenstein JH, Kong CY, Nishioka NS, Provenzale DT, et al. The cost effectiveness of radiofrequency ablation for Barrett's esophagus. Gastroenterology 2012;143:567-75.

44 Phoa KN, van Vilsteren FG, Weusten BL, Bisschops R, Schoon EJ, Ragunath K, et al. Radiofrequency ablation vs endoscopic surveillance for patients with Barrett esophagus and low-grade dysplasia: a randomized clinical trial. JAMA 2014;311:1209-17.

45 Singh S, Manickam P, Amin AV, Samala N, Schouten LJ, Iyer PG, et al. Incidence of esophageal adenocarcinoma in Barrett's esophagus with low-grade dysplasia: a systematic review and meta-analysis. Gastrointestinal endoscopy 2014;79:897-909 e4.

46 Kastelein F, Biermann K, Steyerberg EW, Verheij J, Kalisvaart M, Looijenga LH, et al. Aberrant p53 protein expression is associated with an increased risk of neoplastic progression in patients with Barrett's oesophagus. Gut 2013;62:1676-83.

47 Kastelein F, Spaander MC, Biermann K, Steyerberg EW, Kuipers EJ, Bruno MJ. Nonsteroidal anti-inflammatory drugs and statins have chemopreventative effects in patients with Barrett's esophagus. Gastroenterology 2011;141:2000-8; quiz e13-4.

48 Kastelein F, Spaander MC, Steyerberg EW, Biermann K, Valkhoff VE, Kuipers EJ, et al. Proton pump inhibitors reduce the risk of neoplastic progression in patients with Barrett's esophagus. Clin Gastroenterol Hepatol 2013;11:382-8.

49 Noordzij PG, Poldermans D, Schouten O, Bax JJ, Schreiner FA, Boersma E. Postoperative mortality in The Netherlands: a population-based analysis of surgery-specific risk in adults. Anesthesiology 2010;112:1105-15.

50 Silvis SE, Nebel O, Rogers G, Sugawa C, Mandelstam P. Endoscopic complications. Results of the 1974 American Society for Gastrointestinal Endoscopy Survey. JAMA : the journal of the American Medical Association 1976;235:928-30.

6

Impact of surveillance for Barrett's esophagus on tumor stage and survival of patients with neoplastic progression

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Abstract

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

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

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

Conclusions: EAC is detected at an earlier stage during BE surveillance than in the general population with good survival rates.

Introduction

Barrett's esophagus (BE) is a premalignant condition in which patients have an increased risk of developing esophageal adenocarcinoma (EAC) with an estimated incidence of 0.1 to 0.5% per year [1, 2, 3, 4]. The development of EAC in BE is a gradual process, in which metaplastic epithelium without dysplasia evolves to low-grade dysplasia (LGD), high-grade dysplasia (HGD) and eventually EAC under the influence of esophageal acid exposure [5, 6]. Once a patient has developed EAC the prognosis is poor with a 5-year survival of less than 20% [7, 8, 9]. Endoscopic BE surveillance is therefore recommended to detect EAC at an early stage, when curative treatment is still feasible [10, 11]. Current guidelines recommend endoscopic surveillance every three to five years in patients with non-dysplastic BE, every six to twelve months in patients with LGD and (endoscopic) treatment in patients with established HGD or EAC [11, 12]. A major drawback of endoscopic surveillance is that it is an invasive and expensive procedure which is subject to interobserver variation, sampling error and variation in protocols. However, endoscopic surveillance is the only screening test available for BE. Over the past years there has been a major shift in the treatment of BE patients with neoplastic progression with the introduction of endoscopic mucosal resection (EMR) and ablation techniques such as radiofrequency ablation (RFA), photodynamic therapy (PDT) and argon plasma coagulation (APC) [13]. Endoscopic treatment is effective, less burdensome, associated with low morbidity and mortality rates, and may improve survival [14]. Although esophagectomy is still the mainstay for advanced EAC, esophagectomy is nowadays complemented by neoadjuvant chemotherapy or chemoradiotherapy [15]. Chemotherapy, esophageal stenting and brachytherapy have been added to the palliative treatment of EAC [16].
Recently, the value of endoscopic BE surveillance has been under discussion given the overall low incidence of neoplastic progression and lack of evidence that endoscopic surveillance reduces the risk of advanced EAC and improves survival [17, 18, 19]. These key questions have been evaluated in case-control studies, population-based studies and small prospective cohort studies with conflicting results.[13, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33] Although most studies suggest that endoscopic surveillance enables the detection of early EAC with good survival, some other studies reported no effect on mortality [19]. Furthermore in most studies patients were included independent of BE length. However the risk of neoplastic progression is much lower in patients with short BE [34, 35]. The aim of the present study was to

evaluate the impact of endoscopic surveillance of patients with BE of at least two centimeters according to current guidelines, on tumor stage and survival of patients with EAC.

Methods

Study Design

We conducted a large multicenter prospective cohort study in three academic and twelve regional hospitals throughout the Netherlands. Between November 2003 and December 2004, 783 consecutive patients were included with known or newly diagnosed BE with a maximum length of at least two centimeters according to the Prague C&M criteria [36]. The endoscopic diagnosis was confirmed by the presence of intestinal metaplasia and patients with HGD or EAC in the past or at the index endoscopy were excluded. Endoscopic surveillance was performed according to guidelines of the American College of Gastroenterology [11]. Patients without dysplasia underwent upper endoscopy with biopsy sampling every three years and patients with LGD every year. All endoscopic procedures were performed by experienced gastroenterologists, according to a standardized protocol. At each endoscopy targeted biopsies were taken from mucosal abnormalities and quadrant biopsies were taken every two cm from the most distal to the most proximal part of the BE epithelium, according to the Seattle protocol [37]. Most patients are still under surveillance.

Histology

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

Neoplastic progression

Neoplastic progression was defined as the development of HGD or EAC after inclusion in the study. The diagnosis was made only if at least two pathologists, including an expert pathologist, agreed on the presence of HGD or EAC. Patients with neoplastic progression were treated according to the guidelines of the American College of Gastroenterology. Patients with HGD received intensive endoscopic surveillance or were treated as early EAC with EMR, ablation techniques, or a combination of both depending on local expertise. Patients with advanced EAC received esophagectomy with or without neoadjuvant chemoradiotherapy [11]. EAC staging was performed according to the 7th UICC-AJCC classification. The stage of the primary tumor was based on histological assessment of biopsies, EMR specimens or resection specimens, whichever was available. The highest tumor stage was reported for each patient. After endoscopic or surgical treatment surveillance was resumed. During follow-up occurrence of complications, recurrence and metastasis was recorded.

Survival

Survival data were collected from all patients included in the study. Since surveillance intervals were up to three years and some patients dropped out of surveillance, survival was cross-checked using death registries and municipal administrations. When a patient was deceased, the cause of death was obtained from the attending gastroenterologist or general practitioner. Survival data from patients with EAC in the Netherlands, independent of cause of death and stratified by age, gender, stage, and year of diagnosis, were obtained from the Dutch cancer registry over the same time period [7]. Data on cause of death in the general population, stratified by age, gender and year of death, were obtained from the Dutch central statistical office [38].

Ethics

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

Statistical analysis

The incidence rate of neoplastic progression was calculated by dividing the

number of patients with HGD or EAC by the total person-years of follow-up. Chi-squared tests were used to compare EAC stage at diagnosis in BE patients undergoing surveillance and patients with EAC in the general population. Survival of BE patients with and without neoplastic progression during surveillance was compared in Cox proportional-hazards models adjusted for age and gender, whereby neoplastic progression was modelled as a time-dependent variable. Follow-up time was defined as the time from inclusion in the study to death or 1 January 2014, whichever came first. When no information was available from death or municipal registries, follow-up time was defined as the time from inclusion in the study to the last surveillance endoscopy. Cox-regression models were used to calculate hazard ratios (HR) and 95% confidence intervals (CI). In addition, survival of patients with different EAC stages in the general population was evaluated and compared to survival of patients with neoplastic progression during BE surveillance. To adjust for lead time bias, which is the time between the detection of preclinical EAC during surveillance and the moment EAC becomes symptomatic, we estimated the sojourn time for EAC from the difference in mean age at EAC diagnosis between BE patients undergoing surveillance and patients with EAC in the general population. To adjust for length time bias, which refers to the detection of less aggressive EAC during surveillance, we performed sensitivity analyses in which we only included patients with EAC. The 5-year cumulative survival was estimated using survival tables and Kaplan-Meier curves. In addition, we evaluated cause of death in BE patients and in individuals with similar age and gender in the general population. Two sided P-values <0.05 were considered to be statistically significant. Data were analysed using SPSS Statistics (version 20.0, Chicago, Illinois, USA).

Results

Patient characteristics

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

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

		Cohort n = 783	HGD or EAC n = 53	EAC general population n = 8855
Age	median years (IQR)	61 (53-70)	68 (59-74)	68 (60-77)
	mean (SD)	61 (12)	66 (10)	68 (12)
Male gender	number	573 (73%)	44 (83%)	7164 (81%)
Follow-up	median years (IQR)	7 (4-8)	5 (2-7)	1 (0-2)

HGD, high-grade dysplasia; EAC, esophageal adenocarcinoma

Neoplastic progression

After a median follow-up of 3 years 53 patients (83% male, median age 68 years) developed HGD or EAC with an incidence rate of 1.2 per 100 person-years (IQR 0.9-1.5), which was stable over time (Figure 1). The incidence rate was 0.3 per 100 person-years (IQR 0.2-0.6) for EAC (all stages) and 0.1 per 100 person-years (IQR 0.02-0.2) for advanced EAC (at least stage 2). Thirty-five patients (66%) developed HGD, 12 (22%) T1a EAC, 2 (4%) T1b EAC, 2 (4%) T2 EAC, and 2 (4%) T3 EAC. In 2 patients with T2 EAC, metastases were found in regional lymph nodes (N1). In none of the other patients lymph node metastases were found (N0). At the time of diagnosis, there was no evidence of distant metastases in any of the patients (M0). Thirty-five patients (66%) were classified as stage 0 disease, 14 (26%) as stage 1, and 4 (8%) as stage 2. EAC stage at diagnosis did not significantly change over time. Three patients (75%) with stage 2 EAC were previously diagnosed with LGD, for which they received annual surveillance. The remaining patient was never diagnosed with dysplasia and received surveillance every three years. Two patients (50%) with LGD at inclusion were diagnosed with stage 2 EAC at the first follow-up endoscopy one year later.

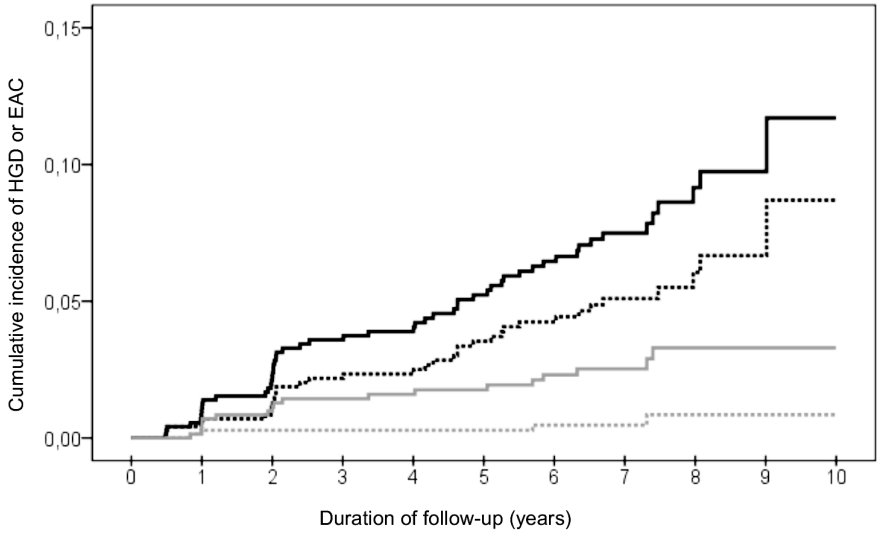

Figure 1. Cumulative incidence of neoplastic progression during Barrett surveillance. ▬ HGD and EAC, ··· HGD, ▬ EAC (all stages), ··· advanced EAC (≥ stage 2)

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

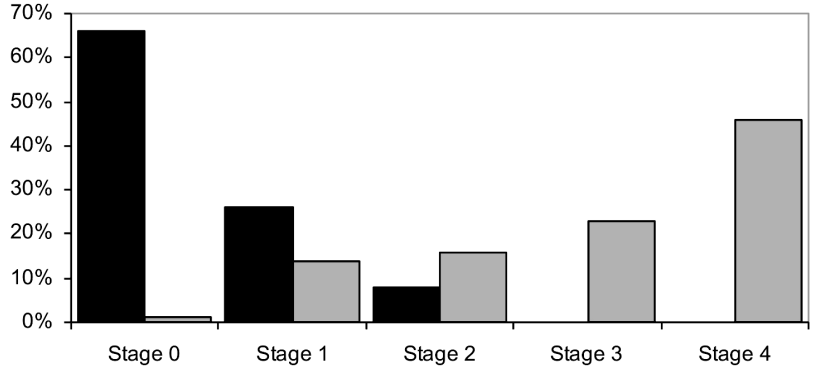

Figure 2. Stage of esophageal adenocarcinoma at the time of diagnosis in BE patients undergoing surveillance (■) and in the general Dutch population (■) ($p<0.001$)

Treatment

During surveillance 10 patients were diagnosed with focal HGD without mucosal abnormalities for which they received intensive surveillance. Although the initial diagnosis of HGD was confirmed by expert pathologists, in none of these patients HGD was confirmed during further follow-up. Therefore it was chosen to refrain from endoscopic treatment and follow a policy of watchful waiting. The remaining 25 patients with HGD received endoscopic treatment. Two patients were treated with PDT, 11 with EMR, 7 with EMR followed by PDT, and 5 with EMR followed by RFA. One patient developed a stenosis after EMR for which dilatation was performed and 1 patient had a perforation for which a stent was placed. Five patients had recurrence of HGD or early EAC during follow-up for which they were treated successfully with EMR and RFA. Of the 12 patients with T1a EAC 2 were treated with EMR, 7 with EMR followed by PDT and 2 with EMR followed by RFA. One patient died prior to treatment, 1 patient developed a stenosis for which dilatation was performed and 2 patients had recurrence for which they were treated successfully with EMR and RFA. The remaining 6 patients with T1b, T2 or T3 EAC were treated with transhiatal esophagectomy, which in 2 patients was complemented by neoadjuvant chemoradiotherapy. Two patients developed postoperative anastomotic leakage. One patient died due to postoperative complications and two patients due to advanced EAC after a median follow-up of 2 years (Figure 3).

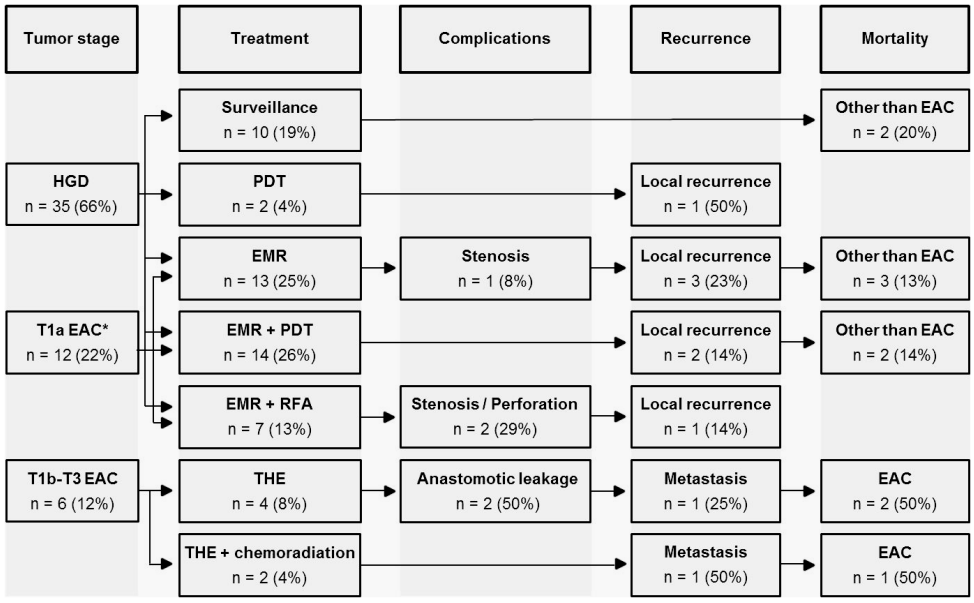

Figure 3. Treatment of patients with neoplastic progression detected during surveillance.
* 1 patient died prior to treatment of a cause not related to Barrett's esophagus, all patients with local recurrence were successfully treated with EMR and RFA
HGD, high-grade dysplasia; EAC, esophageal adenocarcinoma; PDT, photodynamic therapy; EMR, endoscopic mucosal resection; RFA, radiofrequency ablation; THE, transhiatal esophagectomy.

Survival

Of all 53 patients with neoplastic progression during surveillance, 12 patients (23%) (83% male, median age 73 years) died after a median follow-up of 2 years (IQR 1-4 years). The all cause 5-year survival of patients with neoplastic progression during surveillance was 74% (95% CI 60-87%) and was similar for patients in academic and regional hospitals. The 5-year survival was 80% for patients with stage 0 disease (n=35), 68% for stage 1 (n=14), and 33% for stage 2 (n=4). Of the remaining 730 BE patients in the cohort, 100 patients (14%) (76% male, median age 78 years) died after a median follow-up of 7 years (IQR 3-8 years). The all cause 5-years survival of BE patients without neoplastic progression was 94% (95% CI 92-96%). Of the 8855 patients with EAC in the general population, 6352 patients (72%) (81% male, median age 71 years) died

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

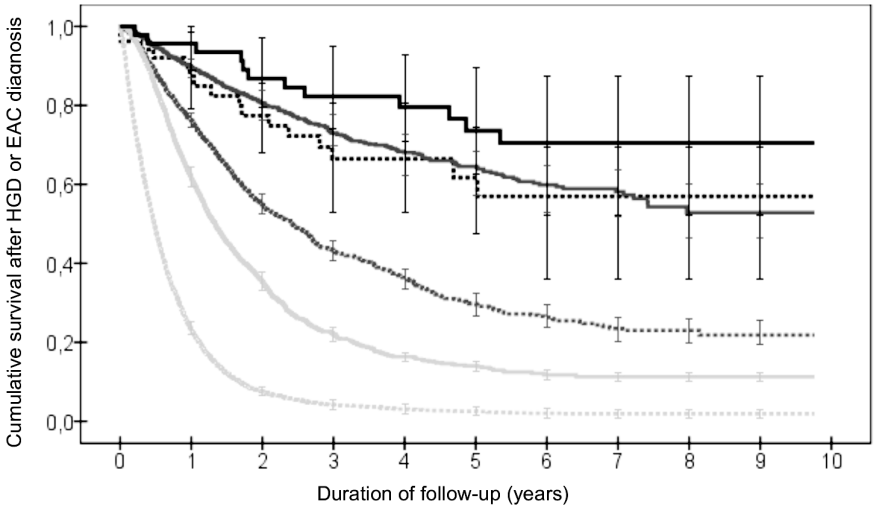

Figure 4. Cumulative survival of Barrett's esophagus (BE) patients with neoplastic progression during surveillance and patients with different stages of esophageal adenocarcinoma (EAC) in the general population.━ BE with neoplastic progression during surveillance, ··· EAC stage 0
━ EAC stage 1, ··· EAC stage 2, ━ EAC stage 3, ··· EAC stage 4

Lead and length time bias

To adjust for lead time bias we estimated the sojourn time for EAC based on the difference in mean age at EAC diagnosis in BE patients undergoing surveillance and patients from the general population (2.2 years). After adjusting for lead time bias, the all cause 5-year survival of patients with neoplastic progression during BE surveillance was 72% and the overall survival was still similar to

those of patients with stage 0 or stage 1 EAC in the general population (HR 0.8, 95% CI 0.4-1.7 and HR 0.9, 95% CI 0.5-1.6 respectively). To adjust for length time bias we performed separate analyses for BE patients with at least stage 1 EAC. The all cause 5-year survival of patients with EAC during BE surveillance was 62% and the overall survival was still similar to those of patients with stage 0 or stage 1 EAC in the general population (HR 0.9, 95% CI 0.3-2.4 and HR 0.9, 95% CI 0.4-2.1 respectively).

Cause of death

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

Table 2. Cause of death in patients with Barrett's esophagus and the general population

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

HGD, high-grade dysplasia; EAC, esophageal adenocarcinoma
* Individuals with similar age and gender in the same period

Discussion

The results of this large multicenter prospective cohort study suggest that EAC is detected at an earlier stage during surveillance than in the general population, independent of age, gender and year of diagnosis, and that endoscopic treatment leads to good survival.

Surveillance is a process of periodic testing in patients at high risk for a certain disease. Key elements in the effectiveness of surveillance are whether disease is detected at an early and curable stage and whether survival is improved. In the present study we showed that EAC was detected at a significantly earlier stage during endoscopic BE surveillance than in the general population. Of all patients with neoplastic progression during BE surveillance, 92% was diagnosed with early EAC (stage 0 or 1), compared to 15% in the general population. These results are in line with those of previous retrospective and small prospective studies, which reported early EAC in 60-95% of BE patients with neoplastic progression during surveillance and 10-40% of patients with EAC in the general population [19, 21, 23, 25, 26, 27, 29, 32]. In two previous studies surveillance failed to detect early EAC [7, 11]. One of the major shortcomings of those studies was that patients were not under strict surveillance, which is crucial for the detection of HGD or early EAC.

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

The overall 5-year survival was 74% in patients with EAC during BE surveillance and 17% in patients with EAC in the general population. Although it is difficult to compare survival of both groups due to different types of bias, including lead and length time bias, this large difference seems clinically relevant. The results correspond to those of previous retrospective and small prospective studies, which report an overall 5-year survival of 65-100% in patients with EAC during surveillance and 0-30% in patients with symptomatic EAC [6, 10, 15, 17, 18, 21, 25, 38, 39]. The majority of patients undergoing BE surveillance died due to cardiovascular diseases or malignancies and only 4% due to EAC, which was comparable to cause of death in individuals with similar age and gender

in the general population. One in four patients with EAC during surveillance died due to EAC or its treatment. Unfortunately, no information was available on cause of death in patients with EAC in the general population. Since the cause of death in patients undergoing BE surveillance was comparable to those of individuals in the general population, it is likely that excess mortality in patients with EAC in the general population is caused by EAC itself or its treatment. This idea is supported by data from the Surveillance, Epidemiology, and End Results (SEER) database, which shows that approximately half of patients with EAC in the United States of America, dies due to EAC or its treatment [40].

The present study shows that EAC is detected at an early stage during BE surveillance, but the cost-effectiveness of BE surveillance is still controversial. Several recent studies among which one of our own study group, have shown that BE surveillance may be cost-effective with intervals of five years for patients with non-dysplastic BE and three years for LGD [41, 42]. Although surveillance intervals were shorter in the current study, a minority of patients still developed advanced EAC. With prolongation of surveillance intervals, the risk of interval carcinomas will increase thereby limiting the protective effect. To improve the cost-effectiveness of endoscopic BE surveillance identification of additional risk factors is needed [39, 43, 44]. Another possibility to improve cost-effectiveness would be a less invasive screening test.

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

municipal administrations.
Our study also has some limitations. Studies evaluating the effect of surveillance may be subject to lead and length time bias [45]. When improved survival is based on earlier detection during surveillance rather than postponement of death this is called lead time bias. Length time bias refers to the fact that surveillance enables the detection of less aggressive disease with a mild course and thereby better survival. Thus even in the absence of a true effect of surveillance it may improve survival due to lead and length time bias. Lead time bias is unlikely to affect the results of our study since improved survival was seen until ten years after diagnosis, while the median survival of patients with symptomatic EAC was only eleven months. To consider lead and length time bias as much as possible we performed additional analyses in which we estimated the sojourn time for EAC based on difference in mean age at EAC diagnosis and excluded patients with HGD, which had no major effect on the results.
Unfortunately we were unable to adjust for differentiation grade, since this information was not available for the majority of patients with EAC in the general population.
Despite our efforts to consider different types of bias as much as possible, we cannot excluded uncontrolled confounding. A randomized controlled trial would be the ideal way to investigate the effect of endoscopic BE surveillance on survival. Although not impossible, it would be difficult to perform such a trial since only a small proportion of patients with EAC is previously known with BE. An alternative would be to perform an observational study including both BE patients undergoing surveillance and BE patients not under surveillance. Unfortunately, all patients participating in our prospective study received endoscopic surveillance and as a result we were only able to compare our data to those of patients with EAC in the general population.
We compared the pathological stage and survival of patients with neoplastic progression during BE surveillance to those of patients with EAC in the general population based on data from the Dutch cancer registry. Since patients are included in this registry based on a clinical or pathological diagnosis of cancer, there is underreporting of HGD. However, since most patients were diagnosed with advanced EAC we assume this is not a major source for bias.
During surveillance 10 BE patients were diagnosed with focal HGD without mucosal abnormalities and although this diagnosis was confirmed by expert pathologists, in none of these patients HGD was confirmed during

further follow-up. An important question is whether this is the result of misclassification or is a reflection of the natural history of focal HGD, since this may result in overtreatment of patients with focal HGD.
Unfortunately, the Dutch cancer registry provides no information on previous participation in surveillance. It is therefore possible that some patients in the control group had previous surveillance, which may result in an underestimation of the surveillance effect. In addition the register provides no information on cause of death in patients with EAC.
Finally, we only included patients with BE of at least two cm in the study and therefore our results cannot be applied universally to all BE patients. Since longer BE length is associated with a higher risk of neoplastic progression we believe that our cohort is representative for the patients who are most likely to benefit from surveillance.
In conclusion, this study suggests that regular endoscopic surveillance of BE patients enables the detection of EAC at an early and curable stage when endoscopic treatment is still feasible and leads to good survival. The results of this study therefore support current guidelines recommending endoscopic surveillance in patients with BE.

Reference

1 Bhat S, Coleman HG, Yousef F, Johnston BT, McManus DT, Gavin AT, et al. Risk of malignant progression in Barrett's esophagus patients: results from a large population-based study. J Natl Cancer Inst 2011;103:1049-57.

2 de Jonge PJ, van Blankenstein M, Looman CW, Casparie MK, Meijer GA, Kuipers EJ. Risk of malignant progression in patients with Barrett's oesophagus: a Dutch nationwide cohort study. Gut 2010;59:1030-6.

3 Hvid-Jensen F, Pedersen L, Drewes AM, Sorensen HT, Funch-Jensen P. Incidence of adenocarcinoma among patients with Barrett's esophagus. N Engl J Med 2011;365:1375-83.

4 Sikkema M, de Jonge PJ, Steyerberg EW, Kuipers EJ. Risk of esophageal adenocarcinoma and mortality in patients with Barrett's esophagus: a systematic review and meta-analysis. Clin Gastroenterol Hepatol 2010;8:235-44; quiz e32.

5 Hameeteman W, Tytgat GN, Houthoff HJ, van den Tweel JG. Barrett's esophagus: development of dysplasia and adenocarcinoma. Gastroenterology 1989;96:1249-56.

6 Kastelein F, Spaander MC, Biermann K, Vucelic B, Kuipers EJ, Bruno MJ. Role of acid suppression in the development and progression of dysplasia in patients with Barrett's esophagus. Dig Dis 2011;29:499-506.

7 http://www.cijfersoverkanker.nl/.

8 Lund O, Kimose HH, Aagaard MT, Hasenkam JM, Erlandsen M. Risk stratification and long-term results after surgical treatment of carcinomas of the thoracic esophagus and cardia. A 25-year retrospective study. The Journal of thoracic and cardiovascular surgery 1990;99:200-9.

9 Pennathur A, Gibson MK, Jobe BA, Luketich JD. Oesophageal carcinoma. Lancet 2013;381:400-12.

10 Fitzgerald RC, di Pietro M, Ragunath K, Ang Y, Kang JY, Watson P, et al. British Society of Gastroenterology guidelines on the diagnosis and management of Barrett's oesophagus. Gut 2013.

11 Wang KK, Sampliner RE, Practice Parameters Committee of the American College of G. Updated guidelines 2008 for the diagnosis, surveillance and therapy of Barrett's esophagus. Am J Gastroenterol 2008;103:788-97.

12 Fitzgerald RC, di Pietro M, Ragunath K, Ang Y, Kang JY, Watson P, et al. British Society of Gastroenterology guidelines on the diagnosis and management of Barrett's oesophagus. Gut 2014;63:7-42.

13 Menon D, Stafinski T, Wu H, Lau D, Wong C. Endoscopic treatments for Barrett's esophagus: a systematic review of safety and effectiveness compared to esophagectomy. BMC gastroenterology 2010;10:111.

14 Pech O, May A, Manner H, Behrens A, Pohl J, Weferling M, et al. Long-term Efficacy

and Safety of Endoscopic Resection for Patients With Mucosal Adenocarcinoma of the Esophagus. Gastroenterology 2013.

15 van Hagen P, Hulshof MC, van Lanschot JJ, Steyerberg EW, van Berge Henegouwen MI, Wijnhoven BP, et al. Preoperative chemoradiotherapy for esophageal or junctional cancer. N Engl J Med 2012;366:2074-84.

16 Homs MY, van Oijen MG, Wijnhoven BP, van Hillegersberg R, de Boer-Dennert M, Siersema PD. Changes in diagnostic and treatment strategies of oesophageal cancer in the period from 2001 to 2009: a survey in the Netherlands. European journal of gastroenterology & hepatology 2012;24:126-33.

17 Ronkainen J, Aro P, Storskrubb T, Johansson SE, Lind T, Bolling-Sternevald E, et al. Prevalence of Barrett's esophagus in the general population: an endoscopic study. Gastroenterology 2005;129:1825-31.

18 Wang JS, Canto MI. Predicting Neoplastic Progression in Barrett's Esophagus. Ann Gastroentol Hepatol 2010;1:1-10.

19 Corley DA, Mehtani K, Quesenberry C, Zhao W, de Boer J, Weiss NS. Impact of endoscopic surveillance on mortality from Barrett's esophagus-associated esophageal adenocarcinomas. Gastroenterology 2013;145:312-9 e1.

20 Aldulaimi DM, Cox M, Nwokolo CU, Loft DE. Barrett's surveillance is worthwhile and detects curable cancers. A prospective cohort study addressing cancer incidence, treatment outcome and survival. European journal of gastroenterology & hepatology 2005;17:943-50.

21 Chang LC, Oelschlager BK, Quiroga E, Parra JD, Mulligan M, Wood DE, et al. Long-term outcome of esophagectomy for high-grade dysplasia or cancer found during surveillance for Barrett's esophagus. Journal of gastrointestinal surgery : official journal of the Society for Surgery of the Alimentary Tract 2006;10:341-6.

22 Conio M, Blanchi S, Lapertosa G, Ferraris R, Sablich R, Marchi S, et al. Long-term endoscopic surveillance of patients with Barrett's esophagus. Incidence of dysplasia and adenocarcinoma: a prospective study. The American journal of gastroenterology 2003;98:1931-9.

23 Cooper GS, Kou TD, Chak A. Receipt of previous diagnoses and endoscopy and outcome from esophageal adenocarcinoma: a population-based study with temporal trends. Am J Gastroenterol 2009;104:1356-62.

24 Corley DA, Levin TR, Habel LA, Weiss NS, Buffler PA. Surveillance and survival in Barrett's adenocarcinomas: a population-based study. Gastroenterology 2002;122:633-40.

25 Ferguson MK, Durkin A. Long-term survival after esophagectomy for Barrett's adenocarcinoma in endoscopically surveyed and nonsurveyed patients. Journal of gastrointestinal surgery : official journal of the Society for Surgery of the Alimentary Tract 2002;6:29-35; discussion 6.

26 Fountoulakis A, Zafirellis KD, Dolan K, Dexter SP, Martin IG, Sue-Ling HM. Effect of surveillance of Barrett's oesophagus on the clinical outcome of oesophageal cancer. Br J Surg 2004;91:997-1003.

27 Grant KS, DeMeester SR, Kreger V, Oh D, Hagen JA, Chandrasoma P, et al. Effect of Barrett's esophagus surveillance on esophageal preservation, tumor stage, and survival with esophageal adenocarcinoma. The Journal of thoracic and cardiovascular surgery 2013;146:31-7.

28 Hoff SJ, Sawyers JL, Blanke CD, Choy H, Stewart JR. Prognosis of adenocarcinoma arising in Barrett's esophagus. The Annals of thoracic surgery 1998;65:176-80; discussion 80-1.

29 Incarbone R, Bonavina L, Saino G, Bona D, Peracchia A. Outcome of esophageal adenocarcinoma detected during endoscopic biopsy surveillance for Barrett's esophagus. Surgical endoscopy 2002;16:263-6.

30 Peters JH, Clark GW, Ireland AP, Chandrasoma P, Smyrk TC, DeMeester TR. Outcome of adenocarcinoma arising in Barrett's esophagus in endoscopically surveyed and nonsurveyed patients. The Journal of thoracic and cardiovascular surgery 1994;108:813-21; discussion 21-2.

31 Roberts KJ, Harper E, Alderson D, Hallissey M. Long-term survival and cost analysis of an annual Barrett's surveillance programme. European journal of gastroenterology & hepatology 2010;22:399-403.

32 van Sandick JW, van Lanschot JJ, Kuiken BW, Tytgat GN, Offerhaus GJ, Obertop H. Impact of endoscopic biopsy surveillance of Barrett's oesophagus on pathological stage and clinical outcome of Barrett's carcinoma. Gut 1998;43:216-22.

33 Bhat SK, McManus DT, Coleman HG, Johnston BT, Cardwell CR, McMenamin U, et al. Oesophageal adenocarcinoma and prior diagnosis of Barrett's oesophagus: a population-based study. Gut 2015;64:20-5.

34 Desai TK, Krishnan K, Samala N, Singh J, Cluley J, Perla S, et al. The incidence of oesophageal adenocarcinoma in non-dysplastic Barrett's oesophagus: a meta-analysis. Gut 2012;61:970-6.

35 Sharma P, Falk GW, Weston AP, Reker D, Johnston M, Sampliner RE. Dysplasia and cancer in a large multicenter cohort of patients with Barrett's esophagus. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association 2006;4:566-72.

36 Sharma P, Dent J, Armstrong D, Bergman JJ, Gossner L, Hoshihara Y, et al. The development and validation of an endoscopic grading system for Barrett's esophagus: the Prague C & M criteria. Gastroenterology 2006;131:1392-9.

37 Abela JE, Going JJ, Mackenzie JF, McKernan M, O'Mahoney S, Stuart RC. Systematic four-quadrant biopsy detects Barrett's dysplasia in more patients than nonsystematic biopsy. The American journal of gastroenterology 2008;103:850-5.

38 http://www.cbs.nl/.

39 Kastelein F, Spaander MC, Biermann K, Steyerberg EW, Kuipers EJ, Bruno MJ. Nonsteroidal anti-inflammatory drugs and statins have chemopreventative effects in patients with Barrett's esophagus. Gastroenterology 2011;141:2000-8; quiz e13-4.

40 http://seer.cancer.gov/.

41 Provenzale D, Schmitt C, Wong JB. Barrett's esophagus: a new look at surveillance based on emerging estimates of cancer risk. The American journal of gastroenterology 1999;94:2043-53.

42 F. Kastelein SvO, E.W. Steyerberg, M. Sikkema, M.C.W. Spaander, C.W.N. Looman, E.J. Kuipers, P.D. Siersema, M.J. Bruno and E.W. de Bekker-Grob. Surveillance in patients with Barrett's oesophagus: a cost-effectiveness analysis.

43 Kastelein F, Biermann K, Steyerberg EW, Verheij J, Kalisvaart M, Looijenga LH, et al. Aberrant p53 protein expression is associated with an increased risk of neoplastic progression in patients with Barrett's oesophagus. Gut 2013;62:1676-83.

44 Kastelein F, Spaander MC, Steyerberg EW, Biermann K, Valkhoff VE, Kuipers EJ, et al. Proton pump inhibitors reduce the risk of neoplastic progression in patients with Barrett's esophagus. Clin Gastroenterol Hepatol 2013;11:382-8.

45 Inadomi JM. Surveillance in Barrett's esophagus: a failed premise. The Keio journal of medicine 2009;58:12-8.

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P53 and SOX2 protein expression predicts esophageal adenocarcinoma response to neoadjuvant chemoradiotherapy

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Abstract

Introduction: Neoadjuvant chemoradiotherapy (nCRT) followed by surgery has become standard of care for esophageal adenocarcinoma (EAC). However, the response to nCRT is highly variable among patients. The aim of this study was to investigate the association between p53, SOX2 and CD44 protein expression and tumor response, and to validate potential predictive biomarker(s) in an independent cohort.

Methods: EAC patients who underwent nCRT plus surgery, between January 2003 and December 2014 at the Erasmus University Medical Center, were included and divided into a primary(n=77) and validation cohort(n=70). P53, SOX2 and CD44 expression was detected by immunohistochemistry in pretreatment tumor biopsies and scored independently by two investigators. Response to nCRT was assessed based on tumor regression grade (TRG) in the resection specimen.

Results: Forty-one(53%) patients in the primary cohort and 33(47%) patients in the validation cohort showed major response (TRG1 or TRG2) in the resection specimen. Aberrant p53 and absence of SOX2 were associated with major response in the primary cohort; adjusted odds ratio(OR) 6.3 (95%CI 1.3–30.1) and adjusted OR 4.1 (95%CI 1.4–12.4), respectively. The same was true for the validation cohort (p53: adjusted OR 8.6; 95%CI 0.93-80.9 and SOX2: adjusted OR 6.1; 95%CI 1.6-23.4). The highest probability of a major response was seen in patients with concurrent aberrant p53 and absence of SOX2 expression, with an OR of 6.7 (95%CI: 2.1-21.4) and 6.2 (95%CI: 1.8-21.2) in the primary and validation cohort.

Conclusions: Pattern of p53 and particularly SOX2 protein expression in EAC predicts response to nCRT. These biomarkers may help to individualize treatment in EAC patients.

Introduction

Over the past decades, the incidence of esophageal adenocarcinoma (EAC) has increased rapidly in the United States and Western Europe [1, 2]. The 5-year overall survival after surgery rarely exceeds 40% and early recurrence is common [2, 3]. Neoadjuvant chemoradiotherapy (nCRT) followed by surgery has recently become standard of care for locally advanced esophageal cancers (EC) and achieves a 5-year overall survival benefit of 10-15% compared to surgery alone [4, 5].

However, response to nCRT is variable, even among patients with a similar disease stage. In the Dutch CROSS trial nearly 25% of the patients with an EAC in the multimodality arm showed a pathological complete response (pCR; i.e. no viable tumor cells) and another 36% of the patients showed a near-complete response (1%-10% residual tumor cells) in the resection specimen [4]. Surgical resection, immediately after nCRT, is still considered the cornerstone of intentionally curative treatment for EC, although associated with significant morbidity and substantial impact on the quality of life [6, 7]. If clinicians were able to accurately identify (near-)complete responders, prior to surgery, these patients might be candidates to postpone or even omit surgical resection. In addition, patients without substantial pathological response do not seem to benefit from nCRT but experience unnecessary side-effects and curative surgery is delayed [8, 9].

Several studies have tried to assess the response to nCRT using conventional endoscopy with biopsy sampling, endoscopic ultrasonography (EUS), computed tomography (CT) and positron emission tomography (PET), but results have been mainly disappointing [10, 11, 12, 13]. Use of predictive biomarkers could improve the ability to predict tumor response and may facilitate individualization of treatment.

The tumor suppressor gene p53 has been identified as an important molecular factor associated with tumor tolerance to chemotherapy and radiation in patients with EC [14, 15, 16]. However, there is no general consensus on the predictive value of p53 status for therapy response. Other potential biomarkers are SOX2 and CD44, both linked to cancer stem cells (CSCs), a small population of cells, found to be more resistant to chemo- and radiotherapy in various malignant tumor types [17, 18]. SOX2 is a transcription factor, related to CSCs and embryonic stem cells, involved in formation and differentiation of esophageal and gastric epithelium [19, 20]. SOX2 expression has been associated with both chemo- and radiotherapy resistance in several malignancies, including

breast cancer and oral squamous cell cancer [21, 22, 23]. CD44 is a membrane glycoprotein involved in cell adhesion and associated with CSCs in gastric and colon cancer [24, 25]. High CD44 expression has been suggested as possible marker of CSCs in EC and may play a role in radiotherapy resistance [26]. Their expression and possible relation with response to nCRT in EAC has barely been investigated. Based on these promising previous publications and our experience with staining and scoring of the selected biomarkers the aim of our study was (I) to investigate the association between p53, SOX2 and CD44 protein expression in pretreatment tumor biopsies and extent of pathological tumor response in the resection specimen of patients with EAC treated with nCRT and (II) to validate potential predictive biomarker(s) for therapy response in an independent cohort of EAC patients treated with nCRT.

Methods

Patients and clinical staging

All consecutive patients with histologically proven EAC who received at least 80% nCRT according to the CROSS regimen followed by esophagectomy, between January 2002 and December 2014 at the Erasmus University Medical Center were eligible for inclusion in the study. Sufficient and representative material of the pretreatment tumor biopsies and resection specimen had to be available. The total cohort of patients who met the selection criteria was divided into a primary and validation cohort based on surgical date (primary cohort surgical date between January 2002 and September 2009, and validation cohort between October 2009 and December 2014). Pretreatment clinical TNM (cTNM) staging included upper gastrointestinal endoscopy with biopsies; EUS (with fine needle aspiration (FNA) when indicated); CT of the neck, chest and abdomen; and external ultrasonography of the neck with FNA in case of suspected lymph nodes. In patients with a high suspicion of metastatic disease a PET-CT was done as well. Tumors were (re-)staged according the 7th UICC-AJCC TNM staging manual [27].

Neoadjuvant treatment and surgery

All patients received nCRT according to the CROSS regimen which consisted of carboplatin (targeted area under the curve = 2) and paclitaxel (50 mg/m2) administered by intravenous infusion on day 1, 8, 15, 22 and 29 with concurrent external beam radiation with a total dose of 41.4 Gy, given in 23

fractions of 1.8 Gy, 5 fractions a week [4]. Esophagectomy was scheduled 4 to 6 weeks after completion of nCRT. For tumors of the intrathoracic esophagus and for junctional tumors with positive lymph nodes at or above the carina, a transthoracic resection with a two-field lymph node dissection was performed. For tumors involving the gastro-esophageal junction, a transhiatal resection was the preferred technique. A lymphadenectomy of the nodes along the celiac axis and its branches was performed in both approaches.

Assessment of treatment response

All pretreatment tumor biopsies and resection specimens (entire primary tumor and all resected lymph nodes) were evaluated by a standardized protocol [28]. Pathological T-stage and N-stage were classified according to the UICC TNM Cancer Staging , 7th edition [29].

In addition, different aspects of tumor response were measured, pathological tumor downstaging and overall tumor regression grade (TRG). For the assessment of the pathological tumor and node downstaging, the initial tumor area, before nCRT, was estimated based on the extent of regressional changes (e.g., fibrosis, keratin pearls, mucous lakes and/or foreign body cell reactions) and on the residual tumor cells in the resection specimen, as has been described previously [30, 31]. These measurements were expressed as prepT and prepN, reflecting the assumed original depth of the primary tumor and the assumed number of originally involved lymph nodes, respectively. PrepT and prepN were compared with the eventual pathological ypT and ypN stage in the resection specimen after nCRT. In case of N-downstaging, patients with N0 stage before and after nCRT were excluded.

The TRG was evaluated using the modified Mandard scoring system [32]. The extent of tumor regression was subdivided in four categories; TRG1: no viable tumor cells; TRG 2: between 1% and 10%; TRG 3: between 11% and 50%; TRG 4: more than 50% residual tumor cells. Patients with TRG 1 or TRG 2 response were classified as major responders (i.e., ≤ 10% of tumor cells remaining), whereas patients with TRG 3 or TRG 4 response were classified as minor responders (i.e., > 10% of tumor cells remaining) [33, 34].

Immunohistochemistry

Sections of 5 mm thickness from paraffin blocks of the complete series of pretreatment tumor biopsies of all included patients were used. Immunohistochemical staining for p53 was performed as a single batch as previously described, using the primary antibody (Clone DO-7, Dako,

Glostrup, Denmark: mouse monoclonal) with a dilution of 1:25 [35]. For SOX2 and CD44 immunohistochemistry, paraffin-embedded tissue sections were deparaffinized in xylene and rehydrated in graded alcohols. Antigen retrieval was enhanced by heating in Tris buffer and endogenous peroxidase activity was blocked by incubating the slides in a solution of 0.3% hydrogen peroxide in phosphate-buffered saline. Primary antibodies, SOX2 (AF2018, dilution 1:800, R&D systems, Abingdon, United Kingdom: goat, polyclonal) and CD44 (clone IM7, dilution 1:1000, BD Biosciences) were applied for 22 hours at 4 degrees Celsius. As for p53, the same batch of SOX2 and CD44 antibodies were used for all included slides. As secondary antibody, a biotinylated horse anti-goat IgG antibody (1:150; BA-950, Vector, Peterborough, United Kingdom) and rabbit anti-rat IgG antibody (1:150; BA-4000, Vector, Peterborough, United kingdom) were used. Visualization was achieved by using the horseradish peroxidase avidin-biotin complex (HRP-ABC) method and diaminobenzidine (DAB). Finally, the slides were counterstained with haematoxylin. A sample of testicular embryonal carcinoma was used as positive control for SOX2 and a sample of tonsil with squamous epithelium for CD44 [36, 37].

Scoring of immunohistochemistry

Immunohistochemically stained slides were examined in tandem with the haematoxylin-eosin stained slides to determine p53, SOX2 and CD44 expression in tumor cells. P53 expression was scored on a three-point scale; normal expression, overexpression or loss of expression [35, 38]. Only intense nuclear staining for p53 was scored as overexpression (see Figure 1 for a representative example). Weak nuclear positivity in tumor cells, comparable to the expression in non-neoplastic squamous epithelium was interpreted as normal and complete loss of nuclear p53 staining was defined as negative expression. Aberrant p53 expression was defined as either overexpression or complete loss of expression in >50% of the tumor cells: this cut-off was chosen based on previous literature and our experience in assessment of p53 staining [35, 39]. Nuclear SOX2 and membranous CD44 expression was scored on a two-point scale; positive or absence of expression. As previously described in esophageal tissue and germ cell malignancies, strong as well as weak nuclear staining, but not cytoplasmatic SOX2 expression was interpret as positive (see Figure 1 for a representative example) [36, 38]. The most optimal cut-off values for SOX2 and CD44 immunohistochemical scoring to predict therapy response were calculated by ROC-curve analysis in the primary cohort, using

the area under the curve (AUC) as the performance measure. Based on these ROC-curve analyses, aberrant SOX2 expression was defined as absence of SOX2 expression in >50% of the tumor cells, whereas positive CD44 expression was defined as membranous CD44 expression of at least one tumor cell. All stained slides were scored independently by two investigators (KB and SvO) who were blinded for clinical outcome. In case of disagreement between the two investigators, slides were reviewed by both investigators simultaneously to reach a consensus diagnosis.

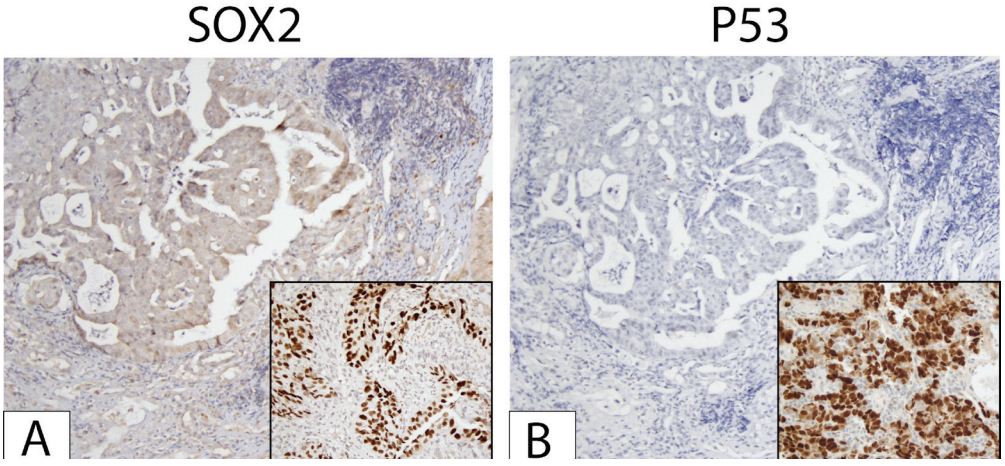

Figure 1. Representative immunohistochemical stained slides of SOX2 negative nuclear expression (A), SOX2 positive nuclear expression (insert), loss of p53 expression (B) and p53 overexpression (insert) in esophageal adenocarcinoma tissue (A-B). Magnification 1:100 A-B, magnification 1:200 inserts.

Ethics

This study was performed on microscopy slides and tissue as used and obtained during regular patient diagnostics. The Ethics council of the Erasmus Medical Center approves research conducted on diagnostic tissue, without special permission. Therefore, no additional ethical approval was required.

Statistical analysis

To evaluate the associations with TRG response and TN-downstaging, clinical, pathological and biomarker data were compared using Kruskal-Wallis tests

for continuous variables and Pearson chi-square tests or Fisher exact test for categorical variables. TRG major response was defined as TRG1 or TRG2 response in the resection specimen (≤ 10% of tumor cells remaining) whereas TRG minor response was defined as TRG3 or TRG 4 response (> 10% of tumor cells remaining). The value of p53 and SOX2 immunohistochemistry to predict TRG major response was estimated in logistic regression models. In multivariable models we adjusted for age, gender, tumor grade, pretreatment clinical T-stage and pretreatment clinical N-stage to estimate adjusted odds ratios (ORs) and 95% Confidence Intervals (CIs). Interobserver agreement for p53 and SOX2 protein expression was determined using Cohen K statistics. Two sided P values of <0.05 were considered statistically significant. Data were analyzed using SPSS statistical software (V.21.0; SPSS, Chicago, IL, USA).

Results

Patients and histopathological characteristics

In total, 258 patients with EAC who received at least 80% nCRT were eligible for inclusion in the study. Of these, 26 patients did not undergo resection and in 85 additional patients not enough representative pretreatment tumor biopsies and/or resection specimen tissue was available, leaving 147 to be included in the study. These 147 patients who met all the selection criteria were subdivided into a primary (n=77) and validation cohort (n=70) (see Table 1 for characteristics). Median age at diagnosis was 64 years (IQR 57-70), predominantly male (88%). Clinical staging revealed most often cT3 (77%), cN+ (69%) also pathological staging revealed most often prepT3 (86%) and prepN+ (57%). Median time between nCRT and subsequent surgical resection was 50 days (IQR 39 – 65 days). The overall distribution of TRG response was TRG1 = 32 (22%), TRG2 = 42 (29%), TRG3 = 43 (29%) and TRG4 = 30 (20%). Aberrant p53 expression and absence of SOX2 expression was seen in 85% (125/147) and 59% (87/147), respectively. Interobserver agreement for both markers was good with a kappa-value of 0.79 (95% CI: 0.74-0.83) for p53 expression and a kappa-value of 0.73 (95% CI: 0.67-0.78) for SOX2 expression. Expression of CD44 was only stained and scored in the primary cohort, in which 50 patients (65%) showed a positive CD44 expression (Table 1).

Table 1. Baseline and histopathological characteristics of primary and validation cohort

Variable	Total n = 147	Primary cohort n = 77	Validation cohort n = 70	P value
Age				
Median, years (IQR)	64 (57 - 70)	61 (55 - 70)	64 (58 - 69)	0.392
Gender				
Woman	18 (12%)	10 (13%)	8 (11%)	0.773
Men	129 (88%)	67 (87%)	62 (89%)	
Median time between nCRT and surgery, days (IQR)	50 (39 - 65)	50 (34 - 62)	50 (40 - 70)	0.212
Grade of differentiation				
Well	10 (7%)	8 (10%)	2 (3%)	0.104
Moderate	83 (56%)	45 (59%)	38 (54%)	
Poor	54 (37%)	24 (31%)	30 (43%)	
cT-stage[a]				
cT1	5 (3%)	3 (4%)	2 (3%)	0.253
cT2	29 (20%)	19 (25%)	10 (14%)	
cT3	113 (77%)	55 (71%)	58 (83%)	
cN-stage[a]				
cN0	46 (31%)	28 (36%)	18 (26%)	0.163
cN1	55 (37%)	31 (40%)	24 (34%)	
cN2	42 (29%)	16 (21%)	26 (37%)	
cN3	4 (3%)	2 (3%)	2 (3%)	
PrepT-stage[b]				
prepT1/T2	21 (14%)	9 (12%)	12 (17%)	0.327
prepT3	125 (86%)	68 (88%)	57 (83%)	
prepN-stage[b]				
prepN0	62 (42%)	37 (48%)	25 (36%)	0.342
prepN1	62 (42%)	29 (38%)	33 (48%)	
prepN2/N3	22 (15%)	11 (14%)	11 (16%)	
ypT-stage[c]				
ypT0	36 (24%)	17 (22%)	19 (27%)	0.095
ypT1	22 (15%)	17 (22%)	5 (7%)	
ypT2	28 (19%)	12 (16%)	16 (23%)	
ypT3	60 (41%)	30 (39%)	30 (43%)	
ypT4a	1 (1%)	1 (1%)	0 (0%)	
ypN-stage[c]				

Variable	Total n = 147	Primary cohort n = 77	Validation cohort n = 70	P value
yNo	95 (65%)	57 (74%)	38 (55%)	0.012
yN+	52 (35%)	20 (26%)	32 (45%)	
Median number of lymph nodes resected (IQR)	15 (11-21)	13 (10-19)	17 (13-22)	<0.001
Total number tumor positive lymph nodes resected	146	48	98	
Radicality[d]				
R0	135 (92%)	73 (95%)	62 (89%)	0.168
R1	12 8%)	4 (5%)	8 (11%)	
TRG[e]				
TRG 1	32 (22%)	15 (19%)	17 (24%)	0.532
TRG 2	42 (29%)	26 (34%)	16 (23%)	
TRG 3	43 (29%)	21 (27%)	22 (31%)	
TRG 4	30 (20%)	15 (20%)	15 (22%)	
P53 expression				
Normal	22 (15%)	13 (17%)	9 (13%)	0.111
P53 overexpression	88 (60%)	40 (52%)	48 (69%)	
Loss of expression	37 (25%)	24 (31%)	13 (18%)	
SOX2 expression				
Positive	60 (41%)	33 (43%)	27 (39%)	0.597
Absence of expression	87 (59%)	44 (57%)	43 (61%)	
CD44 expression				
Positive	50 (65%)	50 (65%)	-	-
Absence of expression	21 (27%)	21 (27%)	-	
Missing	6 (8%)	6 (8%)	-	

Kruskall-Wallis test and Pearson chi-square test or Fisher exact test were used to compare the characteristics of primary and validation cohort.
a Pretreatment T and N-stages as defined by endoscopic ultrasonography
[b] Preoperative T and N-stage estimated based on the extent of regressional changes (eg, fibrosis, keratin pearls, mucous lakes and/or foreign body cell reactions) and on the residual tumor cells in the resection specimen.
[c] Pathological T and N-stage in the resection specimen after neoadjuvante chemoradiotherapy according to UICC TNM Cancer Staging, 7th edition.
[d] R0 was defined as a tumor-free resection margin ≥1 mm. R1 was defined as a macroscopically radical resection, with a microscopically tumor-free resection margin of < 1 mm.
[e] Tumor regression grade (TRG) was defined as; TRG1: no residual tumor cells found; TRG2: 1-10% residual tumor cells; TRG3: 11-50% residual tumor cells; TRG4: >50% residual tumor cells.
Abbreviation: IQR, interquartile range

TRG response

Primary cohort

Forty-one (53%) of the 77 patients had a major pathological response (TRG 1-2) in the resection specimen after nCRT, whereas 36 (47%) patients had a minor response (TRG 3-4). Aberrant p53 expression and absence of SOX2 expression were more common in samples of patients with a major response than in patients with a minor response (p53; 93% vs 72%, p value 0.017 and SOX2; 71% vs 42%, p value 0.010) (Table 2). Aberrant p53 expression was significantly associated with a major response with an OR of 4.9 (95% CI: 1.2–19.4; p value 0.025), even after adjusting for age, gender, tumor grade, pretreatment clinical T-stage and pretreatment clinical N-stage (adjusted OR of 6.3; 95% CI: 1.3–30.1; p value 0.02). Also, absence of SOX2 expression was significantly associated with major response with an OR 3.4 (95% CI: 1.3-8.7; p value 0.011) and adjusted OR of 4.1 (95% CI: 1.4–12.4; p value 0.012) (Table 3).

In addition, the combination of both biomarkers showed that aberrant p53 with concurrent absence of SOX2 expression was seen in 26 (63%) patients with a major response but in only 9 (25%) patients with a minor response (Table 2). Aberrant expression of both biomarkers increased the probability of a major response in the individual patient (adjusted OR of 6.7; 95% CI: 2.1–21.4; p value 0.001) with a sensitivity of 63%, specificity of 75%, positive predictive value (PPV) of 74% and a negative predictive value (NPV) of 64% (Tables 3 and 4). Evaluation of CD44 expression in the pretreatment tumor biopsies showed only a trend of increased possibility in case of absence of expression (p value 0.112). None of the included clinicopathological characteristics were statistically significantly associated with TRG response (Table 2).

Table 2. Clinicopathological characteristics and biomarker expression according to TRG response

	TRG response primary cohort			TRG response validation cohort		
Variable	**Major n = 41**	**Minor n = 36**	**P Value**	**Major n = 33**	**Minor n = 37**	**P Value**
Age						
Median, years (IQR)	62 (56-69)	60 (55-71)	0.967	65 (58-70)	64 (57-69)	0.469
Gender						
Woman	6 (15%)	4 (11%)	0.742	5 (15%)	3 (8%)	0.462
Men	35 (85%)	32 (89%)		28 (85%)	34 (92%)	
Median time between nCRT and surgery, days (IQR)	50 (34-61)	51 (41-66)	0.405	54 (42-67)	49 (39-73)	0.613
Tumor grade						
Well-diff	6 (14%)	2 (6%)	0.392	1 (3%)	1 (3%)	0.369
Moderately-diff	22 (54%)	23 (64%)		15 (46%)	23 (62%)	
Poorly-diff	13 (32%)	11 (30%)		17 (51%)	13 (35%)	
cT-stage [a], [b]						
cT1	2 (5%)	1 (3%)	0.772	2 (6%)	0 (0%)	0.293
cT2	9 (22%)	10 (28%)		4 (12%)	6 (16%)	
cT3	30 (73%)	25 (69%)		27 (82%)	31 (84%)	
cN stage[a]						
cN0	15 (37%)	13 (36%)	0.947	8 (24%)	10 (27%)	0.930
cN1	17 (41%)	14 (39%)		12 (36%)	12 (32%)	
cN2/3	9 (22%)	9 (25%)		13 (39%)	15 (41%)	
PrepT-stage[c]						
prepT1/T2	7 (17%)	2 (6%)	0.162	8 (24%)	4 (11%)	0.151
prepT3	34 (83%)	34 (94%)		25 (76%)	32 (89%)	
PrepN-stage [c]						
prepN0	20 (49%)	17 (47%)	0.448	15 (46%)	10 (28%)	0.294
prepN1	17 (41%)	12 (33%)		14 (42%)	19 (53%)	

Variable	TRG response primary cohort			TRG response validation cohort		
	Major n = 41	Minor n = 36	P Value	Major n = 33	Minor n = 37	P Value
prepN2/3	4 (10%)	7 (20%)		4 (12%)	19 (53%)	
P53 expression						
Normal	3 (7%)	10 (28%)	0.017	1 (3%)	8 (22%)	0.020
Aberrant expressiond	38 (93%)	26 (72%)		32 (97%)	29 (78%)	
SOX2 expression						
Positive	12 (29%)	21 (58%)	0.017	8 (24%)	19 (51%)	0.020
Absence of expression	29 (71%)	15 (42%)		25 (76%)	18 (48%)	
CD44 expression						
Positive	23 (56%)	27 (75%)	0.112	-	-	-
Absence of expression	14 (34%)	7 (19%)		-	-	
Missing	4 (10%)	2 (6%)		-	-	
Number of aberrant biomarkerse						
0 or 1 aberrant biomarker	15 (37%)	27 (75%)	<0.001	9 (27%)	22 (60%)	0.009
2 aberrant biomarkers	26 (63%)	9 (25%)		24 (73%)	15 (40%)	

Kruskall-Wallis test and Pearson chi-square test or Fisher exact test were used to compare the characteristics of patients with major response and patients with minor response.

[a] Pretreatment T and N-stages as defined by endoscopic ultrasonography

[b] When we look at the association between cT-stage and complete pathological response (TRG1) versus all patients with a TRG 2-3-4 response a significant association was observed (p value 0.03).

[c] Preoperative T and N-stage estimated based on the extent of regressional changes (eg, fibrosis, keratin pearls, mucous lakes and/or foreign body cell reactions) and on the residual tumor cells in the resection specimen.

[d] Aberrant p53 expression was defined as either overexpression or complete loss of expression.

[e] Aberrant biomarker expression included aberrant p53 expression and absence of SOX2 expression

Abbreviations: TRG, Tumor Regression Grade; IQR, interquartile range.

Table 3. Results of uni- and multivariate logistic regression analysis of biomarker expression to predict TRG major response in primary and validation cohort.

	Primary cohort						Validation cohort					
	Univariate analysis			Multivariate analysis[a]			Univariate analysis			Multivariate analysis[a]		
Variable	**OR**	**95% CI**	**P Value**	**OR**	**95% CI**	**P Value**	**OR**	**95% CI**	**P Value**	**OR**	**95% CI**	**P Value**
P53 expression												
Normal	1.0			1.0			1.0			1.0		
Aberrant expression[b]	4.9	1.2 - 19	0.025	6.3	1.3 - 30	0.020	8.8	1.04 - 75	0.046	8.6	0.93 - 81	0.058
SOX2 expression												
Positive	1.0			1.0			1.0			1.0		
Absence of expression	3.4	1.3 - 8.7	0.011	4.1	1.4 - 12	0.012	3.3	1.2 - 9.2	0.022	6.1	1.6 - 23	0.008
Number of aberrant biomarkers[c]												
0 or 1 biomarker	1.0			1.0			1.0			1.0		
2 biomarkers	5.2	1.9 - 14	0.001	6.7	2.1 - 21	0.001	3.9	1.4 - 11	0.008	6.2	1.8 - 21	0.004

[a] Adjusted for age, gender, tumor grade, pretreatment clinical T-stage and pretreatment clinical N-stage
[b] Aberrant p53 expression was defined as either overexpression or complete loss of expression
[c] Aberrant biomarker expression included aberrant p53 expression and absence of SOX2 expression.
Logistic regression models were used to calculate odds ratios (ORs) and confidence intervals (CIs) for probability of a major response after nCRT

Table 4. Results of p53 and SOX2 expression as test for predicting major TRG response in EAC patients treated with neoadjuvant chemoradiotherapy.

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Efficiency of the test (%)
Primary cohort					
Aberrant p53 expressiona	93%	28%	59%	77%	62.0
Absence of SOX2 expression	71%	58%	66%	64%	64.9
2 aberrant biomarkers[b]	63%	75%	74%	64%	68.8
Validation cohort					
Aberrant p53 expression*	97%	22%	52%	89%	57.0
Absence of SOX2 expression	76%	51%	58%	70%	62.9
2 aberrant biomarkers[b]	73%	60%	62%	71%	65.7

The efficiency of the test is calculated as (true positive + true negative) / number of samples x 100.
Abbreviations: PPV, positive predictive value; NPV, negative predictive value; TRG, Tumor Regression Grade; EAC, esophageal adenocarcinoma.
[a] Aberrant p53 expression was defined as either overexpression or complete loss of expression
[b] Aberrant biomarker expression included aberrant p53 expression and absence of SOX2 expression.

Validation cohort

CD44 expression showed no significant association with therapy response in the primary cohort and therefore further validation of this biomarker was discontinued. In contrast, validation of the predictive biomarkers p53 and SOX2 was performed in an independent cohort of 70 EAC patients of which 33 patients (47%) showed a major pathological response (TRG 1-2) and 37 patients (53%) a minor response (TRG 3-4). Aberrant p53 expression as individual marker showed borderline significance for predicting therapy response after adjusting for age, gender, tumor grade, pretreatment clinical T-stage and pretreatment clinical N-stage (adjusted OR of 8.6; 95% CI: 0.93-80.9, p value 0.058) (Table 3).
Similarly to the results in the primary cohort, absence of SOX2 expression in the pretreatment tumor biopsies was again significantly associated with major response, with an adjusted OR of 6.1 (95% CI: 1.6-23.4; p value 0.008). Aberrant expression of both biomarkers was associated with a slightly higher probability of a major response with an adjusted OR of 6.2 (95% CI: 1.8-21.2; p value 0.004) as shown in Table 3. The sensitivity of aberrant p53 with concurrent absence of SOX2 expression for predicting a major response was 73%, with a specificity of 60%, PPV of 62% and NPV of 71% (Table 4).

Pathological T- and N-downstaging

Based on the comparison between initial pretreatment pathological tumor staging and evaluation of the residual tumor cells after nCRT, 42 (55%) patients showed pathological T-downstaging and 25 (32%) patients showed pathological N-downstaging in the primary cohort versus 36 (53%) patients with T-downstaging and only 18 (26%) patients with N-downstaging in the validation cohort (Table 5). There was no significant correlation between pathological T- or N-downstaging and p53 expression in both the primary and validation cohort. However, in the validation cohort absence of SOX2 was more common in patients with pathological T-downstaging than in patients without pathological T-downstaging (80% versus 42%) after nCRT (p value of 0.001).

Table 5. Clinicopathological characteristics and biomarker expression according to pathological TN-downstaging.

Variable	Primary cohort					
	Pathological T-downstaging			Pathological N-downstaging[a]		
	Yes n = 42	No n = 35	P Value	Yes n = 25	No n = 16	P Value
Age						
Median, years (IQR)	62 (55 - 68)	62 (55 - 71)	0.910	59 (55 - 71)	59 (55 - 65)	0.843
Gender						
Woman	6 (14%)	4 (11%)	0.748	3 (12%)	4 (25%)	0.401
Men	36 (86%)	31 (89%)		22 (88%)	12 (75%)	
Tumor grade						
Well-diff	6 (14%)	2 (6%)	0.355	2 (8%)	1 (6%)	0.747
Moderately-diff	22 (52%)	23 (66%)		15 (60%)	8 (50%)	
poorly-diff	14 (34%)	10 (28%)		8 (32%)	7 (44%)	
P53 expression						
Normal	4 (10%)	9 (26%)	0.059	4 (16%)	4 (25%)	0.689
Aberrant expression[b]	38 (90%)	26 (74%)		21 (84%)	12 (75%)	
SOX2 expression						
Positive	17 (40%)	16 (46%)	0.644	12 (48%)	9 (56%)	0.606
Absence of expression	25 (60%)	19 (54%)		13 (52%)	7 (44%)	

Variable	Validation cohort					
	Pathological T-downstaging			Pathological N-downstaging[a]		
	Yes n = 36	No n = 33	P Value	Yes n = 18	No n = 26	P Value
Age						
Median, years (IQR)	66 (58 - 71)	64 (58 - 68)	0.307	67 (57 - 73)	64 (58 - 70)	0.304
Gender						
Woman	3 (8%)	5 (15%)	0.466	1 (6%)	3 (11%)	0.634
Men	33 (92%)	28 (85%)		17 (94%)	23 (89%)	
Tumor grade						
Well-diff	2 (6%)	0 (0%)	0.272	0 (0%)	1 (4%)	0.701
Moderately-diff	21 (58%)	17 (52%)		10 (56%)	14 (54%)	
poorly-diff	13 (36%)	16 (48%)		8 (44%)	11 (42%)	
P53 expression						
Normal	4 (11%)	5 (15%)	0.728	3 (17%)	5 (19%)	0.100
Aberrant expression[b]	32 (89%)	28 (85%)		15 (83%)	21 (81%)	

Variable	Validation cohort					
	Pathological T-downstaging			Pathological N-downstaging[a]		
	Yes n = 36	No n = 33	P Value	Yes n = 18	No n = 26	P Value
SOX2 expression						
Positive	7 (20%)	19 (58%)	0.001	6 (33%)	11 (42%)	0.548
Absence of expression	29 (80%)	14 (42%)		12 (67%)	15 (58%)	

For the assessment of pathological TN-downstaging prepT and prepN (estimated based on the extent of regressional changes and the residual tumor cells in the resection specimen) were compared with the eventual pathological ypT and ypN stage after nCRT
[a] Excluding patients with stage N0 before and after neoadjuvant chemoradiotherapy (including primary cohort n = 41 and validation cohort n = 44 patients)
[b] Aberrant p53 expression was defined as either overexpression or complete loss of expression
Abbreviation: IQR, interquartile range

Correlation between biomarker expression and clinicopathological characteristics

Tumor grade was the only clinicopathological characteristic which was significantly correlated with p53 and SOX2; presence of SOX2 expression was associated with a higher tumor grade in analyses of all the pretreatment biopsies (p value of 0.006).

Discussion

The response to nCRT is highly variable among patients with EAC. In our study 50% of the patients showed a major response (TRG1-2) in the resection specimen after nCRT, which is in line with previous publications [4, 40]. The currently available restaging modalities as CT, EUS and PET-CT have limited capacity to assess therapy response prior to surgery [11, 12, 41]. Additionally, a significant proportion of patients do not seem to benefit from neoadjuvant treatment and experience unnecessary side-effects with delayed surgical resection [8, 9]. Therefore, there is a need for informative biomarkers that determine the biological behavior of individual tumors and may contribute to predict individual patient response to nCRT.
Absence of SOX2 expression was seen in 59% of pretreatment tumor biopsies and proved to be an independent predictor for major pathological response in the primary and validation cohort (adjusted OR 4.1 and 6.1, respectively). Only one small study has previously evaluated the value of SOX2 expression for predicting response to nCRT in patients with EC, but no association

was observed [23]. This may be explained by investigation of both EAC and esophageal squamous cell carcinoma (ESCC), differences in assessing therapy response, as well as different interpretation of immunohistochemical staining (cytoplasmatic SOX2 was considered negative in our study). In line with our results, SOX2 expression has been associated with therapy response in several other cancer types. Ovarian and prostate cancer tissue showed a correlation between SOX2 expression and paclitaxel resistance (also part of the CROSS regimen) [21, 22, 42, 43]. The precise mechanism of SOX2 in the context of EAC and response to nCRT is unknown but serves to be investigated.

Aberrant p53 expression was seen in the majority of the pretreatment tumor biopsies (85%) and was significantly associated with major response in the primary cohort, while in the validation cohort only a trend of increased probability was detected. In fact, overall, conflicting data are reported. Kitamura et al. showed that p53 was significantly associated with increased sensitivity to nCRT, whereas other studies could not confirm this correlation [44, 45, 46]. The majority of immunohistochemical studies have been performed in patients with ESCC or mixed population of EAC and ESCC patients, therefore to be interpreted with caution. In addition, in contrast to our study, others scored strong nuclear p53 as aberrant only [39]. Truncating TP53 mutations or epigenetic silencing may result in p53 inactivation by complete absence. Based on our experience, both patterns could be classified as aberrant [35, 38]. Zhang et al. showed in a meta-analysis of in total 28 studies with 1497 patients a significant association between major response to chemotherapy-based treatment in EC with low p53 protein expression or wild-type TP53 [39]. Again, this hampers by tremendous heterogeneity across studies regarding histology, p53 scoring, therapy response, and therapy regimens. A more recent study showed that EC patients with a normal TP53 status may benefit from nCRT with cisplatin/fluorouracil compared to mutant TP53 status (no immunohistochemical analysis performed) [47]. Patients in our study were treated according to the CROSS regimen, consisting of carboplatin plus paclitaxel and radiotherapy. Carboplatin and radiotherapy are likely to act via a p53-controlled pathway, but docetaxel acts differently and it is not clear if and how it interacts with TP53 [48, 49]. Therefore, no general conclusion can be drawn.

SOX2 as a single biomarker may has more power to predict therapy response than p53, but the predictive value slightly increased by the combination of aberrant p53 expression with concurrent absence of SOX2 expression. These

findings might have important and clinically relevant implications. Assessment of p53 and particularly SOX2 immunohistochemistry offers an additional tool, in combination with other diagnostic modalities, to select those patients who are likely to benefit most from this multimodality treatment. Recent data from the CROSS study group showed that a prolonged time to surgery after completion of nCRT up to at least 12 weeks was associated with an increased pathological response, without a significant rise in postoperative complications which supports a more conservative wait-and-see strategy in a selection of patients [9, 50]. However, additional studies are needed to investigate the prognostic value of p53 and SOX2, which is beyond the scope of this study.
Patients in our study were subdivided into TRG major and TRG minor response group based on previous publications [33, 34]. Furthermore, different aspects of tumor response were measured. Besides TRG also pathological TN-downstaging was evaluated and compared with p53 and SOX2 expression. Absence of SOX2 expression was significantly more common in patients with T-downstaging than in patients without T-downstaging, strengthening the association between SOX2 expression and response to nCRT. However, this was only identified in the validation cohort and may be explained by the fact that in the primary cohort several patients showed a TRG 2 response without T-downstaging, so the few residual tumor cells were still present in the initially involved layers of the esophagus. The same was true for p53, where only a trend between p53 expression and T-downstaging was observed in the primary cohort. Our single institution CROSS regimen study has several strengths. Importantly, we identified two biomarkers for predicting therapy response in a primary cohort, used for the determination of the most optimal cut-off value for biomarker expression, and validated these results in a second independent cohort of EAC patients. Additionally, all stained slides were reviewed by at least two experienced observers to obtain a final diagnosis based on consensus.
Our study also has some limitations. We evaluated the predictive value of biomarker expression retrospectively, however all parameters were collected prospectively. Although immunohistochemistry is a standardized method within clinical pathological laboratories, the scoring of the expression can be subjective. However, our study showed a good interobserver agreement for both markers [35, 38]. In conclusion, Expression of p53 and particularly SOX2 in pretreatment tumor biopsies predicts response to nCRT in patients with potentially curable EAC. These biomarkers may contribute, in combination with other modalities, to accurate evaluation of response to nCRT and thereby

help to individualize treatment decision in patients with potentially curable EAC.

Reference

1 Thrift AP, Whiteman DC. The incidence of esophageal adenocarcinoma continues to rise: analysis of period and birth cohort effects on recent trends. Ann Oncol 2012;23:3155-62.

2 Pennathur A, Gibson MK, Jobe BA, Luketich JD. Oesophageal carcinoma. Lancet 2013;381:400-12.

3 Smit JK, Pultrum BB, van Dullemen HM, Van Dam GM, Groen H, Plukker JT. Prognostic factors and patterns of recurrence in esophageal cancer assert arguments for extended two-field transthoracic esophagectomy. Am J Surg 2010;200:446-53.

4 van Hagen P, Hulshof MC, van Lanschot JJ, Steyerberg EW, van Berge Henegouwen MI, Wijnhoven BP, et al. Preoperative chemoradiotherapy for esophageal or junctional cancer. N Engl J Med 2012;366:2074-84.

5 Gebski V, Burmeister B, Smithers BM, Foo K, Zalcberg J, Simes J, et al. Survival benefits from neoadjuvant chemoradiotherapy or chemotherapy in oesophageal carcinoma: a meta-analysis. Lancet Oncol 2007;8:226-34.

6 Donohoe CL, McGillycuddy E, Reynolds JV. Long-term health-related quality of life for disease-free esophageal cancer patients. World J Surg 2011;35:1853-60.

7 Djarv T, Lagergren J, Blazeby JM, Lagergren P. Long-term health-related quality of life following surgery for oesophageal cancer. Br J Surg 2008;95:1121-6.

8 Mariette C, Piessen G, Triboulet JP. Therapeutic strategies in oesophageal carcinoma: role of surgery and other modalities. Lancet Oncol 2007;8:545-53.

9 Noordman BJ, Shapiro J, Spaander MC, Krishnadath KK, van Laarhoven HW, van Berge Henegouwen MI, et al. Accuracy of Detecting Residual Disease After Cross Neoadjuvant Chemoradiotherapy for Esophageal Cancer (preSANO Trial): Rationale and Protocol. JMIR Res Protoc 2015;4:e79.

10 Swisher SG, Maish M, Erasmus JJ, Correa AM, Ajani JA, Bresalier R, et al. Utility of PET, CT, and EUS to identify pathologic responders in esophageal cancer. Ann Thorac Surg 2004;78:1152-60; discussion -60.

11 Schneider PM, Metzger R, Schaefer H, Baumgarten F, Vallbohmer D, Brabender J, et al. Response evaluation by endoscopy, rebiopsy, and endoscopic ultrasound does not accurately predict histopathologic regression after neoadjuvant chemoradiation for esophageal cancer. Ann Surg 2008;248:902-8.

12 Piessen G, Petyt G, Duhamel A, Mirabel X, Huglo D, Mariette C. Ineffectiveness of (1)(8)F-fluorodeoxyglucose positron emission tomography in the evaluation of tumor response after completion of neoadjuvant chemoradiation in esophageal cancer. Ann Surg 2013;258:66-76.

13 Jost C, Binek J, Schuller JC, Bauerfeind P, Metzger U, Werth B, et al. Endosonographic radial tumor thickness after neoadjuvant chemoradiation therapy to predict response and

survival in patients with locally advanced esophageal cancer: a prospective multicenter phase ll study by the Swiss Group for Clinical Cancer Research (SAKK 75/02). Gastrointest Endosc 2010;71:1114-21.

14 Mendonca JA, Francischone CE, Senna PM, Matos de Oliveira AE, Sotto-Maior BS. A retrospective evaluation of the survival rates of splinted and non-splinted short dental implants in posterior partially edentulous jaws. J Periodontol 2014;85:787-94.

15 Vallbohmer D, Holscher AH, Schneider PM, Schmidt M, Dietlein M, Bollschweiler E, et al. [18F]-fluorodeoxyglucose-positron emission tomography for the assessment of histopathologic response and prognosis after completion of neoadjuvant chemotherapy in gastric cancer. J Surg Oncol 2010;102:135-40.

16 Clara A, Fonseca I, Francisca A, Bettencourt A, Vaz F. Unexpected long-term survival in a BRCA2 patient with metastatic carcinosarcoma associated with tamoxifen. Gynecol Oncol Case Rep 2013;4:44-6.

17 Phillips TM, McBride WH, Pajonk F. The response of CD24(-/low)/CD44+ breast cancer-initiating cells to radiation. J Natl Cancer Inst 2006;98:1777-85.

18 Baumann M, Krause M, Hill R. Exploring the role of cancer stem cells in radioresistance. Nat Rev Cancer 2008;8:545-54.

19 Liu K, Lin B, Zhao M, Yang X, Chen M, Gao A, et al. The multiple roles for Sox2 in stem cell maintenance and tumorigenesis. Cell Signal 2013;25:1264-71.

20 Que J, Okubo T, Goldenring JR, Nam KT, Kurotani R, Morrisey EE, et al. Multiple dose-dependent roles for Sox2 in the patterning and differentiation of anterior foregut endoderm. Development 2007;134:2521-31.

21 Chou MY, Hu FW, Yu CH, Yu CC. Sox2 expression involvement in the oncogenicity and radiochemoresistance of oral cancer stem cells. Oral Oncol 2015;51:31-9.

22 Piva M, Domenici G, Iriondo O, Rabano M, Simoes BM, Comaills V, et al. Sox2 promotes tamoxifen resistance in breast cancer cells. EMBO Mol Med 2014;6:66-79.

23 Honing J, Pavlov KV, Mul VE, Karrenbeld A, Meijer C, Faiz Z, et al. CD44, SHH and SOX2 as novel biomarkers in esophageal cancer patients treated with neoadjuvant chemoradiotherapy. Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology 2015.

24 Takaishi S, Okumura T, Tu S, Wang SS, Shibata W, Vigneshwaran R, et al. Identification of gastric cancer stem cells using the cell surface marker CD44. Stem Cells 2009;27:1006-20.

25 Yeung TM, Gandhi SC, Wilding JL, Muschel R, Bodmer WF. Cancer stem cells from colorectal cancer-derived cell lines. Proc Natl Acad Sci U S A 2010;107:3722-7.

26 Smit JK, Faber H, Niemantsverdriet M, Baanstra M, Bussink J, Hollema H, et al. Prediction of response to radiotherapy in the treatment of esophageal cancer using stem cell markers. Radiother Oncol 2013;107:434-41.

27 Rice TW, Blackstone EH, Rusch VW. 7th edition of the AJCC Cancer Staging Manual: esophagus and esophagogastric junction. Ann Surg Oncol 2010;17:1721-4.
28 van Meerten E, van der Gaast A, Tilanus HW, Poley JW, Muller K, van Dekken H. Pathological analysis after neoadjuvant chemoradiotherapy for esophageal carcinoma: the Rotterdam experience. J Surg Oncol 2009;100:32-7.
29 Sobin LH GM, Wittekind C, Union for International Cancer Control U. TNM classification of malignant tumours, seventh edition. 7th ed. Wiley-Blackwell 2010.
30 Wheeler JM, Warren BF, Mortensen NJ, Ekanyaka N, Kulacoglu H, Jones AC, et al. Quantification of histologic regression of rectal cancer after irradiation: a proposal for a modified staging system. Dis Colon Rectum 2002;45:1051-6.
31 Shapiro J, ten Kate FJ, van Hagen P, Biermann K, Wijnhoven BP, van Lanschot JJ. Residual esophageal cancer after neoadjuvant chemoradiotherapy frequently involves the mucosa and submucosa. Ann Surg 2013;258:678-88; discussion 88-9.
32 Mandard AM, Dalibard F, Mandard JC, Marnay J, Henry-Amar M, Petiot JF, et al. Pathologic assessment of tumor regression after preoperative chemoradiotherapy of esophageal carcinoma. Clinicopathologic correlations. Cancer 1994;73:2680-6.
33 Holscher AH, Drebber U, Schmidt H, Bollschweiler E. Prognostic classification of histopathologic response to neoadjuvant therapy in esophageal adenocarcinoma. Ann Surg 2014;260:779-84; discussion 84-5.
34 Francis AM, Sepesi B, Correa AM, Blum MA, Erasmus JJ, Lee JH, et al. The influence of histopathologic tumor viability on long-term survival and recurrence rates following neoadjuvant therapy for esophageal adenocarcinoma. Ann Surg 2013;258:500-7.
35 Kastelein F, Biermann K, Steyerberg EW, Verheij J, Kalisvaart M, Looijenga LH, et al. Aberrant p53 protein expression is associated with an increased risk of neoplastic progression in patients with Barrett's oesophagus. Gut 2013;62:1676-83.
36 de Jong J, Stoop H, Gillis AJ, van Gurp RJ, van de Geijn GJ, Boer M, et al. Differential expression of SOX17 and SOX2 in germ cells and stem cells has biological and clinical implications. J Pathol 2008;215:21-30.
37 Lindquist D, Ahrlund-Richter A, Tarjan M, Tot T, Dalianis T. Intense CD44 expression is a negative prognostic factor in tonsillar and base of tongue cancer. Anticancer Res 2012;32:153-61.
38 van Olphen S, Biermann K, Spaander MC, Kastelein F, Steyerberg EW, Stoop HA, et al. SOX2 as a Novel Marker to Predict Neoplastic Progression in Barrett's Esophagus. Am J Gastroenterol 2015;110:1420-8.
39 Zhang SS, Huang QY, Yang H, Xie X, Luo KJ, Wen J, et al. Correlation of p53 status with the response to chemotherapy-based treatment in esophageal cancer: a meta-analysis. Ann Surg Oncol 2013;20:2419-27.

40 Mariette C, Dahan L, Mornex F, Maillard E, Thomas PA, Meunier B, et al. Surgery alone versus chemoradiotherapy followed by surgery for stage I and II esophageal cancer: final analysis of randomized controlled phase III trial FFCD 9901. J Clin Oncol 2014;32:2416-22.

41 Westerterp M, van Westreenen HL, Reitsma JB, Hoekstra OS, Stoker J, Fockens P, et al. Esophageal cancer: CT, endoscopic US, and FDG PET for assessment of response to neoadjuvant therapy--systematic review. Radiology 2005;236:841-51.

42 Li D, Zhao LN, Zheng XL, Lin P, Lin F, Li Y, et al. Sox2 is involved in paclitaxel resistance of the prostate cancer cell line PC-3 via the PI3K/Akt pathway. Mol Med Rep 2014;10:3169-76.

43 Li Y, Chen K, Li L, Li R, Zhang J, Ren W. Overexpression of SOX2 is involved in paclitaxel resistance of ovarian cancer via the PI3K/Akt pathway. Tumour Biol 2015.

44 Sarbia M, Ott N, Puhringer-Oppermann F, Brucher BL. The predictive value of molecular markers (p53, EGFR, ATM, CHK2) in multimodally treated squamous cell carcinoma of the oesophagus. Br J Cancer 2007;97:1404-8.

45 Kitamura K, Saeki H, Kawaguchi H, Araki K, Ohno S, Kuwano H, et al. Immunohistochemical status of the p53 protein and Ki-67 antigen using biopsied specimens can predict a sensitivity to neoadjuvant therapy in patients with esophageal cancer. Hepatogastroenterology 2000;47:419-23.

46 Bronson NW, Diggs BS, Bakis G, Gatter KM, Sheppard BC, Hunter JG, et al. Molecular Marker Expression Is Highly Heterogeneous in Esophageal Adenocarcinoma and Does Not Predict a Response to Neoadjuvant Therapy. J Gastrointest Surg 2015.

47 Kandioler D, Schoppmann SF, Zwrtek R, Kappel S, Wolf B, Mittlbock M, et al. The biomarker TP53 divides patients with neoadjuvantly treated esophageal cancer into 2 subgroups with markedly different outcomes. A p53 Research Group study. J Thorac Cardiovasc Surg 2014;148:2280-6.

48 Kandioler-Eckersberger D, Ludwig C, Rudas M, Kappel S, Janschek E, Wenzel C, et al. TP53 mutation and p53 overexpression for prediction of response to neoadjuvant treatment in breast cancer patients. Clin Cancer Res 2000;6:50-6.

49 Bonnefoi H, Piccart M, Bogaerts J, Mauriac L, Fumoleau P, Brain E, et al. TP53 status for prediction of sensitivity to taxane versus non-taxane neoadjuvant chemotherapy in breast cancer (EORTC 10994/BIG 1-00): a randomised phase 3 trial. Lancet Oncol 2011;12:527-39.

50 Shapiro J, van Hagen P, Lingsma HF, Wijnhoven BP, Biermann K, ten Kate FJ, et al. Prolonged time to surgery after neoadjuvant chemoradiotherapy increases histopathological response without affecting survival in patients with esophageal or junctional cancer. Ann Surg 2014;260:807-13; discussion 13-4.

8

Loss of SRY-box2 (SOX2) expression predicts adverse survival of patients with esophageal adenocarcinoma

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Abstract

Introduction: Esophageal adenocarcinoma (EAC) is a highly aggressive malignancy with poor survival, which is highly variable amongst patients with comparable conventional prognosticators. Therefore molecular biomarkers are urgently needed to improve survival prediction in these patients. SRY (sex determining region Y)-box 2, also known as SOX2, is a transcription factor involved in embryonal development of the gastrointestinal tract as well as in carcinogenesis. Here we aimed to test whether SOX2 expression is associated with survival in patients with EAC.

Methods: SOX2 was studied by immunohistochemistry in patients who had undergone potentially curative esophagectomy for adenocarcinoma. Protein expression of SOX2 was evaluated using tissue micro arrays from resection specimens and the results were analyzed in relation to the clinical data by Cox regression analysis. SOX2 was evaluated in two independent EAC cohorts (Rotterdam cohort and multicenter UK cohort).

Results: Loss of SOX2 expression was independently predictive for adverse overall survival (OS) in the multivariate analysis adjusted for known factors influencing survival, in Rotterdam as well as in the UK cohort (Rotterdam cohort: HR=1.42, 95% CI 1.07-1.89, p=0.016; UK cohort: HR=1.54, 95% CI 1.08-2.19, p=0.017). When combined with clinico-pathological staging, SOX2 segregated patients into prognostic groups in pT1/2 tumors (p=0.01) and nodal-negative EAC (p=0.038), with incremental adverse effect on OS for stage I EAC with SOX2 loss (HR=3.18, 95% CI 1.18-8.56, p=0.022).

Conclusions: We identified SOX2 as an independent prognostic factor for long-term survival in EAC, in particular in patients with stage I EAC.

Introduction

Esophageal adenocarcinoma (EAC) is an aggressive cancer with steadily increasing incidence [1, 2]. The major risk factors for EAC are gastroesophageal reflux [3], abdominal obesity [4] and Barrett esophagus (BE) [5, 6]. While patients with non-dysplastic BE show a low rate of progression to EAC during surveillance (<1% per year) [7], most patients with EAC exhibit underlying BE at the time of EAC diagnosis and are typically diagnosed at an advanced, frequently incurable stage [8].

Although the addition of (neo-)adjuvant therapy to primary surgical resection improves overall survival (OS) and disease-specific survival in locally advanced tumors, the prognosis of most patients with advanced EAC including those treated with curative intent is dismal, with a 5-year survival of 47% at most [9, 10, 11]. Postsurgical prognostication is currently based on tumor staging according to the American Joint Committee on Cancer staging system, supplemented by pathological criteria [12]. However, even after considering all known parameters including resection margin, nodal status, presence of vascular invasion, tumor grade, and differentiation grade, the course of the disease remains variable [13, 14, 15]. Improving clinical decision making is essential, especially in early EAC. In these patients numerous treatment modalities are available, depending on tumor characteristics, and the best treatment modality for the individual patient is under discussion. One method for a better prognostication in early EAC is the use of biomarkers, which can improve the decision on the optimal treatment strategy.

Various signaling pathways essential for embryonal development are involved in cancer initiation and progression, including the sex determining region Y-box2 (also known as SOX2). SOX2 is a highly conserved gene coded on a single exon which plays a pivotal role in the maintenance of embryonic stem cells [16]. In the gastrointestinal tract it determines the formation and differentiation of esophageal and gastric epithelium during embryogenesis [17, 18]. Besides its role in embryogenesis, SOX2 is involved in various malignancies including squamous cell carcinoma of the esophagus [19], gastric adenocarcinoma [20], prostate cancer [21] and colorectal cancer [22]. SOX2 functions differ depending on the cell of origin and oncogenic as well as tumor suppressive mechanisms have been described. The SOX2 gene can be amplified in squamous cell carcinoma of the esophagus and trachea and acts as a lineage survival oncogene by promoting cell migration and proliferation [23, 24]. Accordingly, upregulation of SOX2 is strongly associated with adverse

outcome in these patients [19]. In contrast, the opposite functions of SOX2 were shown in gastric adenocarcinoma, in which loss of SOX2 expression was correlated with worse prognosis and PTEN has been proposed as a direct target of SOX2 [20].
It is noteworthy that little is known about the role of SOX2 in EAC. Previously, we evaluated SOX2 expression in BE and showed that SOX2 downregulation is highly associated with progression of BE to high grade dysplasia and EAC [25]. Furthermore, SOX2 status was indicative for the pattern of response to neoadjuvant chemoradiotherapy in patients with EAC [26, 27]. Another Dutch group had previously shown that SOX2 may have prognostic effect for disease free survival (DFS) in a small cohort of surgically treated EAC patients [28]. In light of the emerging data on SOX2, the aim of the present study was to assess the role of SOX2 in prognostication of patients with surgically treated EAC with particular emphasis on patients with stage I disease.

Methods

Patient selection

Both, the Rotterdam (N=336) and the UK multicenter cohort (OCCAMS, Oesophageal Cancer Clinical and Molecular Stratification Study cohort, N=420) consisted of patients who underwent esophagectomy with curative intent for pathologically confirmed adenocarcinoma of the esophagus or gastroesophageal junction. Follow-up of all patients was performed in the respective clinical centers and only patients who were alive one month after surgery were included in the analysis. The Rotterdam cohort consisted of patients treated at the Department of Surgery at Erasmus Medical Center, Rotterdam, the Netherlands during the time period between 1995 and 2006. The UK cohort consisted of patients form six tertiary hospitals treated between 1992 and 2000. Within the Rotterdam cohort, 68 (20.2 %) of patients received neoadjuvant chemoradiotherapy (29 patients) or chemotherapy (39 patients). In the OCCAMS cohort, 146 (42.1%) patients received neoadjuvant chemotherapy according to UK guidelines.
The clinical and pathological data of both cohorts were collected and included tumor grade, pathological stage, chemotherapy treatment, age at surgery, comorbidities and OS, amongst others. The TNM system according to the UICC (Union Internationale Contre le Cancer, 2009, 7th edition) was used for pathologic grading and staging [12]. To insure reliable classification, all

tumors were revised by an expert gastrointestinal pathologist.

Tissue micro array

For the construction of a tissue micro array (TMA), formalin-fixed paraffin-embedded tissue from the resection specimens were retrieved from the archives at the Departments of Pathology of the participating institutions. For each tumor, 3 to 6 cores from multiple representative areas of EAC, as identified by a pathologist on H&E slides, were taken from the original paraffin blocks, including the central part and invasive front of the tumor [29, 30].

SOX2 Immunohistochemistry

The SOX2 immunohistochemical staining technique has been described extensively in previous publications [25, 26]. In short, 5µm sections were cut from the TMA and were de-paraffinized and rehydrated. Tissue of squamous cell carcinoma with clear positive staining for SOX2 was placed on each immunohistochemal slide of the TMAs as a positive control. Antigen retrieval was enhanced by heating in a Tris buffer. Endogenous peroxidase activity was blocked by incubating the slides in a solution of 0.3% hydrogen peroxide in phosphate-buffered saline. Primary SOX2 antibody (AF2018, dilution 1:800, R&D systems, Abington, UK: goat, polyclonal) was applied for 22 hours at 4°C. The secondary antibody was a biotinylated horse anti-goat IgG antibody (1:150; BA-4000, Vector, Peterborough, UK). Visualization was achieved using the horseradish peroxidase avidin-biotin complex (HRP-ABC) method and diaminobenzidine (DAB). The slides were counterstained with hematoxylin. The immunohistochemically stained TMA slides from both cohorts were digitalized and scored independently by two investigators (SO and KB), who were blinded to the clinical and pathological outcome. In case of disagreement, the cores were reviewed by both investigators simultaneously and a consensus was achieved.

SOX2 was scored as positive or negative in each of the stained cores. As described previously, weak or strong nuclear expression of at least 50% of the tumor cells was defined as positive, while nuclear expression in less than 50% of the tumor cells as well as cytoplasmatic SOX2 expression were defined as negative [26]. Because SOX2 expression might be heterogeneous in EAC, the overall expression in each tumor was calculated from all corresponding cores. Patients with less than 3 cores containing cells representative for the original EAC were excluded from analysis.

The most optimal cut-off value of immunohistochemistry with SOX2 to predict

survival was calculated by Receiver Operating Characteristics (ROC) curve analysis in the Rotterdam cohort, using the Area Under the Curve (AUC) as the performance measure. Based on this evaluation, absence of SOX2 expression was defined by negative staining of SOX2 in >75% of the cores, and otherwise SOX2 was considered to be present.

Ethics

The investigational protocols of both cohorts were approved by the relevant institutional review boards (MEC-12-469 and LREC 04/Q2006/2).

Statistical analysis

The primary endpoint in this study was 5–year OS, defined as time from surgery until death. The differences between the Rotterdam and the UK cohorts were analyzed using a Students t-test for normal distribution and Mann-Whitney test for non-normal distribution for continuous variables and a $\chi 2$ test for categorical variables. The equality of distribution was tested using a Levene's test. The interobserver variation between the two investigators for scoring of SOX2 (SO, KB) was calculated using Cohen's kappa. Strength of agreement was categorized as follows: 0.00–0.20, poor; 0.21–0.40, fair; 0.41–0.60, moderate; 0.61–0.80, good; and 0.81–1.00, excellent.

Kaplan-Meier curves were used to plot the 5-year survival by SOX2 status. After imputation of missing variables using a linear regression model, univariate and multivariate Cox proportional hazard model was applied to estimate the independent association between the SOX2 immunohistochemical expression and survival. In the multivariate analysis adjustments were made for those clinical and pathological factors which were independently predictive in the univariate analysis. Also, sensitivity analysis using multivariate Cox proportional hazard model excluding all patients with (neo-)adjuvant treatment and adjusting for the clinical and pathological factors was performed to test the SOX2 role in chemoradiotherapy-naïve patients. The pN-stage was dichotomized in a pN0 and a pN+ (pN1-3) group for the multivariate analysis. All analysis were performed using SPSS-software (version 22, SPSS IBM inc, Armonk, NY, USA). A P-value of <0.05 was considered to be statistically significant.

Results

Patient characteristics

The EAC cohort from Rotterdam consisted of 336 patients, while the cohort from the OCCAMS study consisted of 420 patients. Clinical characteristics of the patients from Rotterdam and OCCAMS cohorts as well as for the entire group are listed in Table 1. Patients from the OCCAMS cohort were older compared with those from the Rotterdam cohort (age 66.0 years versus 64.7 years, p=0.01) and had a shorter median follow-up time (18.0 months versus 25.0 months, p<0.01). There was a male predominance in both cohorts as expected. Patients from the Rotterdam cohort more often had a tumor at the esophageal-gastric junction (Siewert type II), a higher degree of differentiation, an earlier T-stage as well as less frequent lymph node metastases (p<0.01). There were fewer patients with (neo)adjuvant treatment in the Rotterdam cohort. Furthermore, loss of SOX2 expression was more common in the OCCAMS cohort compared to the Rotterdam cohort.

Table 1. Clinico-pathological characteristics, combined cohort and specified by Rotterdam and OCCAMS cohort.

Variable	Combined (N=756)		Rotterdam (N=336)		OCCAMS (N=420)		P-value
Age at surgery							
Median	65.4		64.7		66.0		0.01
Range	(33-90)		(33-90)		(33-88)		
Follow-up time, months							
Median	20.9		25.0		18.0		<0.01
Range	(1-199)		(1-199)		(1-193)		
Sex							
Male	602	82.0%	293	87.2%	309	77.6%	<0.01
Female	132	18.0%	43	12.8%	89	22.4%	
Siewert classification							
Type 1	460	69.7%	190	57.1%	270	82.6%	<0.01

Variable	Combined (N=756)		Rotterdam (N=336)		OCCAMS (N=420)		P-value
Type 2	168	25.5%	126	37.8%	42	12.8%	
Type 3	32	4.8%	17	5.1%	15	4.6%	
Recurrence	182	54.2%	182	54.2%	NA		
Resection margin status							
pR0	396	71.0%	245	72.9%	151	68.0%	0.21
pR1	162	29.0%	91	27.1%	71	32.0%	
Histology grade							
Well	52	7.5%	26	7.7%	26	7.3%	0.01
Moderate	248	35.7%	139	41.4%	109	30.4%	
Poor	394	56.8%	171	50.9%	223	62.3%	
Pathologic T-stage							
pT1	79	11.2%	48	14.7%	31	8.2%	<0.01
pT2	132	18.8%	59	18.0%	73	19.4%	
pT3	474	67.3%	218	66.7%	256	67.9%	
pT4	19	2.7%	2	0.6%	17	4.5%	
Pathologic N-stage							
pN0	245	35.9%	142	42.4%	103	29.6%	<0.01
pN1 or more	438	64.1%	193	57.6%	245	70.4%	
(Neo-)adjuvant treatment							
Yes	214	31.3%	68	20.2%	146	42.1%	<0.01
No	469	68.7%	268	79.8%	201	57.9%	
Alive after 60 months							
Yes	234	31.0%	106	31.5%	128	30.5%	0.75
No	522	69.0%	230	68.5%	292	69.5%	

Variable	Combined (N=756)		Rotterdam (N=336)		OCCAMS (N=420)		P-value
SOX2							
Negative	436	66.1%	181	57.1%	255	74.3%	<0.01
Positive	224	33.9%	136	42.9%	88	25.7%	

Association between SOX2 expression and survival

The interobserver agreement for the assessment of SOX2 immunohistochemistry between the two observers was excellent (kappa-value= 0.92; p<0.001). After exclusion of patients with less than 3 representative cores available for the SOX2 evaluation, 539 patients remained from the total of 756 patients for the final analysis of SOX2 immunohistochemistry (288 in Rotterdam cohort and 251 in the OCCAMS cohort). In total, SOX2 was positive in 186 EAC while 351 EAC were SOX2 negative.

In the Rotterdam cohort, negative SOX2 was associated with a shorter median OS compared to patients with positive SOX2: 19.5 versus 32.9 months (p<0.01). Survival in the OCCAMS cohort was similar to the Rotterdam cohort with a median survival of 15.0 months in SOX2 negative versus 26.0 months (p<0.01) in SOX2 positive tumors (supplemental Table 1). Corresponding Kaplan-Meier curves of both cohorts separately and the combined group are depicted in Figure 1.

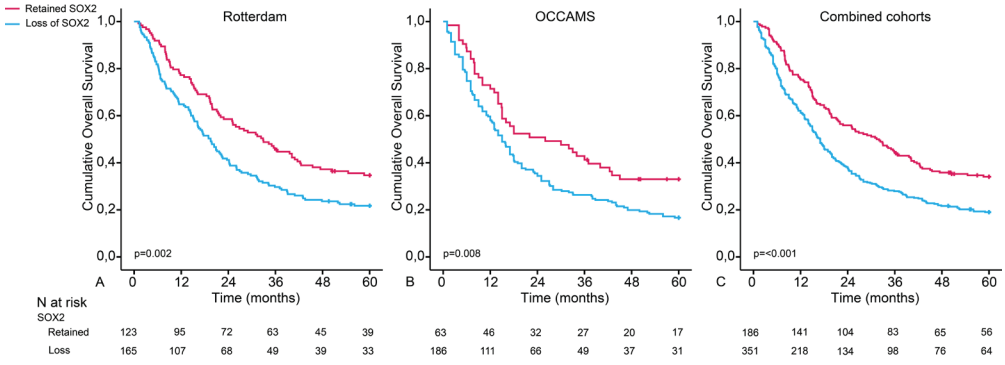

Figure 1: Expression of SOX2 is prognostic for overall survival; Rotterdam cohort (A), OCCAMS cohort (B) and combined cohort (C) (p-values are indicated in the left lower corner of each graph).

Next, univariate analysis was performed according to the Cox proportional hazard ratio model which demonstrated HR for death in patients with SOX2 loss of 1.54 (95% CI 1.16-2.04, p=0.003) for the Rotterdam cohort, HR of 1.58 (95% CI 1.12-2.22, p=0.009) for the OCCAMS cohort and HR of 1.55 (95% CI 1.25-1.93, p<0.001) for the combined cohort.

SOX2 is independent of conventional clinico-pathological parameters for patient prognosis

Multivariate Cox-regression analysis was performed in the Rotterdam, OCCAMS and combined cohort to test the independent value of SOX2 in relation to other clinical parameters. SOX2 remained significant for OS, as summarized in Table 2, in both cohorts separately as well as in the combined cohort (HR=1.42, 95% CI 1.14-1.77, p=0.002).
Information about the DFS was only available in the Rotterdam cohort. Here, SOX2 was independently predictive for recurrence of the disease with a HR of 1.37 (95% CI 1.01-1.86, p= 0.045) in a multivariate analysis (see Table 2). Chemotherapy-naïve patients were selected for further sub-analysis, in which SOX2 loss was confirmed as statistically significant prognostic indicator for worse OS (Table 3).

Table 2. Multivariate survival analysis, for all patients (specified in Rotterdam and OCCAMS cohort). Positive SOX2 expression was used as reference.

SOX2 (positive ref)		HR	95% CI	P-value	N
Combined cohort	OS	1.42	1.14-1.77	0.002	402
Rotterdam	OS	1.42	1.07-1.89	0.016	287
	DFS	1.37	1.01-1.86	0.045	287
OCCAMS	OS	1.54	1.08-2.19	0.017	115

HR=Hazard ratio, CI=Confidence Interval. OS=Overall Survival, DFS=Disease Free Survival.

SOX2 loss predicts worse outcome in chemotherapy-naïve patients with stage I EAC

Next, we evaluated the prognostic value of SOX2 in chemotherapy naïve patients in relation to the clinic-pathological staging. SOX2 showed segregation in prognostic groups in pT1/pT2 tumors (HR=2.36, 95% CI 1.23-4.51, p=0.01) but not in pT3/pT4 tumors (Figure 2a, Table 3). When combining SOX2 with the nodal status, patients with pT1 EAC and loss of SOX2 had a trend for pN+ (p=0.070). For pT2-pT4 tumors no correlation of SOX2 and nodal status was

found (data not shown). When combining SOX2 and pN-stage, a significant segregation into prognostic groups was detected in pN0-patients (HR=1.71, 95% CI 1.03-2.85, p=0.038) while in pN1-pN3 patients no effect of SOX2 was visible (Table 3 and Figure 2b).

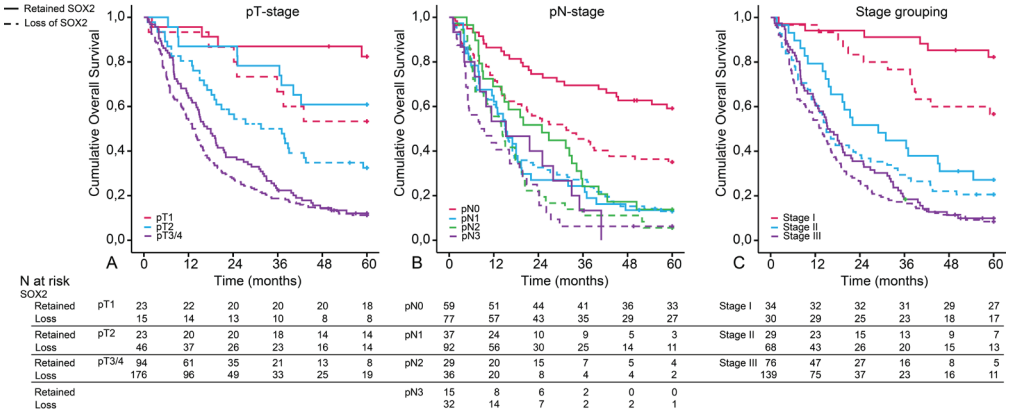

Figure 2. SOX2 expression in combination to clinico-pathological staging (a: pT-stage, b: pN-stage and c: stage groupings) segregates chemotherapy naïve patients, into prognostic groups in early EAC (pT1/pT2 tumors, pN-tumors and stage I tumors, p<0.05).

Based on the findings on the pT- and pN-stage, Kaplan-Meier curve was constructed for the differentiating effects of SOX2 for each of the stage groupings as mentioned in the TNM classification, in which the sub-stages were combined in stage I, stage II and stage III. Here a differentiating effect for stage I was found only, with HR a for death of 3.18 (95% CI 1.18-8.56, p=0.022) (Table 3 and Figure 2c).

Table 3. Multivariate survival analysis for SOX2-expression in chemotherapy naïve patient, for all patients and specified for pT1/pT2-tumors, pN0-tumors and stage I-tumors. Positive SOX2 expression is used as reference.

SOX2 (positive ref)	HR	95% CI	P-value	N
Overall	1.35	1.04-1.75	0.026	297
pT1/2	2.36	1.23-4.51	0.010	105
pN0	1.71	1.03-2.84	0.038	112
Stage I	3.18	1.18-8.56	0.022	64

HR=Hazard ratio, CI=Confidence Interval.

Discussion

In the present study we demonstrated that SOX2 immunohistochemistry adds to the prognostication of patients with EAC. SOX2 loss was predictive for adverse outcome in both of the independent cohorts (Rotterdam and OCCAMS) with significant incremental adverse effect for OS, especially in pN0 and stage I EAC.

Besides prediction of response to neoadjuvant treatment, improved early detection and individualized targeted treatment, prognostication is an important current challenge in the clinical management of patients with EAC. Earlier studies attempted to identify clinically applicable predictive biomarkers for treatment response and overall prognosis, but most studies were underpowered [31] or included heterogeneous patient populations with squamous cell carcinoma and adenocarcinoma which have different biology [32]. Furthermore, biomarker analysis might be hampered by different neoadjuvant regimens used for treatment of advanced EAC making the comparison between relevant studies difficult [33]. Large collaborative projects using standardized methodology are required to generate a clinically useful approach. Using this strategy, a three-gene immunohistochemical panel was previously shown to be useful in a large multicenter study [34]. Hereby, combining TNM staging with this immunohistochemical panel of EGFR, TRIM44 and SIRT2 allowed segregation of patients with stages II and III disease into distinct prognostic groups, while the effect in stage I was minimal [34]. This is different from the SOX2 findings reported here.

The transcription factor SOX2 not only plays an essential role in the embryological formation of the stomach and esophagus [18], but is also involved in pathogenesis of gastric [35, 36] and esophageal (squamous cell) carcinoma [19]. Little is known so far about the role of SOX2 in EAC and BE. We previously detected gradually decreased SOX2 expression in low- and high grade dysplasia of patients with BE which is possibly related to the loss of epithelial identity during neoplastic BE progression [25]. In advanced EAC, retained expression of SOX2 has previously been related to resistance to neoadjuvant chemo-radiation therapy in patients treated according to the CROSS-regime [26, 27]. Also, an earlier small Dutch study on 94 patients with surgically treated EAC suggested SOX2 loss to be a predictor of impaired DFS but was underpowered to establish incremental value of SOX2 for OS [28]. In the present study we focused on surgically treated EAC and analyzed immunohistochemical SOX2 expression in relation to the clinic-pathological

parameters and OS in two well-characterized and independent EAC cohorts. In fact, we could not only confirm prognostic value of SOX2 for the DFS (HR 1.37, p=0.045) but also showed for the first time that SOX2 loss predicts adverse OS in patients with EAC. Importantly, SOX2 status was independent of all clinical and histological parameters known to influence survival including neoadjuvant treatment (HR 1.42, p=0.002).

Among all patients with EAC, patients with stage I disease are in a prognostic favorable group with a 5-years survival of 87.7% and 73.3% for stage Ia and Ib respectively [37]. In these patients prediction of survival is difficult while numerous treatment modalities are available, including endomucosal resection and surgical treatment, with or without neoadjuvant treatment. At this moment the optimal treatment strategy for patients with stage I EAC is not known because of the increased but highly variable risk of lymph-node metastasis [38]. Also, the beneficial effects of neoadjuvant therapy in these patients are not clear [39]. In the present study we showed an adverse incremental value for OS in chemotherapy-naive patients with stage I EAC with loss of SOX2 (HR 3.18, p=0.022). Furthermore, our results suggest that SOX2 might predict lymph node metastases in pT1 EAC, however, further studies, preferably in specific target group of patients with pT1b EAC would be valuable to confirm this finding.

The role of SOX2 in the pathogenesis of EAC is still poorly understood. Significant association of retained SOX2 expression and favorable survival could be explained by SOX2 function as a tumor suppressor gene in parallel to the findings in gastric carcinoma. Lower mitotic rate, increased apoptosis and reduced invasion and dissemination were detected in gastric cancer with retained SOX2 expression compared to those with SOX2 loss [36, 40, 41]. In line with its tumor suppressive role, several downstream targets of SOX2 were identified in gastric cancer including CCND1, pRB1, CDKN1B as wells as PTEN and pAKT [36, 41, 42]. Given the lineage specific SOX2 function in formation of the stomach and esophagus during embryogenesis, the role of SOX2 in EAC might be similar to that in gastric cancer.

The current study has some limitations including its retrospective design and the fact that since we included all patients with surgically resected EAC, the subgroup of stage I tumors was limited. Another limitation is that we tested the expression of SOX2 on TMAs constructed from resection specimens and not in preoperative biopsies of patients with EAC. Further validation of our results in the prospective setting and on preoperative tumor material would therefore

be valuable. Another potential bias is the SOX2 immunohistochemistry and interpretation of the results. SOX2 detection in this study was performed by standardized immunohistochemical technique which is easily reproducible in the pathology laboratory. However, because the interpretation might be subjective, all SOX2 stained TMAs were scored by two investigators, showing an excellent interobserver agreement (kappa 0.92), indicating that accurate classification of SOX2 pattern is possible in general practice.

In conclusion, our study shows that immunohistochemical detection of SOX2 provides prognostic information in patients with EAC independently of clinical parameters. Using this marker in addition to the current staging systems could be of particular relevance in selected populations of nodal-negative tumors and stage I EAC. Identification of patients with absent SOX2 expression and hence a worse prognosis compared to tumors with retained SOX2 might prompt for a change in patient management. Since SOX2 loss is indicative for a more aggressive tumor biology, a more extensive therapy might be justified for patients with stage I EAC and loss of SOX2 expression. The precise biological role of SOX2 in EAC requires further elucidation.

Reference

1 Rubenstein JH, Shaheen NJ. Epidemiology, Diagnosis, and Management of Esophageal Adenocarcinoma. Gastroenterology 2015;149:302-17 e1.

2 Bosetti C, Levi F, Ferlay J, Garavello W, Lucchini F, Bertuccio P, et al. Trends in oesophageal cancer incidence and mortality in Europe. Int J Cancer 2008;122:1118-29.

3 Lagergren J, Bergstrom R, Lindgren A, Nyren O. Symptomatic gastroesophageal reflux as a risk factor for esophageal adenocarcinoma. N Engl J Med 1999;340:825-31.

4 Singh S, Sharma AN, Murad MH, Buttar NS, El-Serag HB, Katzka DA, et al. Central adiposity is associated with increased risk of esophageal inflammation, metaplasia, and adenocarcinoma: a systematic review and meta-analysis. Clin Gastroenterol Hepatol 2013;11:1399-412.

5 Desai TK, Krishnan K, Samala N, Singh J, Cluley J, Perla S, et al. The incidence of oesophageal adenocarcinoma in non-dysplastic Barrett's oesophagus: a meta-analysis. Gut 2012;61:970-6.

6 Hvid-Jensen F, Pedersen L, Drewes AM, Sorensen HT, Funch-Jensen P. Incidence of adenocarcinoma among patients with Barrett's esophagus. N Engl J Med 2011;365:1375-83.

7 Thomas T, Abrams KR, De Caestecker JS, Robinson RJ. Meta analysis: Cancer risk in Barrett's oesophagus. Aliment Pharmacol Ther 2007;26:1465-77.

8 Aida J, Vieth M, Shepherd NA, Ell C, May A, Neuhaus H, et al. Is carcinoma in columnar-lined esophagus always located adjacent to intestinal metaplasia?: a histopathologic assessment. Am J Surg Pathol 2015;39:188-96.

9 van Hagen P, Hulshof MC, van Lanschot JJ, Steyerberg EW, van Berge Henegouwen MI, Wijnhoven BP, et al. Preoperative chemoradiotherapy for esophageal or junctional cancer. N Engl J Med 2012;366:2074-84.

10 van der Woude SO, Hulshof MC, van Laarhoven HW. CROSS and beyond: a clinical perspective on the results of the randomized ChemoRadiotherapy for Oesophageal cancer followed by Surgery Study. Chin Clin Oncol 2016;5:7.

11 Shapiro J, van Lanschot JJ, Hulshof MC, van Hagen P, van Berge Henegouwen MI, Wijnhoven BP, et al. Neoadjuvant chemoradiotherapy plus surgery versus surgery alone for oesophageal or junctional cancer (CROSS): long-term results of a randomised controlled trial. Lancet Oncol 2015;16:1090-8.

12 Sobin L.H. GMK, Wittekind C. TNM Classification of Malignant Tumours, 7th Edition: Wiley-Blackwell, 2010.

13 O'Neill JR, Stephens NA, Save V, Kamel HM, Phillips HA, Driscoll PJ, et al. Defining a positive circumferential resection margin in oesophageal cancer and its implications for adjuvant treatment. Br J Surg 2013;100:1055-63.

14 Schoppmann SF, Jesch B, Zacherl J, Riegler MF, Friedrich J, Birner P.

Lymphangiogenesis and lymphovascular invasion diminishes prognosis in esophageal cancer. Surgery 2013;153:526-34.

15 Yendamuri S, Huang M, Malhotra U, Warren GW, Bogner PN, Nwogu CE, et al. Prognostic implications of signet ring cell histology in esophageal adenocarcinoma. Cancer 2013;119:3156-61.

16 Wegner M. From head to toes: the multiple facets of Sox proteins. Nucleic Acids Res 1999;27:1409-20.

17 Liu K, Lin B, Zhao M, Yang X, Chen M, Gao A, et al. The multiple roles for Sox2 in stem cell maintenance and tumorigenesis. Cell Signal 2013;25:1264-71.

18 Que J, Okubo T, Goldenring JR, Nam KT, Kurotani R, Morrisey EE, et al. Multiple dose-dependent roles for Sox2 in the patterning and differentiation of anterior foregut endoderm. Development 2007;134:2521-31.

19 Wang Q, He W, Lu C, Wang Z, Wang J, Giercksky KE, et al. Oct3/4 and Sox2 are significantly associated with an unfavorable clinical outcome in human esophageal squamous cell carcinoma. Anticancer Res 2009;29:1233-41.

20 Zhang X, Yu H, Yang Y, Zhu R, Bai J, Peng Z, et al. SOX2 in gastric carcinoma, but not Hath1, is related to patients' clinicopathological features and prognosis. J Gastrointest Surg 2010;14:1220-6.

21 Jia X, Li X, Xu Y, Zhang S, Mou W, Liu Y, et al. SOX2 promotes tumorigenesis and increases the anti-apoptotic property of human prostate cancer cell. J Mol Cell Biol 2011;3:230-8.

22 Neumann J, Bahr F, Horst D, Kriegl L, Engel J, Luque RM, et al. SOX2 expression correlates with lymph-node metastases and distant spread in right-sided colon cancer. BMC Cancer 2011;11:518.

23 Hussenet T, Dali S, Exinger J, Monga B, Jost B, Dembele D, et al. SOX2 is an oncogene activated by recurrent 3q26.3 amplifications in human lung squamous cell carcinomas. PLoS One 2010;5:e8960.

24 Bass AJ, Watanabe H, Mermel CH, Yu S, Perner S, Verhaak RG, et al. SOX2 is an amplified lineage-survival oncogene in lung and esophageal squamous cell carcinomas. Nat Genet 2009;41:1238-42.

25 van Olphen S, Biermann K, Spaander MC, Kastelein F, Steyerberg EW, Stoop HA, et al. SOX2 as a novel marker to predict neoplastic progression in Barrett's esophagus. Am J Gastroenterol 2015;110:1420-8.

26 van Olphen SH, Biermann K, Shapiro J, Wijnhoven BP, Toxopeus EL, van der Gaast A, et al. P53 and SOX2 protein expression predicts esophageal adenocarcinoma in response to neoadjuvant chemoradiotherapy. Ann Surg 2016.

27 Honing J, Pavlov KV, Mul VE, Karrenbeld A, Meijer C, Faiz Z, et al. CD44, SHH

and SOX2 as novel biomarkers in esophageal cancer patients treated with neoadjuvant chemoradiotherapy. Radiother Oncol 2015;117:152-8.

28 Honing J, Pavlov KV, Meijer C, Smit JK, Boersma-van Ek W, Karrenbeld A, et al. Loss of CD44 and SOX2 expression is correlated with a poor prognosis in esophageal adenocarcinoma patients. Ann Surg Oncol 2014;21 Suppl 4:S657-64.

29 De Bruijn K, Biermann K, Shapiro J, Dogan F, Spaander M, Janssen J, et al. Absence or low IGF-1R-expression in esophageal adenocarcinoma is associated with tumor invasiveness and radicality of surgical resection. J Surg Oncol 2015;111:1047-53.

30 Peters CJ, Rees JR, Hardwick RH, Hardwick JS, Vowler SL, Ong CA, et al. A 4-gene signature predicts survival of patients with resected adenocarcinoma of the esophagus, junction, and gastric cardia. Gastroenterology 2010;139:1995-2004.

31 McCormick Matthews LH, Noble F, Tod J, Jaynes E, Harris S, Primrose JN, et al. Systematic review and meta-analysis of immunohistochemical prognostic biomarkers in resected oesophageal adenocarcinoma. Br J Cancer 2015;113:107-18.

32 Gowryshankar A, Nagaraja V, Eslick GD. HER2 status in Barrett's esophagus & esophageal cancer: a meta analysis. J Gastrointest Oncol 2014;5:25-35.

33 Noble F, Nolan L, Bateman AC, Byrne JP, Kelly JJ, Bailey IS, et al. Refining pathological evaluation of neoadjuvant therapy for adenocarcinoma of the esophagus. World J Gastroenterol 2013;19:9282-93.

34 Ong CA, Shapiro J, Nason KS, Davison JM, Liu X, Ross-Innes C, et al. Three-gene immunohistochemical panel adds to clinical staging algorithms to predict prognosis for patients with esophageal adenocarcinoma. J Clin Oncol 2013;31:1576-82.

35 Tian T, Zhang Y, Wang S, Zhou J, Xu S. Sox2 enhances the tumorigenicity and chemoresistance of cancer stem-like cells derived from gastric cancer. J Biomed Res 2012;26:336-45.

36 Wang S, Tie J, Wang R, Hu F, Gao L, Wang W, et al. SOX2, a predictor of survival in gastric cancer, inhibits cell proliferation and metastasis by regulating PTEN. Cancer Lett 2015;358:210-9.

37 Talsma K, van Hagen P, Grotenhuis BA, Steyerberg EW, Tilanus HW, van Lanschot JJ, et al. Comparison of the 6th and 7th Editions of the UICC-AJCC TNM Classification for Esophageal Cancer. Ann Surg Oncol 2012;19:2142-8.

38 Gamboa AM, Kim S, Force SD, Staley CA, Woods KE, Kooby DA, et al. Treatment allocation in patients with early-stage esophageal adenocarcinoma: Prevalence and predictors of lymph node involvement. Cancer 2016;122:2150-7.

39 Lutz MP, Zalcberg JR, Ducreux M, Ajani JA, Allum W, Aust D, et al. Highlights of the EORTC St. Gallen International Expert Consensus on the primary therapy of gastric, gastroesophageal and oesophageal cancer - differential treatment strategies for subtypes of

early gastroesophageal cancer. Eur J Cancer 2012;48:2941-53.

40 Chen Y, Huang Y, Zhu L, Chen M, Huang Y, Zhang J, et al. SOX2 inhibits metastasis in gastric cancer. J Cancer Res Clin Oncol 2016.

41 Otsubo T, Akiyama Y, Yanagihara K, Yuasa Y. SOX2 is frequently downregulated in gastric cancers and inhibits cell growth through cell-cycle arrest and apoptosis. Br J Cancer 2008;98:824-31.

42 Zhang X, Hua R, Wang X, Huang M, Gan L, Wu Z, et al. Identification of stem-like cells and clinical significance of candidate stem cell markers in gastric cancer. Oncotarget 2016.

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General discussion

S.H. van Olphen

Introduction

In order to detect high-grade dysplasia (HGD) or esophageal adenocarcinoma (EAC) at an early stage when curative treatment is still feasible, endoscopic surveillance is recommended in patients with Barrett's esophagus (BE). In the Netherlands, recommendations for BE surveillance are based on the guidelines of the American College of Gastroenterology, which recommend endoscopic surveillance with biopsy sampling every 3-5 years for BE without dysplasia, endoscopic surveillance every 6-12 months for BE with low-grade dysplasia (LGD), and (endoscopic) treatment for patients with established HGD or EAC [1, 2, 3, 4]. However, the value of endoscopic BE surveillance (based on histological diagnosis alone) has been under discussion given the overall low incidence of neoplastic progression, lack of discriminative tests for adequate risk stratification and limited evidence that surveillance prevent advanced EAC and improves survival. Initially, the estimated incidence of EAC in patients with BE was believed to be between 0.5 and 1% per year [5, 6, 7, 8]. However, more recently population-based studies and two meta-analyses have set this risk around 0.12% to 0.38% per year [9, 10, 11, 12]. These relatively low annual risk values reinforce the need for better risk stratification tools in BE patients to make BE surveillance truly cost-effective. The aim of this thesis was to investigate whether biomarkers can contribute to improved risk stratification in BE in order to optimize surveillance strategies and to evaluate the effectiveness of surveillance according to the current guidelines in terms of cost-effectiveness and survival. In addition, we investigated the value of biomarkers on prediction of prognosis and therapy response in EAC patients.

Biomarkers in Barrett's esophagus surveillance

Nowadays, histological diagnosis of LGD is used for the risk assessment of neoplastic progression in BE surveillance and more intensive follow-up is recommended in LGD patients (yearly instead of every 3 years) [1, 2, 13]. However, the histological diagnosis of LGD has a low predictive value, owing to sample error and a considerable inter- and intraobserver variation [14, 15, 16]. Curvers et al. demonstrated that when LGD diagnosed by community hospital pathologist was reviewed by a panel of expert gastro-intestinal pathologists, 85% was downstaged. Patients with a confirmed diagnosis of LGD had a markedly increased annual progression risk of 9% versus the patients whose diagnosis of LGD was downstaged (0.9%). So in our opinion

all patients diagnosed with LGD should undergo revision of the histological diagnosis by an expert panel [14]. The use of (a panel of) objective biomarkers (in addition to histology) may improve risk stratification in all BE patients. Many immunohistochemical (IHC) biomarkers have been studied in BE progression mainly because the ability to directly visualize stains as applied to intact histological morphology. Besides, IHC is relatively cheap and easy applicable to clinical practice compared to other techniques. Currently, p53 IHC staining is only recommended in the guideline of the British Society of Gastroenterology to improve the diagnostic reproducibility of the histological diagnosis of dysplasia [1]. However, thus far in routine clinical care, neither p53 nor other IHC biomarkers are used as predictors of neoplastic progression. We conducted a systematic review and meta-analyses to investigate the value of different IHC biomarkers for predicting neoplastic progression in BE, it showed that only four IHC biomarkers have been investigated more than once. The biomarker investigated most frequently is p53 which was assessed in 12 studies, totaling 2023 patients. The meta-analysis showed that aberrant p53 expression is associated with neoplastic progression with an OR of 4.15 (95% CI 1.96 to 8.81) and is predictive of progression in patients with both non-dysplastic BE and BE with LGD. IHC p53 staining in addition to the histological diagnosis can be implemented to improve risk stratification in BE surveillance, but consensus formation amongst pathologists concerning the appropriate staining method and cut-off value is necessary. Our research group previously reported in a large case-control study that aberrant p53 expression was significantly associated with an increased risk of neoplastic progression in BE. However, only 40% of the BE patients with progression to HGD or EAC showed an aberrant p53 protein expression during surveillance, indicating that additional biomarkers are needed [17].

A potential biomarker for BE progression is the transcription factor SOX2 (sex determining region Y-box2), a highly conserved single exon gene which plays a pivotal role in the maintenance of embryonic stem cells and also determines formation and differentiation of esophageal and gastric epithelium during embryogenesis [18, 19, 20]. SOX2 is involved in various malignancies including squamous cell carcinoma of the esophagus, gastric adenocarcinoma, as well as colorectal cancer [21, 22, 23]. In cancer, SOX2 functions are cell-dependent and oncogenic as well as tumor suppressive mechanisms have been described. Using IHC, SOX2 protein was shown to be progressively downregulated in intestinal metaplasia and adjacent gastric cancer [24, 25]. Recent studies

in gastric cancer cells revealed a role of SOX2 in growth inhibition through cell-cycle arrest and induction of apoptosis, indicating cancer-suppressive functions [26]. However, the predictive value of SOX2 in BE and its expression during the metaplasia-dysplasia-adenocarcinoma sequence is largely unknown. Another promising biomarker is cyclin A, a protein that plays an important role in the G1-S transition of the cell cycle. The results of previous studies evaluating the value of cyclin A expression for predicting neoplastic progression in BE are conflicting. A relative small case-control study (48 patients) showed that cyclin A surface expression was significantly associated with an increased risk of neoplastic progression (OR 7.6; 95% CI 1.6 to 37.0), whereas a more recent larger population-based study (380 patients) could not confirm this correlation and only found a trend towards an increased risk of progression (OR 1.32; 95% CI 0.66 to 2.66) [27, 28]. The value of cyclin A has not yet been investigated in large prospective studies. In addition, there is a lack of studies testing performance of multiple biomarker simultaneously in the same cohort of BE patient. To investigate the predictive value of SOX2 and cyclin A we performed a case-control study within a large multicenter prospective cohort of 720 BE patients (Probar cohort) and combined these results with our previously reported p53 and AMACR immunohistological data in the same cohort, to identify an informative panel of biomarkers for predicting neoplastic progression in BE. SOX2 and cyclin A protein expression was evaluated in more than 12.000 biopsies from 625 patients.

SOX2 expression was progressively lost in dysplastic BE and was seen in only 2% of biopsy series without dysplasia, in contrast to 28% in LGD and 67% in HGD or EAC. Loss of SOX2 expression was significantly associated with an almost 5-fold increased risk of neoplastic progression, independent of age, gender, BE length and esophagitis. Also Cyclin A surface positivity significantly increased throughout the metaplasia-dysplasia-carcinoma progression steps and was associated with a 2-fold increased risk of neoplastic progression. However, in a fully adjusted model (including histological diagnosis and the biomarkers p53, SOX2, cyclin A and AMACR), aberrant p53 expression showed the highest change in AUC (0.05) after exclusion, to a lesser extent aberrant SOX2 expression (0.014) and histological diagnosis of LGD (0.005). The biomarkers cyclin A only showed a minimal drop in AUC after exclusion (0.003). The highest predictive value was achieved by concurrent loss of SOX2 expression and aberrant p53 expression in BE patients with LGD. The incremental value of cyclin A was limited. We have also shown good interobserver agreement

for the assessment of SOX2 expression and p53 expression, which indicates that both markers are clinically suitable markers to predict progression in BE. Use of these markers could significantly improve risk stratification and has the potential to improve cost-effectiveness of BE surveillance. In daily routine practice, these biomarkers would not necessarily replace current clinical and histological variables, but it is envisaged that a panel of biomarker, combined with relevant clinical and histological factors may improve our ability to objectively assess a patient's individual risk. Although routine p53 and SOX2 staining incur higher costs than histology alone, application of this panel of biomarkers has the potential to reduce the overall costs of Barrett surveillance. Patients at low-risk of neoplastic progression, i.e. the majority of the patients with LGD, might be follow-up less intensively with the potential to eventually discharge them. However, a more detailed cost-effectiveness analysis should be performed to evaluate the economic value of p53 and SOX2 IHC.

Cost-effectiveness Barrett surveillance

BE patients have a higher risk of developing EAC compared to the general population, but the absolute risk of neoplastic progression is relatively low. One of the key questions in the discussion about BE surveillance is whether is it cost-effective. Previous studies investigating the cost-effectiveness of BE surveillance have shown variable results. Surveillance was reported to be cost-effective in four studies with surveillance intervals ranging from two to five years [29, 30, 31, 32]. However, in four other studies surveillance was not cost-effective with sometimes even higher costs and less quality of life than without surveillance [33, 34, 35, 36]. These highly variable results were mainly due to different assumptions about progression rates and quality of life associated with different health states. In most studies, the incidence of EAC was estimated based on pooled literature data and esophagectomy was performed in case of both HGD and EAC. Importantly, over the past years there has been a major shift in the treatment of BE patients with the introduction of endoscopic treatment modalities such as endoscopic mucosal resection (EMR) and radiofrequency ablation (RFA) for patients with HGD or early EAC. Esophagectomy is still the cornerstone for curative treatment of advanced EAC, but is nowadays combined with neoadjuvant chemoradiotherapy (nCRT) [37, 38]. To investigate the cost-effectiveness of BE surveillance according to the current guidelines, we performed a cost-effectiveness analysis within a large multicenter prospective cohort of 714 patients with BE. A multi-

state-Markov model was used to calculate progression rates based on the prospective follow-up data. These progression rates were incorporated in a decision-analytic model, including costs and quality of life data. We evaluated different surveillance intervals and different therapeutic modalities for BE without dysplasia and LGD. The incremental cost-effectiveness ratio (ICER) was calculated in costs per quality-adjusted life year (QALY) gained. Assuming a willingness-to-pay threshold of €35.000 per QALY, surveillance with EMR and RFA for HGD or early EAC, and esophagectomy for advanced EAC is cost-effective every 5-years for no dysplasia and every 3-years for LGD. For patients with ND, the results of our study correspond to recommendations made in current guidelines [39, 40]. For patients with LGD, surveillance is recommended with intervals of six to twelve months, while according to our study intervals should be at least three years in order to be cost-effective. Identification and incorporation of additional biomarkers/risk factors besides histological diagnosis of LGD may improve risk stratification and eventually cost-effectiveness of Barrett surveillance with shorter intervals.

Survival

Another key element in the discussion of Barrett surveillance is whether surveillance is able to reduce the occurrence of advanced EAC and improves survival. These key questions have been investigated in case-control studies, population-based studies and small prospective cohort studies with conflicting results [41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56]. Most studies have shown that patients diagnosed with EAC in BE surveillance programs have earlier-stage tumors and better survival rates than those diagnosed with EAC after the onset of symptoms. In contrast, some other studies reported no effect on mortality [56]. However, in these studies BE patients were not under strict surveillance, which is crucial for the detection of early EAC. For this purpose, we evaluated the impact of endoscopic surveillance on tumor stage and survival, of patients with BE of at least two centimeters according to current guidelines and incorporating of the new (endoscopic) treatment modalities in case of HGD or EAC, within a large prospective cohort of 783 BE patients. During surveillance incident cases of HGD or EAC were identified. Survival data were collected and cross-checked using death and municipal registries and compared to data of patients with EAC in the general Dutch population. Thirty-three BE patients developed HGD or EAC during surveillance, which was diagnosed at a significantly earlier stage than in the general population

(P<0.001). Survival of BE patients with neoplastic progression diagnosed during surveillance was not significantly worse than those of patients without progression (HR 1.8, 95% CI 0.9-3.3), and was similar to those of patients with stage 0 or stage 1 EAC in the general population (HR 0.8, 95% CI 0.3-1.8 and HR 0.7, 95% CI 0.4-1.2). This study suggests that regular endoscopic surveillance of BE patients enables the detection of EAC at an early and curable stage when endoscopic treatment is still feasible and leads to good survival. The results of this study therefore support current guidelines recommending endoscopic surveillance in patients with BE. Although a randomized controlled trial would be the ideal way to investigate the effect of endoscopic BE surveillance on survival, but ethically impossible.

Biomarker in Esophageal adenocarcinoma (EAC)

Prediction of response to neoadjuvant treatment, improved early detection and prognostication, as well as individualized targeted treatment are the current challenges in the clinical management of EAC patients. Earlier studies attempted to identify clinically applicable prognostic biomarkers for treatment response and overall prognosis in EAC but most of the performed studies were underpowered or consisted of highly heterogeneous patient population with squamous carcinoma and adenocarcinoma, and lacked standardized interpretation of immunohistochemical staining [57, 58, 59, 60, 61, 62]. Furthermore, biomarker analysis in EAC patients might be obscured by various neoadjuvant regimens used for advanced EAC making the comparison between studies difficult[63]. Large (collaborative) projects using standardized methodology are required to generate clinically useful approaches. Therefore, we investigate the association between p53, SOX2 and CD44 protein expression in pretreatment tumor biopsies and tumor response in a primary cohort (77 patients), and validated these result in a second independent cohort of EAC patients (70 patients). In a subsequent study, we evaluated the prognostic value of SOX2 in two independent cohorts after assessment of tissue micro arrays from resected EAC specimens of in total 756 patients. Expression of p53 but in particularly SOX2 was significantly associated with response to neo-adjuvant chemo-radiotherapy (nCRT). Loss of SOX2 expression indicates a high probability of a major response to nCRT with an adjusted OR of 4.1 (95%CI 1.4–12.4) in the primary cohort and an adjusted of OR 6.1 (95%CI 1.6-23.4) in the validation cohort, respectively. SOX2 expression was not only predictive for therapy response but also improved prognostication in patients

undergoing resection of EAC. We showed that loss of SOX2 is an independent predictor of adverse outcome with median survival of 19.7 months after 5 years in contrast to 31.7 months in patients with retained SOX2 expression. Importantly, most significant effects of SOX2 for predicting prognosis were detected in pT1 (HR of 3.53, p=0.018) and pN0 (HR of 1.61, p<0.001) tumors in our study.

These finding might have clinical impact and could be of particular relevance in selected populations of patients with pT1pN0 tumors. Identification of poor prognostic group among these otherwise good-prognosis patients could lead to a choice of more intensive surveillance or adjustment of (neo)adjuvant treatment. Hence, SOX2 could identify pT1b patients who have a high risk of disease recurrence, and at the same time could profit the most from nCRT. Since the prediction of prognosis is very limited in pT1b EAC, SOX2 may assist in clinical decision-making in this group of patients.

On the molecular level, our clinical findings might be explained by the differentially regulated SOX2 targets [26, 64, 65, 66]. SOX2 loss might lead to loss of PTEN and upregulation of pAKT, with decrease of sensitivity to apoptosis of the tumor cells, while the mitotic activity, invasive growth and metastatic capability are increased, a mechanism previously identified in gastric cancer cells. Given the lineage specific SOX2 function in formation of stomach and esophagus during embryogenesis, the SOX2 role in EAC might be similar to that observed in gastric adenocarcinoma. However, further clinical and fundamental studies would be valuable to confirm our findings and to establish cellular SOX2 functions in EAC.

Conclusions

BE surveillance might enable the detection of EAC at an early and curable stage when endoscopic treatment with EMR and RFA is still feasible, and which eventually leads to improves survival. Although surveillance according to the current guidelines is cost-effective for patients without dysplasia, for patients with LGD, surveillance is recommended with intervals of six to twelve months, while according to our study intervals should be at least three years in order to be cost-effective. Identification and incorporation of additional biomarkers may improve risk stratification and eventually cost-effectiveness of BE surveillance. A panel of biomarkers with p53 and SOX2 immunohistochemistry appears to be more predictive than the histological diagnosis of LGD alone. P53 and SOX2 IHC have a good interobserver

agreement, are relatively cheap and easy applicable to clinical practice compared to other techniques, which makes them clinically suitable markers. SOX2 expression is not only predictive for neoplastic progression in BE but also might improve prognostication in patients undergoing resection for EAC and is significantly associated with therapy response in potential curable EAC patients. SOX2 may help to individualize (treatment) decision-making in EAC patients.

Reference

1 Fitzgerald RC, di Pietro M, Ragunath K, Ang Y, Kang JY, Watson P, et al. British Society of Gastroenterology guidelines on the diagnosis and management of Barrett's oesophagus. Gut 2014;63:7-42.

2 Wang KK, Sampliner RE, Practice Parameters Committee of the American College of G. Updated guidelines 2008 for the diagnosis, surveillance and therapy of Barrett's esophagus. Am J Gastroenterol 2008;103:788-97.

3 Sampliner RE, Practice Parameters Committee of the American College of G. Updated guidelines for the diagnosis, surveillance, and therapy of Barrett's esophagus. The American journal of gastroenterology 2002;97:1888-95.

4 Spechler SJ, Sharma P, Souza RF, Inadomi JM, Shaheen NJ, American Gastroenterological A. American Gastroenterological Association technical review on the management of Barrett's esophagus. Gastroenterology 2011;140:e18-52; quiz e13.

5 Reid BJ, Levine DS, Longton G, Blount PL, Rabinovitch PS. Predictors of progression to cancer in Barrett's esophagus: baseline histology and flow cytometry identify low- and high-risk patient subsets. The American journal of gastroenterology 2000;95:1669-76.

6 Sikkema M, de Jonge PJ, Steyerberg EW, Kuipers EJ. Risk of esophageal adenocarcinoma and mortality in patients with Barrett's esophagus: a systematic review and meta-analysis. Clin Gastroenterol Hepatol 2010;8:235-44; quiz e32.

7 Yousef F, Cardwell C, Cantwell MM, Galway K, Johnston BT, Murray L. The incidence of esophageal cancer and high-grade dysplasia in Barrett's esophagus: a systematic review and meta-analysis. American journal of epidemiology 2008;168:237-49.

8 Hage M, Siersema PD, van Dekken H, Steyerberg EW, Dees J, Kuipers EJ. Oesophageal cancer incidence and mortality in patients with long-segment Barrett's oesophagus after a mean follow-up of 12.7 years. Scandinavian journal of gastroenterology 2004;39:1175-9.

9 Bhat S, Coleman HG, Yousef F, Johnston BT, McManus DT, Gavin AT, et al. Risk of malignant progression in Barrett's esophagus patients: results from a large population-based study. J Natl Cancer Inst 2011;103:1049-57.

10 Desai TK, Krishnan K, Samala N, Singh J, Cluley J, Perla S, et al. The incidence of oesophageal adenocarcinoma in non-dysplastic Barrett's oesophagus: a meta-analysis. Gut 2012;61:970-6.

11 Desai TK, Singh J, Samala N, Subbiah P. The incidence of esophageal adenocarcinoma in Barrett's esophagus has been overestimated. The American journal of gastroenterology 2011;106:1364-5; author reply 5-6.

12 Hvid-Jensen F, Pedersen L, Drewes AM, Sorensen HT, Funch-Jensen P. Incidence of adenocarcinoma among patients with Barrett's esophagus. The New England journal of medicine 2011;365:1375-83.

13 Sikkema M, Looman CW, Steyerberg EW, Kerkhof M, Kastelein F, van Dekken H, et al. Predictors for neoplastic progression in patients with Barrett's Esophagus: a prospective cohort study. Am J Gastroenterol 2011;106:1231-8.

14 Curvers WL, ten Kate FJ, Krishnadath KK, Visser M, Elzer B, Baak LC, et al. Low-grade dysplasia in Barrett's esophagus: overdiagnosed and underestimated. The American journal of gastroenterology 2010;105:1523-30.

15 Kerkhof M, van Dekken H, Steyerberg EW, Meijer GA, Mulder AH, de Bruine A, et al. Grading of dysplasia in Barrett's oesophagus: substantial interobserver variation between general and gastrointestinal pathologists. Histopathology 2007;50:920-7.

16 Skacel M, Petras RE, Gramlich TL, Sigel JE, Richter JE, Goldblum JR. The diagnosis of low-grade dysplasia in Barrett's esophagus and its implications for disease progression. Am J Gastroenterol 2000;95:3383-7.

17 Kastelein F, Biermann K, Steyerberg EW, Verheij J, Kalisvaart M, Looijenga LH, et al. Aberrant p53 protein expression is associated with an increased risk of neoplastic progression in patients with Barrett's oesophagus. Gut 2013;62:1676-83.

18 Wegner M. From head to toes: the multiple facets of Sox proteins. Nucleic Acids Res 1999;27:1409-20.

19 Liu K, Lin B, Zhao M, Yang X, Chen M, Gao A, et al. The multiple roles for Sox2 in stem cell maintenance and tumorigenesis. Cell Signal 2013;25:1264-71.

20 Que J, Okubo T, Goldenring JR, Nam KT, Kurotani R, Morrisey EE, et al. Multiple dose-dependent roles for Sox2 in the patterning and differentiation of anterior foregut endoderm. Development 2007;134:2521-31.

21 Wang Q, He W, Lu C, Wang Z, Wang J, Giercksky KE, et al. Oct3/4 and Sox2 are significantly associated with an unfavorable clinical outcome in human esophageal squamous cell carcinoma. Anticancer Res 2009;29:1233-41.

22 Zhang X, Yu H, Yang Y, Zhu R, Bai J, Peng Z, et al. SOX2 in gastric carcinoma, but not Hath1, is related to patients' clinicopathological features and prognosis. J Gastrointest Surg 2010;14:1220-6.

23 Neumann J, Bahr F, Horst D, Kriegl L, Engel J, Luque RM, et al. SOX2 expression correlates with lymph-node metastases and distant spread in right-sided colon cancer. BMC Cancer 2011;11:518.

24 Tsukamoto T, Inada K, Tanaka H, Mizoshita T, Mihara M, Ushijima T, et al. Down-regulation of a gastric transcription factor, Sox2, and ectopic expression of intestinal homeobox genes, Cdx1 and Cdx2: inverse correlation during progression from gastric/intestinal-mixed to complete intestinal metaplasia. J Cancer Res Clin Oncol 2004;130:135-45.

25 Tsukamoto T, Mizoshita T, Mihara M, Tanaka H, Takenaka Y, Yamamura Y, et al. Sox2 expression in human stomach adenocarcinomas with gastric and gastric-and-intestinal-mixed

phenotypes. Histopathology 2005;46:649-58.
26 Otsubo T, Akiyama Y, Yanagihara K, Yuasa Y. SOX2 is frequently downregulated in gastric cancers and inhibits cell growth through cell-cycle arrest and apoptosis. Br J Cancer 2008;98:824-31.
27 Bird-Lieberman EL, Dunn JM, Coleman HG, Lao-Sirieix P, Oukrif D, Moore CE, et al. Population-based study reveals new risk-stratification biomarker panel for Barrett's esophagus. Gastroenterology 2012;143:927-35 e3.
28 Lao-Sirieix P, Lovat L, Fitzgerald RC. Cyclin A immunocytology as a risk stratification tool for Barrett's esophagus surveillance. Clin Cancer Res 2007;13:659-65.
29 Inadomi JM, Sampliner R, Lagergren J, Lieberman D, Fendrick AM, Vakil N. Screening and surveillance for Barrett esophagus in high-risk groups: a cost-utility analysis. Annals of internal medicine 2003;138:176-86.
30 Inadomi JM, Somsouk M, Madanick RD, Thomas JP, Shaheen NJ. A cost-utility analysis of ablative therapy for Barrett's esophagus. Gastroenterology 2009;136:2101-14 e1-6.
31 Sonnenberg A, Fennerty MB. Medical decision analysis of chemoprevention against esophageal adenocarcinoma. Gastroenterology 2003;124:1758-66.
32 Sonnenberg A, Soni A, Sampliner RE. Medical decision analysis of endoscopic surveillance of Barrett's oesophagus to prevent oesophageal adenocarcinoma. Alimentary pharmacology & therapeutics 2002;16:41-50.
33 Das A, Wells C, Kim HJ, Fleischer DE, Crowell MD, Sharma VK. An economic analysis of endoscopic ablative therapy for management of nondysplastic Barrett's esophagus. Endoscopy 2009;41:400-8.
34 Garside R, Pitt M, Somerville M, Stein K, Price A, Gilbert N. Surveillance of Barrett's oesophagus: exploring the uncertainty through systematic review, expert workshop and economic modelling. Health technology assessment (Winchester, England) 2006;10:1-142, iii-iv.
35 Gordon LG, Mayne GC, Hirst NG, Bright T, Whiteman DC, Watson DI. Cost-effectiveness of endoscopic surveillance of non-dysplastic Barrett's esophagus. Gastrointestinal endoscopy 2013.
36 Provenzale D, Schmitt C, Wong JB. Barrett's esophagus: a new look at surveillance based on emerging estimates of cancer risk. The American journal of gastroenterology 1999;94:2043-53.
37 van Hagen P, Hulshof MC, van Lanschot JJ, Steyerberg EW, van Berge Henegouwen MI, Wijnhoven BP, et al. Preoperative chemoradiotherapy for esophageal or junctional cancer. N Engl J Med 2012;366:2074-84.
38 Gebski V, Burmeister B, Smithers BM, Foo K, Zalcberg J, Simes J, et al. Survival benefits from neoadjuvant chemoradiotherapy or chemotherapy in oesophageal carcinoma: a

meta-analysis. Lancet Oncol 2007;8:226-34.
39 Fitzgerald RC, di Pietro M, Ragunath K, Ang Y, Kang JY, Watson P, et al. British Society of Gastroenterology guidelines on the diagnosis and management of Barrett's oesophagus. Gut 2013.
40 American Gastroenterological A, Spechler SJ, Sharma P, Souza RF, Inadomi JM, Shaheen NJ. American Gastroenterological Association medical position statement on the management of Barrett's esophagus. Gastroenterology 2011;140:1084-91.
41 Aldulaimi DM, Cox M, Nwokolo CU, Loft DE. Barrett's surveillance is worthwhile and detects curable cancers. A prospective cohort study addressing cancer incidence, treatment outcome and survival. European journal of gastroenterology & hepatology 2005;17:943-50.
42 Chang LC, Oelschlager BK, Quiroga E, Parra JD, Mulligan M, Wood DE, et al. Long-term outcome of esophagectomy for high-grade dysplasia or cancer found during surveillance for Barrett's esophagus. Journal of gastrointestinal surgery : official journal of the Society for Surgery of the Alimentary Tract 2006;10:341-6.
43 Conio M, Blanchi S, Lapertosa G, Ferraris R, Sablich R, Marchi S, et al. Long-term endoscopic surveillance of patients with Barrett's esophagus. Incidence of dysplasia and adenocarcinoma: a prospective study. The American journal of gastroenterology 2003;98:1931-9.
44 Cooper GS, Kou TD, Chak A. Receipt of previous diagnoses and endoscopy and outcome from esophageal adenocarcinoma: a population-based study with temporal trends. The American journal of gastroenterology 2009;104:1356-62.
45 Corley DA, Levin TR, Habel LA, Weiss NS, Buffler PA. Surveillance and survival in Barrett's adenocarcinomas: a population-based study. Gastroenterology 2002;122:633-40.
46 Ferguson MK, Durkin A. Long-term survival after esophagectomy for Barrett's adenocarcinoma in endoscopically surveyed and nonsurveyed patients. Journal of gastrointestinal surgery : official journal of the Society for Surgery of the Alimentary Tract 2002;6:29-35; discussion 6.
47 Fountoulakis A, Zafirellis KD, Dolan K, Dexter SP, Martin IG, Sue-Ling HM. Effect of surveillance of Barrett's oesophagus on the clinical outcome of oesophageal cancer. The British journal of surgery 2004;91:997-1003.
48 Grant KS, DeMeester SR, Kreger V, Oh D, Hagen JA, Chandrasoma P, et al. Effect of Barrett's esophagus surveillance on esophageal preservation, tumor stage, and survival with esophageal adenocarcinoma. The Journal of thoracic and cardiovascular surgery 2013;146:31-7.
49 Hoff SJ, Sawyers JL, Blanke CD, Choy H, Stewart JR. Prognosis of adenocarcinoma arising in Barrett's esophagus. The Annals of thoracic surgery 1998;65:176-80; discussion 80-1.
50 Incarbone R, Bonavina L, Saino G, Bona D, Peracchia A. Outcome of esophageal

adenocarcinoma detected during endoscopic biopsy surveillance for Barrett's esophagus. Surgical endoscopy 2002;16:263-6.

51 Peters JH, Clark GW, Ireland AP, Chandrasoma P, Smyrk TC, DeMeester TR. Outcome of adenocarcinoma arising in Barrett's esophagus in endoscopically surveyed and nonsurveyed patients. The Journal of thoracic and cardiovascular surgery 1994;108:813-21; discussion 21-2.

52 Roberts KJ, Harper E, Alderson D, Hallissey M. Long-term survival and cost analysis of an annual Barrett's surveillance programme. European journal of gastroenterology & hepatology 2010;22:399-403.

53 van Sandick JW, van Lanschot JJ, Kuiken BW, Tytgat GN, Offerhaus GJ, Obertop H. Impact of endoscopic biopsy surveillance of Barrett's oesophagus on pathological stage and clinical outcome of Barrett's carcinoma. Gut 1998;43:216-22.

54 Menon D, Stafinski T, Wu H, Lau D, Wong C. Endoscopic treatments for Barrett's esophagus: a systematic review of safety and effectiveness compared to esophagectomy. BMC gastroenterology 2010;10:111.

55 Bhat SK, McManus DT, Coleman HG, Johnston BT, Cardwell CR, McMenamin U, et al. Oesophageal adenocarcinoma and prior diagnosis of Barrett's oesophagus: a population-based study. Gut 2015;64:20-5.

56 Corley DA, Mehtani K, Quesenberry C, Zhao W, de Boer J, Weiss NS. Impact of endoscopic surveillance on mortality from Barrett's esophagus-associated esophageal adenocarcinomas. Gastroenterology 2013;145:312-9 e1.

57 McCormick Matthews LH, Noble F, Tod J, Jaynes E, Harris S, Primrose JN, et al. Systematic review and meta-analysis of immunohistochemical prognostic biomarkers in resected oesophageal adenocarcinoma. Br J Cancer 2015;113:107-18.

58 Gowryshankar A, Nagaraja V, Eslick GD. HER2 status in Barrett's esophagus & esophageal cancer: a meta analysis. J Gastrointest Oncol 2014;5:25-35.

59 Honing J, Pavlov KV, Mul VE, Karrenbeld A, Meijer C, Faiz Z, et al. CD44, SHH and SOX2 as novel biomarkers in esophageal cancer patients treated with neoadjuvant chemoradiotherapy. Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology 2015.

60 Sarbia M, Ott N, Puhringer-Oppermann F, Brucher BL. The predictive value of molecular markers (p53, EGFR, ATM, CHK2) in multimodally treated squamous cell carcinoma of the oesophagus. Br J Cancer 2007;97:1404-8.

61 Kitamura K, Saeki H, Kawaguchi H, Araki K, Ohno S, Kuwano H, et al. Immunohistochemical status of the p53 protein and Ki-67 antigen using biopsied specimens can predict a sensitivity to neoadjuvant therapy in patients with esophageal cancer. Hepatogastroenterology 2000;47:419-23.

62 Bronson NW, Diggs BS, Bakis G, Gatter KM, Sheppard BC, Hunter JG, et al. Molecular

Marker Expression Is Highly Heterogeneous in Esophageal Adenocarcinoma and Does Not Predict a Response to Neoadjuvant Therapy. J Gastrointest Surg 2015.

63 Noble F, Nolan L, Bateman AC, Byrne JP, Kelly JJ, Bailey IS, et al. Refining pathological evaluation of neoadjuvant therapy for adenocarcinoma of the esophagus. World J Gastroenterol 2013;19:9282-93.

64 Chen Y, Huang Y, Zhu L, Chen M, Huang Y, Zhang J, et al. SOX2 inhibits metastasis in gastric cancer. J Cancer Res Clin Oncol 2016.

65 Wang S, Tie J, Wang R, Hu F, Gao L, Wang W, et al. SOX2, a predictor of survival in gastric cancer, inhibits cell proliferation and metastasis by regulating PTEN. Cancer Lett 2015;358:210-9.

66 Zhang X, Hua R, Wang X, Huang M, Gan L, Wu Z, et al. Identification of stem-like cells and clinical significance of candidate stem cell markers in gastric cancer. Oncotarget 2016.

10

Summary
Nederlandse samenvatting

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Barrett's surveillance

Barrett's esophagus (BE) is a premalignant condition in which patients have an increased risk to develop esophageal adenocarcinoma (EAC) with an estimated incidence of 0.1 to 0.4% per year. The transition from BE to EAC is a gradual process, in which intestinal metaplasia evolves via low-grade dysplasia (LGD), to high-grade dysplasia (HGD) and finally to EAC. Endoscopic surveillance is recommended for BE patients to detect HGD or EAC at an early and potentially curable stage. Histological diagnosis of LGD is used for the risk assessment of neoplastic progression in BE surveillance and more intensive follow-up is recommended in LGD patients (yearly instead of every 3 years). However, diagnosis of LGD has a low predictive value, owing to sample error and a considerable inter- and intraobserver variation. Identification of additional predictors could improve risk stratification and hence cost-effectiveness of BE surveillance.

The aim of part I of this thesis was to evaluate whether use of biomarkers can improve risk stratification in BE in order to optimize surveillance. In addition, the cost-effectiveness of BE surveillance according to the current guidelines and impact on survival was assessed.

Many immunohistochemical (IHC) biomarkers have been studied in BE progression mainly because it's relatively easy applicable for daily practice but so far none have progressed to the stage of routine clinical use. In **chapter 2** the existing literature is systematically reviewed regarding to the value of IHC biomarkers for predicting neoplastic progression in BE patients and meta-analysis was performed. The biomarker investigated most frequently is p53 and the performed meta-analysis showed that aberrant p53 expression is significantly associated with neoplastic progression in both non-dysplastic BE and LGD. P53 staining in addition to the histological diagnosis, can be implemented to improve risk stratification in BE surveillance. However, our group previously reported that only 40% of the BE patients with progression to HGD or EAC showed an aberrant p53 protein expression during surveillance, suggesting that additional biomarkers are needed. Another potential biomarker is the transcription factor SOX2. In **chapter 3** the value of SOX2 IHC is investigated to predict neoplastic progression in BE. A case-control study was conducted with a large multicenter prospective cohort of 720 BE patients. Patients with neoplastic progression, defined as development of HGD or EAC, were classified as cases and patients without neoplastic progression as controls. SOX2 expression was determined in more than 12,000 biopsies.

SOX2 expression was progressively lost in dysplastic BE and was significantly associated with an almost 5-fold increased risk of neoplastic progression, independent of age, gender, BE length and esophagitis. The use of this marker, in combination with p53, has the potential to significantly improve risk stratification by selecting truly high risk BE patients for neoplastic progression, and hence the cost-effectiveness of Barrett surveillance.
Another potential biomarker for predicting neoplastic progression in BE is cyclin A. In **chapter 4** the value of cyclin A to predict neoplastic progression in BE is investigated. Because there is a lack of studies testing performance of multiple biomarkers simultaneously in the same cohort of BE patients, we combined these results with our previously reported p53, AMACR and SOX2 IHC data. Cyclin A surface positivity was associated with a 2-fold increased risk of neoplastic progression. However, in a fully adjusted model, aberrant p53 expression showed the highest change in area under the curve (AUC) after exclusion (0.050 specified), to a lesser extent aberrant SOX2 expression (0.014) and histological diagnosis of LGD (0.005). The biomarkers cyclin A only showed a minimal change in AUC after exclusion (0.003). The highest predictive value was achieved by concurrent loss of SOX2 expression and aberrant p53 expression in BE patients with LGD (being AUC 0.72; 95% CI 0.67 to 0.77). The incremental value of cyclin A was limited.
As indicated, BE patients have a higher risk of developing EAC compared to the general population, but the absolute risk of neoplastic progression is relative low. One of the key questions in the discussion about BE surveillance is whether it is cost-effective. In **chapter 5** the cost-effectiveness of BE surveillance according to the current guidelines is investigated. A cost-effectiveness analysis was preformed within a large multicenter prospective cohort of 714 patients with BE. A multi-state-Markov model was used to calculate progression rates based on the prospective follow-up data, which were incorporated in a decision-analytic model, including costs and quality of life data. Different surveillance intervals and different therapeutic modalities for BE without dysplasia and LGD were evaluated. Assuming a willingness-to-pay threshold of €35.000 per quality-adjusted life year gained, surveillance with endoscopic mucosal resection (EMR) and radiofrequency ablation (RFA) for HGD or early EAC, and esophagectomy for advanced EAC is cost-effective every 5-years for no-dysplasia and every 3-years for LGD.
Other key elements in the discussion of Barrett surveillance are whether surveillance is able to reduce the risk of advanced EAC and improve survival.

In **Chapter 6** the impact of BE surveillance according to the current guidelines is investigated on tumor stage and survival of EAC patients, within a large prospective cohort of 783 BE patients. During surveillance incident cases of HGD or EAC were identified. Survival data were collected and cross-checked using death and municipal registries and compared to data of patients with EAC in the general Dutch population. EAC was diagnosed at a significantly earlier stage during BE surveillance than in general population. Survival of BE patients with neoplastic progression diagnosed during surveillance was not significantly worse than those of patients without and similar to those of patients with stage 0 or stage 1 EAC in the general population. This suggests that BE surveillance enables the detection of EAC at an early stage with good survival rates. The results of this study therefore support current guidelines recommending endoscopic surveillance in patients with BE.

Esophageal adenocarcinoma (EAC) and biomarkers

Neoadjuvant chemoradiotherapy (nCRT) followed by surgery has recently become standard of care for locally advanced esophageal cancers and achieves survival benefit. Still, the overall prognosis for most patients including those treated with curative intent is dismal with a 5-year survival of 47% at most. However, response to nCRT and overall prognosis is highly variable in EAC patients. Prediction of response to neoadjuvant treatment, improved pre-operative prognostication, as well as individualized targeted treatment are the current challenges in the clinical management of EAC patients. The aim of part II of this thesis was to evaluate the value of biomarkers on prediction of prognosis and therapy response in EAC patients. In **chapter 7** the association between p53, SOX2 and CD44 protein expression in pretreatment tumor biopsies for tumor response is investigated. Expression of p53 but in particularly SOX2 was significantly associated with response to nCRT in primary and validation cohort. Loss of SOX2 expression indicated a high probability of a major response to nCRT. In **chapter 8** the prognostic value of SOX2 is investigated in two independent cohorts after assessment of TMAs from resected EAC specimens of in total 756 patients. Loss of SOX2 expression was an independent predictor of adverse outcome with median survival of 19.7 months after 5 years in contrast to 31.7 months in patients with retained SOX2. Importantly, most significant effects of SOX2 for predicting prognosis were detected in pT1N0 tumors. Assessment of SOX2 may help to individualize (treatment) decision-making in EAC patients.

In conclusion, BE surveillance might enable the detection of EAC at an early and curable stage when endoscopic treatment with EMR and RFA is still feasible, and which eventually leads to improves survival. Although surveillance according to the current guidelines is cost-effective for patients without dysplasia, for patients with LGD, surveillance is recommended with intervals of six to twelve months, while according to our study intervals should be at least three years in order to be cost-effective. Identification and incorporation of additional biomarkers may improve risk stratification and eventually cost-effectiveness of BE surveillance. A panel of biomarkers with p53 and SOX2 immunohistochemistry appears to be more predictive than the histological diagnosis of LGD alone. P53 and SOX2 IHC have a good interobserver agreement, are relatively cheap and easy applicable to clinical practice compared to other techniques, which makes them clinically suitable markers. SOX2 expression is not only predictive for neoplastic progression in BE but also might improve prognostication in patients undergoing resection for EAC and is significantly associated with therapy response in potential curable EAC patients.

Nederlandse samenvatting

Barrett slokdarm is een premaligne aandoening waarbij patiënten een verhoogd risico hebben op het ontwikkelen van een adenocarcinoom van de slokdarm met een geschatte incidentie van 0.1 tot 0.4% per jaar. De ontwikkeling van een adenocarcinoom in Barrett slokdarm is een stapsgewijs proces waarbij metaplastisch cilinder epitheel veranderd in laaggradige dysplasie, hooggradige dysplasie en uiteindelijk adenocarcinoom. Endoscopische surveillance is geadviseerd in patiënten met een Barrett slokdarm in de hoop zo hooggradige dysplasie of slokdarm adenocarcinoom in een vroegtijdig stadium op te sporen wanneer curatieve behandeling nog mogelijk is. De histologische diagnose van laaggradige dysplasie wordt gebruikt voor de risico inschatting voor maligne ontaarding in Barrett slokdarm surveillance en een meer intensieve follow-up is geadviseerd in patiënten met laaggradige dysplasie (jaarlijks in plaats van elke drie jaar). De diagnose van laaggradige dysplasie is echter onderhevig aan steekproeffouten en inter- en intraobserver variatie wat de voorspellende waarde beperkt. Identificeren van andere voorspellers voor maligne ontaarding zou risicostratificatie van patiënten met een Barrett slokdarm kunnen verbeteren en daarmee de kosteneffectiviteit van Barrett slokdarm surveillance.

Het doel van deel I van dit proefschrift was om te onderzoeken of het gebruik van biomarkers kan bijdragen aan een verbeterde risicostratificatie van patiënten met een Barrett slokdarm om zo surveillance te optimaliseren. Daarnaast evalueerde we het effect van surveillance volgens de huidige richtlijnen op kosteneffectiviteit en overleving. Vele immunohistochemische (IHC) biomarkers zijn onderzocht in Barrett slokdarm surveillance omdat de techniek van immunohistochemie relatief makkelijke en toepasbaar is in de dagelijkse praktijk, maar tot op heden wordt nog geen biomarker routinematig gebruikt in de kliniek. In **hoofdstuk 2** wordt de volledige literatuur systematisch geëvalueerd met betrekking tot de waarde van IHC biomarkers voor het voorspellen van maligne ontaarding in Barrett slokdarm patiënten en is een meta-analyse verricht. De biomarker p53 is het meest frequent onderzocht en de verrichte meta-analyse toont dat een afwijkende p53 expressie significant geassocieerd is met maligne ontaarding in zowel niet dysplastisch Barrett epitheel als in laaggradige dysplasie. P53 IHC kleuring in toevoeging op de histologische diagnose van dysplasie, kan geïmplementeerd worden om zo risico stratificatie binnen Barrett surveillance te verbeteren. Echter, onze onderzoeksgroep heeft eerder laten zien dat maar in 40% van

de Barrett patiënten met maligne ontaarding tot hooggradige dysplasie of slokdarm adenocarcinoom er sprake is van een afwijkende p53 expressie bij de voorafgaande surveillance, dit suggereert dat er aanvullende biomarkers nodig zijn. Een andere potentiële biomarker is de transcriptie factor SOX2. In **hoofdstuk 3** is de waarde van SOX IHC onderzocht voor het voorspellen van maligne ontaarding in Barrett slokdarm. Hiervoor is een case-control studie verricht binnen een groot multicenter prospectieve cohort studie van 720 Barrett patiënten. Patiënten met maligne ontaarding, gedefinieerd als hooggradige dysplasie of adenocarcinoom, werden geclassificeerd als cases en overige patiënten zonder maligne ontaarding als controles. SOX2 expressie werd bepaald in meer dan 12.000 biopten. SOX2 expressie was toenemend afwezig in dysplastisch Barrett epitheel en was geassocieerd met een bijna 5 keer verhoogd risico op maligne ontaarding, onafhankelijk van leeftijd, geslacht, Barrett lengte en aanwezigheid van oesofagitis. Het gebruik van deze biomarker, in combinatie met de biomarker p53, heeft de potentie om risicostratificatie significant te verbeteren door het selecteren van de daadwerkelijk hoog risico Barrett patiënten voor het ontwikkelen van maligne ontaarding, en daarmee ook de kosteneffectiviteit van Barrett surveillance.
Een andere potentiële biomarker voor het voorspellen van maligne ontaarding in Barrett slokdarm is cyclin A. In **hoofdstuk 4** is de waarde van cyclin A voor het voorspellen van maligne ontaarding in Barrett slokdarm onderzocht. Omdat er een gebrek is aan studies die de waarden van verschillende biomarkers in eenzelfde cohort van Barrett patiënten onderzoekt, zijn de resultaten van de biomarker cyclin A gecombineerd met de eerder gepubliceerde p53, AMACR en SOX2 IHC gegevens. Cyclin A positiviteit was geassocieerd met een 2 keer verhoogd risico op maligne ontaarding. Echter, in een volledig gecorrigeerd model toonde een afwijkende p53 de grootste verandering in 'gebied onder de curve' na uitsluiting (0.050 specifiek), in mindere mate was dit het geval voor een afwijkende SOX2 expressie (0.014) en de histologische diagnose van laaggradige dysplasie (0.005). De biomark cyclin A toonde alleen een minimaal verandering in 'gebied onder de curve' na uitsluiting (0.003). De hoogste voorspellende waarde werd gezien bij gelijktijdig verlies van SOX2 expressie en een afwijkende p53 expressie in Barrett patiënten met laaggradige dysplasie (AUC 0.72; 95% CI 0.67 to 0.77). De toegevoegde waarde van cyclin A was gelimiteerd.
Zoals aangegeven, hebben patiënten met een Barrett slokdarm een verhoogd risico op het ontwikkelen van slokdarm adenocarcinoom in vergelijking

met de algemene populatie, maar het absolute risico op maligne ontaarding is relatief laag. Een van de kernvragen binnen de discussie van Barrett surveillance is of Barrett surveillance kosteneffectief is. In **hoofdstuk 5** werd de kosteneffectiviteit van Barrett surveillance volgend de huidige richtlijnen onderzocht. Een kosteneffectiviteitsanalyse werd verricht binnen een groot multicenter prospectieve cohort studie met daarin 714 Barrett patiënten. Een Multi-state-Markov model werd gebruikt om progressie kansen te berekenen op basis van prospectieve follow-up gegevens, welke vervolgens werden opgenomen in een beslismodel met daarin gegevens over kosten en kwaliteit van leven. We evalueerde verschillende surveillance intervallen voor patiënten zonder dysplasie of met laaggradige dysplasie en verschillende behandelmogelijkheden voor patiënten met hooggradige dysplasie of adenocarcinoom. Uitgaande van een drempel van €35.000 per gewonnen levensjaar, lijkt surveillance elke 5 jaar, met endoscopische behandeling voor zowel hooggradige dysplasie als vroegcarcinomen, en een slokdarmresectie met neoadjuvante chemoradiotherapie voor een gevorderd adenocarcinoom kosteneffectief te zijn in patiënten zonder dysplasie en surveillance elke 3 jaar in patiënten met laaggradige dysplasie.
Een ander kernonderwerp binnen de discussie van Barrett surveillance is of surveillance daadwerkelijk het risico op het ontwikkelen van een gevorderd adenocarcinoom verminderd en de overleving verbeterd. In **hoofdstuk 6** hebben we onderzocht wat de invloed is van Barrett surveillance volgends de huidige richtlijnen op tumor stadium en overleving van patiënten met een adenocarcinoom van de slokdarm, binnen een groot prospectieve cohort van 783 Barrett patiënten. Overlevingsdata werden verzameld en vergeleken met overlevingsdata van patiënten met een adenocarcinoom van de slokdarm in de algemene Nederlandse bevolking. Slokdarm adenocarcinoom werd in een significant vroeger stadium gevonden tijdens Barrett surveillance dan in de algemene bevolking. De overleving van Barrett patiënten met maligne ontaarding tijdens surveillance was overeenkomstig met de overleving van patiënten met een stadium 0 of 1 adenocarcinoom in de algemene bevolking. Dit suggereert dat Barrett surveillance detectie van adenocarcinoom in een vroeg stadium mogelijk maakt met daarbij goede overlevingskansen. De resultaten van deze studie ondersteunen de huidige richtlijnen de endoscopische surveillance in patiënten met een Barrett slokdarm adviseren.

Slokdarm adenocarcinoom en biomarkers

Neoadjuante chemoradiotherapie (nCRT) gevolgd door een operatie is sinds recent de standaard behandeling voor lokaal gevorderd slokdarmkanker en zorgt voor een betere overleving. Toch is de prognose voor de meeste patiënten, inclusief de patiënten die met curatieve intenties behandeld worden somber met een 5-jaars overleving van 47% maximaal. Echter, de response op nCRT en overall prognose is zeer variabel in patiënten met een slokdarm adenocarcinoom. Het voorspellen van response op neoadjuvante behandeling, het beter bepalen van de prognose voor de operatie, als geïndividualiseerde gerichte behandeling zijn de huidige uitdagingen binnen de management van slokdarm adenocarcinoom patiënten.

Het doel van deel II van dit proefschrift was om de waarde van biomarkers voor het voorspellen van prognose en therapie response in patiënten met een slokdarm adenocarinoom te onderzoeken. In **hoofdstuk 7** wordt de associatie tussen de biomarkers p53, SOX2 en CD44 expressie in biopten en tumorresponse onderzocht. Expressie van p53, maar in het bijzonder expressie van SOX2 was significant geassocieerd met response op nCRT in twee onafhankelijk cohorten. Verlies van SOX2 expressie geeft een grotere kans op een goede response op nCRT. In **hoofdstuk 8** wordt de prognostische waarden van SOX2 onderzocht in twee onafhankelijke cohorten na beoordeling van slokdarm adenocarcinoom resectiepreparaten van in totaal 756 patiënten. Verlies van SOX2 was een onafhankelijke voorspeller voor een ongunstige overleving met een gemiddelde overleving van 19.7 maanden na 5 jaar in tegenstelling tot 31.7 maanden bij patiënten met een behouden SOX2 expressie. Belangrijker, het meest uitgesproken effect van SOX2 expressie voor het voorspellen van prognose werd gezien in pT1N0 tumoren. Dus het bepalen van SOX2 eiwitexpressie kan helpen bij de besluitvorming voor behandeling in patiënten met een slokdarm adenocarcinoom.

Concluderend, Barrett surveillance maakt het opsporen van slokdarm adenocarcinoom in een vroeg stadium mogelijk wanneer endoscopische behandeling nog tot de mogelijkheden behoord, en uiteindelijk leidt tot de verbetering van de overleving. Hoewel follow-up volgends de huidige richtlijnen kosteffectief is voor patiënten zonder dysplasie, voor patiënten met laaggradige dysplasie, is surveillance met een interval van 6 maanden tot 1 jaar geadviseerd, terwijl volgens onze studie het interval tenminste 3 jaar moet zijn om kosteffectief te zijn. Identificeren van aanvullende biomarkers kan risicostratificatie en daarmee uiteindelijk kosteneffectiviteit van Barrett

surveillance verbeteren. Een panel van biomarkers met p53 en SOX2 lijkt meer voorspellend dan de histologische diagnose van laaggradige alleen. P53 en SOX2 hebben een goede interobserver overeenkomst, zijn relatief goedkoop en makkelijk toepasbaar in de kliniek vergeleken met andere technieken, wat er voor zorgt dat beide klinisch geschikte markers zijn. SOX2 expressie is niet alleen voorspellend voor maligne ontaarding in Barrett surveillance maar verbeterd ook het voorspellen van de prognose in patiënten die een slokdarmresectie ondergaan en is significant geassocieerd met therapie response in patiënten met een potentieel te genezen adenocarcinoom van de slokdarm.

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Curriculum Vitae
PhD portfolio

S.H. van Olphen

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Sophie

Appendix: Deelnemende centra CYBAR en ProBar studies

Erasmus MC	
Afdeling Maag-, Darm- en Leverziekten	M.Kerkhof, M. Sikkema, F. Kastelein, S. van Olphen, C. Roumans, P. Siersema, E. Kuipers, M. Bruno, M. Spaander
Afdeling Patholgie	H. van dekken, K. Biermann, F. ten Kate, M. Doukas
Ijsselland ziekenhuis	
Afdeling Maag-, Darm- en Leverziekten	W. Bode, H.Geldof, T. Tang
Afdeling Patholgie	H. van der Valk
Ikazia ziekenhuis	
Afdeling Maag-, Darm- en Leverziekten	R. Ouwendijk, C. Leunis, P. ter Borg
Afdeling Patholgie	R. Giard, M. Bakker
VUMC	
Afdeling Maag-, Darm- en Leverziekten	E. Klinkenberg, R. Felt
Afdeling Patholgie	G. Meijer, M. Broeckaert
Albert Schweitzer ziekenhuis	
Afdeling Maag-, Darm- en Leverziekten	W. Lesterhuis, R. Beukers, J. Alderliesten, J. Meijer, S. Hollaar
Afdeling Patholgie	R. Heinhuis
Deventer ziekenhuis	
Afdeling Maag-, Darm- en Leverziekten	F. ter Borg, I. van Zon, Y. Dieterman
Afdeling Patholgie	J. Arends, R. Smits, M. Louwen
ZGT Hengelo	
Afdeling Maag-, Darm- en Leverziekten	C. Wielens, G. tan
Afdeling Patholgie	J. van Baarlen

Rijnstate ziekenhuis	
Afdeling Maag-, Darm- en Leverziekten	R. de Vries, P. van Embden, B. den hartog, J. Burger, P. Poort
Afdeling Patholgie	A. Mulder, M. Rijnders, J. Meijer
Sint Franciscus gasthuis	
Afdeling Maag-, Darm- en Leverziekten	A. van Tilburg, E. Luthart, B. Ferreira, S. Mulder
Afdeling Patholgie	H. van der Valk
Medisch spectrum Twente	
Afdeling Maag-, Darm- en Leverziekten	J. Kolkman
Afdeling Patholgie	J. van Baarlen
Orbis MC Sittard	
Afdeling Maag-, Darm- en Leverziekten	L. Engels, G. Remeeus, M. Romberg- Camps
Afdeling Patholgie	W. Vos
UMC Groningen	
Afdeling Maag-, Darm- en Leverziekten	F. Peters
Afdeling Patholgie	A. Karrenbeld
Zaans MC	
Afdeling Maag-, Darm- en Leverziekten	R. Loffeld
Afdeling Patholgie	M. Flens
Franciscus ziekenhuis Roosendaal	
Afdeling Maag-, Darm- en Leverziekten	H. van Roermund
Afdeling Patholgie	F. Lockefeer
Isala ziekenhuis Zwolle	
Afdeling Maag-, Darm- en Leverziekten	J. Kastermans, B. Schenk
Afdeling Patholgie	F. Moll

Maasstad ziekenhuis	
Afdeling Maag-, Darm- en Leverziekten	M. Hadithi
Afdeling Patholgie	M. Bakker
Reinier de Graaf	
Afdeling Maag-, Darm- en Leverziekten	R. Quispel, M. Goudzwaard en P. Van der Kraaij- Mertens
Afdeling Patholgie	C. Meijers

Curriculum Vitae

Sophie van Olphen werd op 8 December 1985 geboren te Breda. In 2004 behaalde zij haar VWO diploma aan het Carolus Borromeus College te Helmond. Vervolgens begon zij in datzelfde jaar aan de studie Geneeskunde aan de Erasmus universiteit Rotterdam, waar zij in 2008 haar doctoraal behaalde. In 2010 doorliep zij haar keuze coschap op de afdeling interne geneeskunde en Maag-, Darm- en Leverziekten van het St. Elisabeth ziekenhuis te Tilburg en haar oudste coschap op de afdeling Maag-, Darm- en Leverziekten van het Amphia ziekenhuis te Breda, waarna zij in oktober 2010 haar artsenexamen behaalde. Hierna werkte zij een kleine twee jaar als ANIOS op de afdelingen interne geneeskunde en Maag-, darm en Leverziekten van het Amphia ziekenhuis. In augustus 2012 startte zij met promotieonderzoek op het gebied van Barrett slokdarm en slokdarm adenocarcinoom vanuit de afdeling Maag-, Darm en Leverziekten onder begeleiding van prof. dr. M.J. Bruno en dr. M.C.W. Spaander en afdeling pathologie van het Erasmus Medisch Centrum onder begeleiding van prof. dr. L.H.J. Looijenga en dr. B. Biermann. Per 1 januari 2016 is zij met veel plezier gestart met haar opleiding tot Maag-, Darm- en Leverarts vanuit het Universitair Medisch Centrum Utrecht (opleider: Dr. B. Oldenburg). De vooropleiding Interne geneeskunde volgt zij gedurende anderhalf jaar in het Sint Antonius ziekenhuis te Nieuwegein/ Utrecht (opleiders: dr. A.B.M. Geers, dr. P.C. de Jong).

Publications

S.H. van Olphen, V.T. Janmaat, K. Biermann, L.H.J. Looijenga, M.B. Bruno, M.C.W. Spaander
Immunohistochemical biomarkers for risk stratification of neoplastic progression in Barrett esophagus: a systematic review and meta-analysis
Submitted for publication

S.H. van Olphen, K. Biermann, M.C.W Spaander, F. Kastelein, E.W. Steyerberg, J.A Stoop, M.J. Bruno, and L.H.J. Looijenga on behalf of the ProBar-study group.
SOX2 as a novel marker to predict neoplastic progression in Barrett esophagus
American Journal of Gastroenterology, 2015 Oct;110(10):1420-8

S.H. van Olphen, F.J.C ten Kate, M. Doukas, F. Kastelein, E.W. Steyerberg, J.A. Stoop, M.C.W. Spaander, L.H.J. Looijenga, M.J. Bruno and K. Biermann on behalf of the ProBar-study group.
Value of cyclin A immohistochemistry for cancer risk-stratification in Barrett esophagus surveillance
Medicine, 2016 Nov;95(47):e5402.

F. Kastelein, **S.H. van Olphen**, E.W. Steyerberg, M. Sikkema, M.C.W. Spaander, C.W.N. Looman, E.J. Kuipers, P.D. Siersema, M.J. Bruno and E.W. de Bekker-Grob on behalf of the ProBar-study group.
Surveillance in patients with long-segment Barrett's esophagus: a cost-effectiveness analysis.
Gut. 2015 Jun;64(6):864-71.

F. Kastelein, **S.H. van Olphen**, E.W. Steyerberg, M.C.W. Spaander and M.J. Bruno on behalf of the ProBar-study group.
Impact of surveillance for Barrett's oesophagus on tumor stage and survival of patients with neoplastic progression
Gut. 2015 Apr 22. pii: gutjnl-2014-308802

S.H. van Olphen, K. Biermann, J. Shapiro, B.P.L. Wijnhoven, E.L.A. Toxopeus, A. van der Gaast, J.A. Stoop, J.J.B. van Lanschot, M.C.W. Spaander, M.J. Bruno, L.H.J. Looijenga
P53 and SOX2 protein expression predicts esophageal adenocarcinoma response to neoadjuvant chemoradiotherapy
Ann Surg. 2016 Jan 15

F.J.C. ten Kate, **S.H. van Olphen**, M.J. Bruno, B.P.L. Wijnhoven, J.J.B. van Lanschot, L.H.J. Looijenga, R.C. Fitzgerald, K. Biermann
Loss of SRY-box2 (SOX2) expression and its impact on survival of patients with oesophageal adenocarcinoma
British Journal of Surgery, 2017

PhD Portfolio

	Oral presentations	Workload (ECTS)
2014	SOX2 as a novel marker to predict neoplastic progression in barrett's esophagus *Molmed Day Erasmus MC, Rotterdam, the Netherlands*	0.6
2014	SOX2 as a novel marker to predict neoplastic progression in barrett's esophagus *Dutch Society of gastroenterology, Veldhoven, the Netherlands*	0.6
2014	Surveillance in patients with long-segment Barrett's esophagus: a cost-effectiveness analysis *Dutch Society of gastroenterology, Veldhoven, the Netherlands*	0.6
2014	Surveillance in patients with long-segment Barrett's esophagus: a cost-effectiveness analysis *Dutch Society of gastroenterology, Veldhoven, the Netherlands*	0.6
2014	SOX2 as a novel marker to predict neoplastic progression in barrett's esophagus *Digestive Diseases Week, Chicago, United States*	0.6
2014	Impact of surveillance for Barrett's oesophagus on tumor stage and survival of patients with neoplastic progression multicentre prospective cohort. *United European Gastroenterology week, Vienna, Austria*	0.6

2014	Barrett's esophagus surveillance, ProBar cohort *Department of Public Health, Erasmus MC, Rotterdam, the Netherlands*	0.6
2014	SOX2 as a novel marker to predict neoplastic progression in barrett's esophagus *United European Gastroenterology week, Vienna, Austria*	0.6
2015	SOX2 and P53 protein expression predicts response to preoperative chemoradiotherapy in patients with esophageal adenocarcinoma. *Dutch Society of gastroenterology, Veldhoven, the Netherlands*	0.6
2015	Prediction of malignant transformation in Barrett's esophagus *PALM meeting, Josephine Nefkens Instituut, Erasmus MC, Rotterdam, the Netherlands*	0.6
2013 - 2016	Presentations at the LEPO meetings, department of Pathology, Erasmus MC, Rotterdam, the Netherlands	4.0

Poster presentations		Workload (ECTS)
2014	Surveillance in patients with Barrett's esophagus: Cost-effectiveness analysis *United European Gastroenterology week, Vienna, Austria*	0.6
2014	Surveillance in patients with Barrett's esophagus: Cost-effectiveness analysis *Digestive Diseases Week, Chicago, United States*	0.6
2014	SOX2 as a novel marker to predict neoplastic progression in barrett's esophagus *Molmed Day Erasmus MC, Rotterdam, the Netherlands*	0.6

Attended seminars and workshops		Workload (ECTS)
2012	The introduction Course on statistics & survival analysis for research *Molecular Medicine school, Rotterdam, the Netherlands*	0.5
2012	Basic introduction course on SPSS *Molecular Medicine school, Rotterdam, the Netherlands*	0.5
2012	Cursus Endnote *Medische bibliotheek Erasmus MC, Rotterdam, the Netherlands*	0.2

2012	Cursus Pubmed *Medische bibliotheek Erasmus MC, Rotterdam, the Netherlands*	0.2
2013	Photoshop & Illustrator CS6 workshop 0.3 *Molecular Medicine school, Rotterdam, the Netherlands*	0.3
2013	Course biomedical research techniques *Molecular Medicine school, Rotterdam, the Netherlands*	1.5
2013	Basiscursus Regelgeving en Organisatie voor Klinisch onderzoekers (BROK), *Erasmus University Medical Center, Rotterdam, the Netherlands.*	2.5
2013	Post-DDW symposium *Society of Gastroenterology, Utrecht, the Netherlands*	0.3
2014	The 100th anniversary of the Daniel den Hoed Oncology Centre, Rotterdam, the Netherlands	0.3
2013 - 2016	Seminars at the department of Pathology, Erasmus Medical Center, Rotterdam	4.0
2013 - 2016	Seminars at the department of Gastroenterology and Hepatology, Erasmus Medical Center, Rotterdam	4.0

Educational activities		Workload (ECTS)
2013	Supervising graduation project Vezna de Maare, student Biomedical laboratory research, Avans, Breda, the Netherlands	2.0
2014	Supervising training project Marloes de Wijngaert, student laboratory technology, ROC, Utrecht, the Netherlands	2.0
2014	Supervising training project Quincy van den Bosch, student laboratory technology, ROC, Leiden, the Netherlands	2.0
2015	Supervising graduation project Yadira, student Biomedical laboratory research, Leiden, the Netherlands	2.0

(Inter)national conferences	
2012	United European Gastroenterology week, Amsterdam, the Netherlands
2013	Congress of the European society for diseases of the esopahgus, Rotterdam, the Netherlands
2013	Dutch Society of gastroenterology (spring), Veldhoven, the Netherlands
2014	Digestive Diseases Week, Chicago, United States
2014	United European Gastroenterology week, Vienna, Austria

2014 Dutch Society of gastroenterology (spring and autumn), Veldhoven, the Netherlands

2015 Dutch Society of gastroenterology (spring), Veldhoven, the Netherlands

Membership

2012 - current Dutch society of gastroenterology