Prognostication and New Treatment Strategies for Esophageal and Junctional Cancer

Joël Shapiro

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Prognostication and New Treatment Strategies for Esophageal and Junctional Cancer

Prognosticatie en nieuwe behandelstrategieën voor slokdarm- en junctiecarcinomen

Thesis

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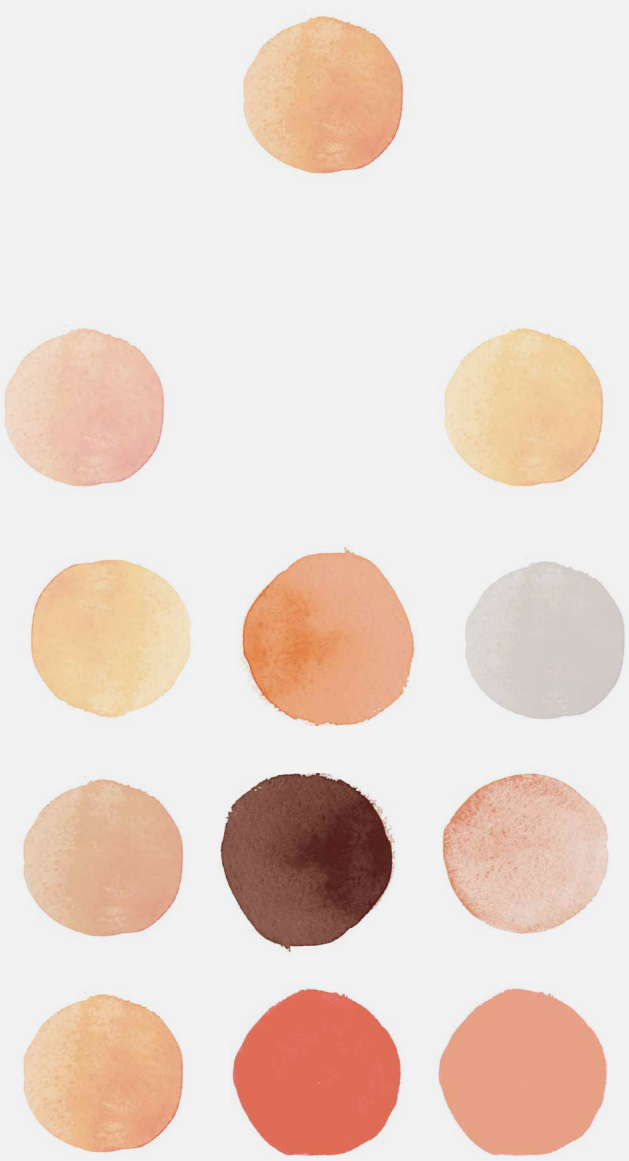
Voor mijn familie

Table of Contents

Chapter 1
General introduction



Esophageal cancer

Worldwide, esophageal cancer is the eighth most common cancer and the sixth most common cause of death from cancer, with an estimated 456,000 new cases (3.2% of total) and 400,000 deaths (4.9% of total) in 2012.[1] Esophageal cancer has two main histological subtypes — esophageal squamous cell carcinoma and esophageal adenocarcinoma. Esophageal squamous cell carcinoma accounts for approximately 90% of esophageal cancer cases worldwide. While historically esophageal adenocarcinoma has been rare, it has been associated with a rapid rise in incidence over the last decades, especially in Western countries.[2-4] Consequently, esophageal adenocarcinoma is now the most prevalent esophageal cancer subtype in Western European and North American countries. In The Netherlands, approximately 2,550 new cases of esophageal cancer were reported in 2014, of which approximately 1,750 (69%) cases were of the adenocarcinoma subtype.[5]

Prognostication

In the field of oncology, prognosis usually relates to the probability of survival or disease recurrence over a specific period, based on patient, tumor and treatment related characteristics. Therefore, prognostication deals with estimating how long a patient is expected to survive (or be without disease recurrence), given the presence (or absence) of certain prognostic characteristics. Medical prognostication and prognostic models are mainly used to inform patients and their treating physicians about the expected future course of their illness and to guide decisions on further treatment, if any.[6]

In patients with cancer of the esophagus or esophagogastric junction, several prognostic factors for long-term survival after primary surgical resection have been identified. Important, patient related prognostic factors include age[7,8], gender[8-11] and pretreatment weight loss[12, 13]. The extent of esophageal cancer progression is usually classified using the Union for International Cancer Control (UICC) tumor (T), node (N) and metastasis (M) staging system.[14] Where the T-stage indicates the extent of the primary tumor depth of invasion in the esophageal wall, the N-stage indicates the number of tumor positive regional lymph nodes and the M-stage indicates the presence of distant dissemination to other organs. This categorization corresponds with a decreasing probability of survival (*i.e.* M1 is associated with a worse prognosis than N3, and N1 is associated with a worse prognosis than T3). The TNM-stage is used to direct treatment choices before start of treatment and to estimate the probability of survival after completion of treatment.
An important treatment related prognostic factor is surgical radicality[15-17], *i.e.* the completeness of surgical tumor removal. However, most of these well-established prognostic factors have been identified and validated in the era of primary surgical resection.

Treatment of esophageal cancer

Presently, surgical resection is considered the cornerstone of intentionally curative treatment in the Netherlands for patients eligible (*i.e.* stages cT1b-4aN0-3M0) to undergo surgical resection. In the international literature reported 5-year survival rates for patients treated with primary surgical resection range from 6 to 50%, but rarely exceed 35%.[18-22] However, esophageal resections are associated with perioperative mortality rates of 1-5% in high-volume centers, severe postoperative morbidity and a substantial impact on the quality of life.[23-28] In order to improve the radicality of surgical resection and the long term survival after surgical resection many trials have been performed to study the additional value of (neo-) adjuvant chemo- and/or radiation therapy.[29-32] One of the largest and most recent of these trials is the Dutch multicenter randomized CROSS trial (ChemoRadiotherapy for Oesophageal cancer followed by Surgery Study), which compared neoadjuvant chemoradiotherapy plus surgery to surgery alone.[33]

The recently completed CROSS trial was a multicenter, randomized phase III trial,[33] which started in March 2004 and included and analyzed 366 patients during a 5-year period. It included patients from 5 academic and 2 non-academic high-volume teaching hospitals in The Netherlands. The study compared neoadjuvant chemoradiotherapy (nCRT) followed by surgery with surgery alone in patients with potentially curable esophageal cancer (cT2-3 N0-1 M0 and cT1 N1 M0, according to the UICC TNM classification, 6th ed.[33]), with a planned inclusion of 175 patients per arm. The neoadjuvant regimen consisted of carboplatin (AUC=2) and paclitaxel (50 mg/m^2) given by intravenous infusion on days 1, 8, 15, 22 and 29, combined with concurrent radiation therapy delivered using a multiple field technique. A total dose of 41.4 Gy was given in 23 fractions of 1.8 Gy, 5 fractions per week, starting on the first day of the first cycle of chemotherapy. The aim of this trial was to compare overall survival between patients treated with nCRT followed by surgery and patients treated with surgery alone for potentially curable, esophageal adenocarcinoma or squamous cell carcinoma. The most common toxic effects in the chemoradiotherapy followed by surgery group were leukopenia (6%), anorexia (5%), fatigue (3%) and neutropenia (2%). Median overall survival of patients who received nCRT plus surgery was 49 months, compared to 24 months for those who received surgery alone. With a median follow-up of 32 months, 70 patients had died in the nCRT group versus 97 in the surgery-alone group. Three-year overall survival was superior in the nCRT arm (HR 0.66; 95% confidence interval 0.50-0.87; p=0.003).

Results from the CROSS trial show that the addition of neoadjuvant chemoradiotherapy (carboplatin, paclitaxel and 41.4 Gy of concurrent radiotherapy) to surgery significantly increases survival as compared to surgery alone. Therefore, neoadjuvant chemoradiation plus surgery is now considered the standard of care in The Netherlands for potentially curable esophageal cancer (cT2-4a N0-3 M0 and cT1 N1-3 M0, according to the UICC TNM classification, 7th ed.[14]) in patients fit to undergo this treatment.

New treatment strategy

In subsequent analyses of secondary endpoints of the CROSS trial a striking finding was made.[33] In the nCRT group 49% of patients with a squamous cell carcinoma and 23% of patients with an adenocarcinoma had a pathologically complete response (pCR) in the resection specimen (*i.e.* no viable tumor cells were found at the site of the primary tumor or in the resected regional lymph nodes, as determined by conventional histological examination). Therefore, these results impose an ethical imperative to reconsider and study the necessity of standard esophagectomy, which is associated with severe postoperative morbidity and a substantial impact on the quality of life[23,25-27], in all patients after application of the CROSS-regimen. Under analogous conditions, a non-operative management in rectal cancer patients with a clinically complete response after nCRT has been shown feasible and safe, leading to organ-sparing treatment with low morbidity and mortality rates and favorable long-term survival in a subset of these rectal cancer patients.[34-36]

Outline of the thesis

The research in this thesis addresses issues concerning prognostication and a new treatment strategy for potentially curable esophageal or esophagogastric junction cancer. The thesis is divided into two parts. Part I focusses on the prediction of survival using conventional and more novel prognostic factors in patients with esophageal or junctional cancer. While Part II focusses on the long-term survival benefit of neoadjuvant chemoradiotherapy plus surgery (as compared to surgery alone) and on the feasibility of a new treatment strategy for a subset of patients with potentially curable esophageal or junctional cancer.

Part I

Prognostication for esophageal and junctional cancer

In patients with cancer of the esophagus or esophagogastric junction, several prognostic factors for long-term survival after primary surgical resection have been identified. These include age[7,8], gender[8-11], weight loss[12,13], histological tumor subtype[37,38], tumor location[39-41], tumor length[42-44], clinical TNM-stage[45-47], tumor grade[37,48], surgical radicality[15-17] and pathological TNM-stage[41,49]. Prediction models have been developed to predict overall survival in individual patients, based on these prognostic factors.[50,51] However, most of these well-established prognostic factors have been identified and validated in the era of primary surgical resection. In **chapter 1** we quantify the impact of nCRT on these well-established prognostic factors, and develop and validate a prognostic model for patients treated with nCRT plus surgery for esophageal or junctional cancer. In patients undergoing nCRT, pretreatment stage can only be estimated using endoscopic ultrasonography (EUS), computed tomography (CT) and more recently positron emission tomography (PET) for the T- and N-stages and is known to be relatively inaccurate, especially for the N-stage.[52,53] In **chapter 2** we introduce and validate a novel method of determining pretreatment tumor extent, based on the extent of regressional changes (*e.g.* fibrosis, mucous lakes, keratin pearls, and/or foreign body giant cell reactions) and on the presence of residual tumor cells in the resection specimen. We determine the interobserver reproducibility of this new pretreatment pathological staging system, we compare this pretreatment pathological staging system with the pretreatment clinical staging system and we determine the value of this new pretreatment pathological staging system for posttreatment prognostication. As nCRT is known to frequently 'sterilize' regional lymph nodes, it is unclear whether extended lymphadenectomy after nCRT is still indicated for prognostic and therapeutic reasons. In **chapter 3** we compare the prognostic impact of total number of resected nodes and the number of resected positive nodes between patients who underwent nCRT plus surgery or surgery alone for esophageal or junctional cancer. In **chapter 4** we create and validate a small optimized panel of immunohistochemistry markers that could be used to segregate patients with esophageal adenocarcinoma into different prognostic groups.

Part II

New treatment strategy for esophageal and junctional cancer

The CROSS trial compared nCRT plus surgery to surgery alone in squamous cell carcinoma and adenocarcinoma of the esophagus or esophagogastric junction. Initial results, after a median follow-up 45 months, showed an absolute increase in five-year overall survival of 13% in favor of the nCRT plus surgery group. In **chapter 5** we report the long-term results of the CROSS trial and we analyze secondary end-points, such as progression-free survival and recurrence patterns. It is questionable whether patients with a pathologically complete response (pCR) in the resection specimen after nCRT have sufficient additional benefit to justify subsequent standard esophagectomy. Therefore, these high complete response rates impose a strong ethical imperative to clinically identify patients with pCR after nCRT. Several studies have tried to identify patients with pCR after nCRT using conventional endoscopy with histological biopsies, endoscopic ultrasonography (EUS), computed tomography (CT) and positron emission tomography (PET)[54-60]. However, results from these studies have been mostly disappointing. Before a watchful waiting policy can be safely considered in a subgroup of patients with esophageal cancer after nCRT, a better insight into the exact location of residual tumor in the esophageal wall and regional lymph nodes is needed. Therefore, in **chapter 6** we describe the exact location of residual tumor in the esophageal wall and resected lymph nodes after nCRT and we describe the tumor regression pattern of esophageal cancer as induced by nCRT. It remains unclear, however, whether time to surgery (TTS; *i.e.* the interval between the last day of nCRT and the day of surgery) has an impact on pCR rate, on short-term surgical outcome and on long-term survival. Theoretically, prolonged TTS might increase pCR rate and possibly improve disease-free survival because of a prolonged effect of nCRT. Conversely, prolonged TTS might lead to residual tumor outgrowth, increased difficulty of surgical resection with a higher postoperative complication rate and possibly a worse overall survival. In **chapter 7** we investigate the impact of TTS after nCRT on pCR rate, short-term surgical outcome and disease-free and overall survival in a cohort of patients with potentially curable esophageal or junctional cancer, who underwent nCRT according to CROSS[33] followed by surgical resection. Finally, in **chapter 8**, we describe the study protocol of a single arm diagnostic feasibility trial (preSANO, Dutch Trial Register NTR4834)[61] which is currently running in several Dutch high volume centers and aims to determine the accuracy of clinically detecting or predicting the presence of residual disease after nCRT.

Table 1 — Overview of studies in the thesis

Part I

Prognostication for esophageal and junctional cancer

Chapter	Design	Sample	Population	Research focus
2	Retrospective cohort The Netherlands	Patients with esophageal or junctional cancer, treated with surgery alone or nCRT plus surgery (CROSS-I, CROSS-II and post-CROSS)	1.017	To determine the prognostic value of patient, disease and treatment related characteristics
3	Retrospective cohort Rotterdam and Amsterdam	Patients with esophageal or junctional cancer, treated with nCRT plus surgery (CROSS-II and post-CROSS)	180	To determine the prognostic value of pre-treatment pathological tumor extent
4	RCT The Netherlands	Patients with esophageal or junctional cancer, treated with surgery alone or nCRT plus surgery, who underwent surgical resection (CROSS-II)	320	To determine the prognostic value of number of lymph nodes resected and number of positive lymph nodes resected
5	Retrospective cohort Rotterdam, Pittsburgh and Cambridge	Patients with esophageal or junctional adenocarcinoma, treated with surgery alone	1.040	To validate the prognostic value of epidermal growth factor receptor (EGFR), tripartite motif-containing 44 (TRIM44), and sirtuin 2 (SIRT2)

Part II

New treatment strategies for esophageal and junctional cancer

Chapter	Design	Sample	Population	Research focus
6	RCT The Netherlands	Patients with esophageal or junctional cancer, treated with surgery alone or nCRT plus surgery (CROSS-II)	366	To determine the long-term overall and progression-free survival benefit of nCRT plus surgery as compared to surgery alone
7	Retrospective cohort Rotterdam	Patients with esophageal or junctional cancer, treated with nCRT plus surgery (CROSS-I, CROSS-II and post-CROSS)	102	To describe the exact location of residual tumor in the esophageal wall and resected lymph nodes after nCRT
8	Retrospective cohort Rotterdam	Patients with esophageal or junctional cancer, treated with nCRT plus surgery (CROSS-I, CROSS-II and post-CROSS)	325	To determine the impact of time to surgery after nCRT on pCR rate, surgical outcome and survival

RCT: randomized controlled trial

CROSS-I cohort: nCRT plus surgery, single center non-randomized feasibility trial[62] (n=51, 2001–2004)

CROSS-II cohort: nCRT plus surgery or surgery alone, Dutch multicenter randomized controlled trial[33] (n=178/188, 2004–2009)

post-CROSS cohort: nCRT plus surgery, standard of care, 2009–2013).

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Chapter 2

Published:

An abbreviated version of this manuscript was accepted for publication.

Prediction of survival in patients with esophageal or junctional cancer: impact of neoadjuvant chemoradiotherapy on conventional prognostic factors

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Abstract

Background
The value of conventional prognostic factors is unclear in the era of multimodality treatment for esophageal cancer. This study aimed to quantify the impact of neoadjuvant chemoradiotherapy (nCRT) on well-established prognostic factors and to develop and validate a prognostic model.

Methods
Patients treated with surgery alone or with nCRT plus surgery were included. Multivariable Cox modeling was used to identify prognostic factors for overall survival, with treatment included as an interaction. A prediction model for individual survival was developed using stepwise backward selection in nCRT plus surgery patients. The model was internally and cross-validated and a nomogram was designed for use in clinical practice.

Results
In total, 1017 patients were included, 391 in the surgery alone group and 626 in the nCRT plus surgery group. Independent prognostic factors in the surgery alone group were age, tumor histology, surgical approach, radicality, pT-stage and pN-stage. Whereas, in the nCRT plus surgery group, only cN-stage and pN-stage remained. Tumor histology, surgical approach and pT-stage were significantly less prognostic, while cN-stage was significantly more prognostic in patients treated with nCRT plus surgery. The final prognostic model included cN-stage, pT-stage and pN-stage and had moderate discrimination (c-index at internal validation 0.63).

Conclusion
In nCRT plus surgery patients, only pretreatment cN-stage and post-treatment pN-stage remain as independent prognostic factors. The final prediction model, based on cN-stage, pT-stage and pN-stage, has moderate discriminatory ability. These results strengthen the need for new prognostic factors to improve survival prediction in the era of multimodality treatment for esophageal cancer.

Introduction

In patients with cancer of the esophagus or esophagogastric junction, several pretreatment prognostic factors for long-term survival after primary surgical resection have been identified. These include age[1,2], gender[2-5], weight loss[6,7], histological tumor subtype[8,9], tumor location[10-12], tumor length[13-15] and clinical TNM-stage[16,17]. Well-established prognostic factors which become available after esophagectomy include surgical approach[18,19], surgical radicality[20-22], tumor grade[8,23] and pathological TNM-stage.[12,24] Prediction models have been developed to predict overall survival in individual patients, based on these prognostic factors.[25,26] However, most of these well-established prognostic factors have been identified and validated in the era of primary surgical resection.

Recent studies show that the addition of neoadjuvant chemoradiotherapy (nCRT) to surgery substantially improves locoregional control and long-term survival as compared to surgery alone.[27,28] In many countries, nCRT plus surgery is now standard of care for these patients. However, the value of conventional prognostic factors and the accuracy of models for individual survival prediction are still unclear in the context of current multimodality treatment strategies and have not yet been investigated in a large patient cohort.

We aim (I) to quantify the impact of nCRT on several well-established prognostic factors, and (II) to develop and validate a prognostic model in patients treated with nCRT plus surgery for esophageal or junctional cancer.

Methods

Patient selection
Patients were included, who were treated with surgery alone as standard of care (pre-CROSS, 1993–2001), with nCRT plus surgery as part of the single-center non-randomized CROSS-I trial[29] (2001–2004), with surgery alone or nCRT plus surgery as part of the multicenter randomized controlled CROSS-II trial[28] (2004–2009), or with nCRT plus surgery as standard of care at the Erasmus MC, Rotterdam, The Netherlands or at the Academic Medical Center, Amsterdam, The Netherlands (post-CROSS, 2009–2013). Both squamous cell carcinoma (SCC) and adenocarcinoma (AC) histologies were included. Patients who did not receive at least 80% of the planned dose of chemoradiotherapy, who received a different nCRT regimen or in whom surgical resection was not completed were excluded. Inclusion criteria of the randomized CROSS-II trial[28] were retrospectively applied to patients treated with

surgery alone as part of standard of care, *i.e.* only those patients were included, who underwent the complete staging protocol and who had locally advanced disease (cT2-T4a or cT1N+).

Clinical staging
In all patients, pretreatment staging included endoscopy with biopsy, endoscopic ultrasonography (with fine needle aspiration [FNA] when indicated), CT scan of the neck, chest and abdomen and external ultrasonography of the neck (with FNA when indicated). PET scans were not routinely performed during this study period but were performed in some patients when available and indicated. Tumor location and tumor length were determined by pretreatment endoscopy. Clinical T-stage and N-stage were determined by endoscopic ultrasonography and CT-scanning with or without FDG-PET-scanning according to the Union for International Cancer Control (UICC) TNM Cancer Staging, 6th edition.[30]

Neoadjuvant and surgical treatment
Neoadjuvant chemoradiotherapy (nCRT) was given according to the CROSS regimen.[28,29] For carcinomas at or above the level of the carina a transthoracic esophageal resection (TTE) with a two-field lymph node dissection was performed. For carcinomas located well below the level of the carina, either TTE with two-field lymph node dissection or a transhiatal esophageal resection (THE) was performed depending on fitness of the patient and preference of the surgeon. For carcinomas involving the esophagogastric junction, THE was the preferred technique. In both the transthoracic and the transhiatal approach, an upper abdominal lymphadenectomy was performed, including resection of nodes along the hepatic artery, splenic artery and origin of the left gastric artery. Open- as well as minimally invasive techniques were used.

Pathological assessment
All histopathological parameters were prospectively collected. Tumor histology was determined based on the pretreatment biopsy, while tumor grade was determined in the resection specimen only. In the absence of residual tumor in the resection specimen, tumor grade was scored as 'non determinable' (Gx). A microscopically radical resection (R_0) was defined as a tumor-free resection margin ≥1 mm. R_1 was defined as a macroscopically radical resection, with a microscopically tumor-free resection margin <1 mm. Pathological T-stage and N-stage were (re)scored according to the UICC TNM Cancer Staging, 7th edition[31]. The tumor regression grade (TRG) was scored using the system as reported by Chirieac *et al*.[32,33].

Follow-up and data collection
Clinical and surgical characteristics were collected from prospectively maintained institutional databases. Survival was determined using hospital records and municipal registers. Survival was limited at five years to reduce the effect of death by other causes.

Data analysis
Data were described as medians with an interquartile range in case of continuous variables and frequencies with percentages in case of categorical variables. Grouped data were compared using Student's t-test and Pearson's chi-squared test. An advanced multiple imputation approach was used to impute missing data, resulting in five separate datasets.[34] Categories with less than 20 cases were combined with related categories. Weight loss was truncated at 10 kilograms (=p95). During imputation pT0 was set to combine with TRG1 and Gx. In the total patient cohort, hazard ratios (HRs), with corresponding 95% confidence intervals (CIs) were calculated using a multivariable Cox proportional-hazards model, with treatment (*i.e.* surgery alone or nCRT plus surgery) included as an interaction in the analysis. The following characteristics were included: age, gender, weight loss, tumor histology, tumor location, tumor length, clinical T-stage (cT), clinical N-stage (cN), surgical approach, radicality of resection, tumor grade in the resection specimen, pathological T-stage (pT), pathological N-stage (pN) and tumor regression grade (TRG). Survival was calculated from the end of therapy (day of surgery in both treatment groups) until death or end of follow-up. Differences in prognostic impact of the various characteristics were quantified by including statistical interaction terms for the two treatment groups ('treatment interaction'), defined as the HR associated with a characteristic among patients undergoing nCRT plus surgery divided by the HR for the same characteristic among patients undergoing surgery alone (*i.e.* HR_{nCRT+S}/HR_S). Significance was set to $p<0.05$.

Development, validation and visualization of prognostic model
A prognostic model was developed in the nCRT plus surgery group, using stepwise backward selection, where variables were excluded from the model in a stepwise manner, testing for the significance of elimination per variable[35], until no further improvement was achieved. The prognostic model was internally validated by correcting for optimism and cross-validated by dividing the total nCRT plus surgery cohort into a cohort with patients from the Erasmus MC, Rotterdam, and a cohort with patients from all other centers. Where the prognostic was developed in one cohort and validated in the other, and *vice versa*. The model was tested for prognostic accuracy, using Harrell's concordance-index (c-index).[36] The c-index determines for two randomly chosen subjects the probability that the model predicts a higher risk for the subject with poorer outcome. Analyses were performed using the

following R-packages[37]: 'multivariate imputation by chained equations' (mice)[34], 'regression modeling strategies' (rms)[38].

The prognostic strength of individual risk factors in the prognostic model were visualized in a nomogram. The weights for each category within an individual risk factor were calculated by multiplying the original coefficients of the multivariable Cox model with ten and rounding the result to the lowest whole number. The total number of points derived from all predictors was used to calculate the expected one-year and five-year overall survival rates.

Results

Patient, tumor and treatment related characteristics

In total, 1017 patients were included, of whom 391 were treated with surgery alone and 626 were treated with nCRT plus surgery. Median age at diagnosis was 63 years (table 1). Most patients were male (79%), had an adenocarcinoma (77%), most often clinically staged as cT3 (77%). Significant differences were found between surgery alone patients and nCRT plus surgery patients for weight loss (p=0.001), tumor location (p=0.045) and clinical N-stage (p<0.001) (table 1). Also, a transhiatal approach was performed significantly more often in the surgery alone group as compared to the nCRT plus surgery group (p<0.001).

Prognostic factors in patients treated with surgery alone or nCRT plus surgery

In patients treated with surgery alone, independent prognostic factors for overall survival were age (HR per decade=1.21 95%CI 1.05–1.40 p=0.009), tumor histology (HR SCC *vs.* AC=1.93 95%CI 1.36–2.75 p<0.001), surgical approach (HR TTE or other *vs.* THE=0.74 95%CI 0.55–0.98 p=0.036), radicality (HR R_1-R_2 *vs.* R_0=1.63 95%CI 1.25–2.13 p<0.001), pT-stage (HR pT3-pT4 *vs.* pT1=3.35 95%CI 1.64–6.85 p=0.001) and pN-stage (HR pN1 *vs.* pN0=2.07 95%CI 1.41–3.04 p<0.001, HR pN2 *vs.* pN0=2.99 95%CI 2.01–4.46 p<0.001 and pN3 *vs.* pN0=4.57 95%CI 3.01–6.95 p<0.001) (table 2). Whereas, in patients treated with nCRT plus surgery, the only independent prognostic factors were cN-stage (HR cN1 *vs.* cN0=1.46 95%CI 1.09–1.95 p=0.012) and pN-stage (HR pN1 *vs.* pN0=1.78 95%CI 1.32–2.39 p<0.001, HR pN2 *vs.* pN0=1.98 95%CI 1.29–3.02 p<0.001 and pN3 *vs.* pN0=4.34 95%CI 2.38–7.93 p<0.001). Specifically, TRG was not prognostic for survival (HR TRG1 *vs.* TRG2=0.77 95%CI 0.52–1.12, HR TRG3 *vs.* TRG2=1.21 95%CI 0.85–1.72 and TRG4 *vs.* TRG2=1.03 95%CI 0.69–1.54). This was also not the case when different groupings of TRG[39-41] were tested (data not shown).

A significant difference in prognostic value between the two treatment groups (*i.e.* treatment interaction) was identified for tumor histology (HR_{nCRT}+S/HRS SCC *vs.* AC=0.40 95%CI 0.24–0.67, $P_{interaction}$=0.001), indicating that tumor histology significantly decreased in prognostic value in the nCRT plus surgery group (*i.e.* the HR between subgroups decreased). Significant treatment interaction was also identified for cN-stage (HR_{nCRT}+S/HRS cN1 *vs.* cN0=1.55 95%CI 1.04–2.31, $P_{interaction}$=0.030), surgical approach (HR_{nCRT}+S/HRS TTE *vs.* THE=1.56 95%CI 1.04–2.33, $P_{interaction}$=0.030) and pT-stage (HR_{nCRT}+S/HRS pT3-pT4 *vs.* pT1=0.34 95%CI 0.15–0.78, $P_{interaction}$=0.011). These results indicate that clinical N-stage significantly improved in prognostic value in the nCRT plus surgery group, while surgical approach and pT-stage significantly decreased in prognostic value.

Prediction model for survival in patients treated with nCRT plus surgery

After stepwise backward selection, the final prediction model included cN-stage, pT-stage and pN-stage. Discrimination of the prediction model was moderate (c-index at internal validation 0.63). Cross-validation between the Erasmus MC cohort (n=246) and the other centers (n=380) was comparable (c-index 0.62 and 0.63, resp.). Discrimination of the prediction model was higher in surgery alone patients (c-index 0.66). Finally, a nomogram was constructed (figure 1) to allow for individual one-year and five-year overall survival estimations, based on the three variables included in the final prediction model. As an example, patients with pretreatment suspicion of nodal disease (cN1) and a complete response in the resection specimen (pT0, pN0) would have a total of two points, which corresponds with an estimated one-year and five-year survival rate of 88% and 62%, respectively.

Discussion

In this large and comprehensive study on patients with esophageal or junctional cancer, only clinical N-stage (cN-stage) and pathological N-stage (pN-stage) remained as independent prognostic factors in patients treated with neoadjuvant chemoradiotherapy (nCRT) plus surgery. Pathological T-stage (pT-stage) was added to the final prediction model for these patients after backward selection. Tumor histology, surgical approach and pT-stage were significantly less prognostic, while cN-stage was significantly more prognostic in patients treated with nCRT plus surgery as compared to patients who underwent surgery alone.

By using a single statistical model, which included both treatment groups, specific effects of nCRT on prognostic factors could be determined, independent from the effects of surgery. Results indicate an overall decrease in significance and in number of independent prognostic factors in patients treated with nCRT plus surgery. Interestingly, there was no overlap in significant independent prognostic factors between the two treatment groups, except for pN-stage, confirming previous reports[42,43,] and thus underlining the continued significance of pN-stage as an important prognostic factor in the era of multimodality treatment.[42-45]

Surprisingly, pretreatment clinical N-stage (cN-stage) increased in prognostic value in patients treated with nCRT plus surgery. In patients treated with surgery alone, cN-stage and pathological N-stage (pN-stage) are different estimations of the same disease state (*i.e.* pretreatment clinical estimation and posttreatment pathological estimation, resp.). Clinical N-stage is known to be relatively inaccurate[46,47] and therefore has little additional prognostic value (on top of pN-stage) in patients treated with surgery alone. However, in patients treated with nCRT plus surgery, cN-stage is no longer necessarily similar to pN-stage. By definition, cN-stage is an estimation of nodal involvement before nCRT, while pN-stage is an estimation of nodal involvement after nCRT. Therefore, cN-stage and pN-stage represent different disease states in these patients and cN-stage, although relatively inaccurate, had additional prognostic value, as was seen in these analyses.

Another important finding is that surgical approach lost prognostic value in patients treated with nCRT plus surgery. In patients treated with surgery alone, a transthoracic approach was associated with a significantly more favorable prognosis as compared to a transhiatal approach, whereas in patients treated with nCRT plus surgery, a transthoracic approach was associated with a non-significantly less favorable prognosis. These findings suggest that in patients treated with nCRT plus surgery, the benefit of a transthoracic approach is at best limited and the necessity for maximization of surgical lymph node retrieval should be questioned. However, only a new randomized trial, comparing these two surgical approaches (with their inherent differences in extent of lymphadenectomy) after neoadjuvant treatment will offer a more definitive answer.

The final prognostic model had moderate discriminatory ability in patients treated with nCRT plus surgery, which is lower than what is generally reported for other tumor types after neoadjuvant treatment.[48-50] Interestingly, the model (although developed in patients who underwent nCRT plus surgery) performed better in patients who underwent surgery alone. This indicates that from a prognostic perspective, neoadjuvant chemoradiotherapy has a strong equalizing effect on patients, making individual survival predictions in the era of nCRT less reliable.

Unfortunately, this study could not identify any additional factors outside of the already well-established TNM-staging system that might contribute to more accurate prognostication in the era of multimodality treatment. Even the much studied and widely applied tumor regression grading (TRG) systems[32,33,39-41] were not significantly associated with survival in this large and homogeneous patient cohort, thus questioning the usefulness of TRG as an independent prognostic factor in esophageal or junctional cancer patients. These results, therefore, strengthen the need for new prognostic factors, such as genetic and molecular markers, to improve the accuracy of individual survival prediction in the era of multimodality treatment for esophageal and junctional cancer.

Limitations

Although this study only included parameters that have been collected prospectively, the time period was relatively long (1993-2013), which might have caused bias in the comparison of patients treated with surgery alone or with nCRT plus surgery despite the selection of all patients according to the same inclusion criteria as applied in the CROSS-I and CROSS-II trials. A further limitation is that not all recognized prognostic factors in esophageal and junctional cancer could be included in this study, such as extracapsular lymph node involvement[51-53], signet cell features in esophageal adenocarcinomas[54,55] and genetic and molecular markers.[56,57]

Conclusions

Most conventional prognostic factors lose their prognostic significance in patients with potentially curable esophageal or junctional cancer, when treated with neoadjuvant chemoradiotherapy (nCRT) plus surgery. In the era of nCRT, clinical N-stage and pathological N-stage remain as independent prognostic factors. Surgical approach, which is of prognostic relevance in patients treated with surgery alone, loses its prognostic significance after nCRT, thus questioning the necessity of maximization of surgical lymph node retrieval. Furthermore, tumor regression grading is not independently associated with survival in patients treated with nCRT plus surgery. The final prediction model, based on clinical N-stage, pathological T-stage and pathological N-stage, has moderate discriminatory ability. These results strengthen the need for new prognostic factors to improve survival prediction in the era of multimodality treatment for esophageal and junctional cancer.

Table 1 — Clinical, surgical and histopathological characteristics in 1017 patients with potentially curable carcinoma of the esophagus or esophagogastric junction, treated with surgery alone or neoadjuvant chemoradiotherapy (nCRT) plus surgery.

	Total	Surgery alone	nCRT plus surgery	
	(n=1017) 1993 – 2013	(n=391) 1993 – 2009	(n=626) 2001 – 2013	
	n (%)*	**n** (%)*	**n** (%)*	**p****
Age [years]				0.275
Median (p25 – p75)	63 (56 – 69)	63 (55 – 69)	63 (56 – 69)	
Gender				0.578
Female	212 (21)	78 (20)	129 (22)	
Male	805 (79)	313 (80)	451 (78)	
Weight loss [kg]				**0.001**
Median (p25 – p75)	3 (0 – 6)	2 (0 – 5)	3 (0 – 6)	
Missing (%)	42 (4)	23 (6)	19 (3)	
Tumor histology[€]				0.866
Squamous cell carcinoma	224 (22)	85 (22)	139 (22)	
Adenocarcinoma	783 (77)	302 (77)	481 (78)	
Undeterminable	10 (1)	4 (1)	6 (1)	
Tumor location[§]				0.045
Cervical	1 (0)	1 (0)	– –	
Upper third esophagus	12 (1)	9 (2)	3 (1)	
Middle third esophagus	129 (13)	48 (13)	81 (13)	
Lower third esophagus	653 (65)	240 (63)	413 (66)	
Esophagogastric junction	211 (21)	86 (22)	125 (20)	
Missing	11	7	4	
Tumor length[§] [cm]				0.262
Median (p25 – p75)	5 (3 – 6)	4 (3 – 6)	5 (3 – 6)	
Missing (%)	85 (9)	38 (10)	47 (8)	
cT-stage[‡]				0.739
cT1	20 (2)	8 (2)	12 (2)	
cT2	193 (20)	80 (21)	113 (19)	
cT3	757 (77)	281 (75)	476 (78)	
T4	16 (2)	6 (2)	10 (2)	
Missing	31	16	15	
cN-stage[‡]				**<0.001**
cN0	387 (39)	205 (55)	182 (30)	
cN1	599 (61)	169 (45)	430 (70)	
Missing	31	17	14	

	Total	Surgery alone	nCRT plus surgery	
	(n=1017) 1993 – 2013	(n=391) 1993 – 2009	(n=626) 2001 – 2013	
	n (%)*	**n** (%)*	**n** (%)*	**p****
Surgical approach				**<0.001**
Transhiatal approach	487 (48)	263 (67)	6224 (36)	
Transthoracic approach	525 (52)	128 (33)	397 (63)	
Other	5 (1)	– –	5 (1)	
Radicality◇				**<0.001**
R_0	851 (84)	262 (67)	589 (94)	
R_1	163 (16)	126 (32)	37 (6)	
R_2	3 (0)	3 (1)	– –	
Tumor grade£				**<0.001**
Gx (undeterminable)	171 (20)	– –	171 (36)	
G1	40 (5)	31 (8)	9 (2)	
G2	327 (38)	194 (51)	133 (28)	
G3	313 (37)	155 (41)	158 (34)	
Missing	166	11	155	
pT-stageΔ	187 (19)			**<0.001**
pT0	128 (13)	– –	187 (30)	
pT1, includes pTis	169 (17)	39 (10)	89 (14)	
pT2	518 (51)	63 (16)	106 (17)	
pT3	9 (1)	278 (72)	240 (38)	
pT4	6	5 (1)	4 (1)	
Missing		6	–	
pN-stageΔ	523 (52)			**<0.001**
pN0	247 (24)	123 (32)	400 (64)	
pN1	149 (15)	101 (26)	146 (23)	
pN2	93 (9)	89 (23)	60 (10)	
pN3	5	73 (19)	20 (3)	
Missing		5	–	
Tumor regression grade¥ (TRG)	187 (30)			**–**
TRG1	135 (22)	– –	187 (30)	
TRG2	175 28)	– –	135 (22)	
TRG3	124 (20)	– –	175 (28)	
TRG4	5	– –	124 (20)	
Missing		–	5	

Legend table 1

* Data presented as median (interquartile range) or number (%). Percentages may not add up to 100 due to rounding.

** Data were compared between the surgery alone and nCRT plus surgery groups using Student's t-test for continuous variables and Pearson's chi-squared test for categorical variables.

€ Tumor histology was determined in the pretreatment biopsy.

§ Tumor location and tumor length were determined by endoscopy.

‡ Clinical T-stage and N-stage were determined by endoscopic ultrasonography and CT-scanning with or without FDG-PET-scanning according to the Union for International Cancer Control (UICC) TNM Cancer Staging, 6th edition.[30] cT1: (sub) mucosal involvement, cT2: proper muscle layer involvement, cT3: surrounding stroma involvement.

◇ R_0 was defined as a tumor-free resection margin ≥ 1 mm. R_1 was defined as a macroscopically radical resection, with a microscopically tumor-free resection margin < 1 mm.

£ Tumor grade was determined in the resection specimen only. Histological tumor grade was not determined in the pretreatment biopsy.

Δ Pathological T-stage and N-stage, as measured in the resection specimen were (re)scored according to UICC TNM Cancer Staging, 7th edition[31]; pT1: (sub)mucosal involvement, pT2: proper muscle layer involvement, pT3: surrounding stroma involvement; pN0: no lymph node positivity, pN1: 1-2 lymph nodes positive, pN2: 3-6 lymph nodes positive, pN3: ≥7 lymph nodes positive.

¥ Tumor regression grade was scored as defined by Chirieac *et al.*[32,33] : TRG1: no residual tumor cells found; TRG2: 1-10% residual tumor cells; TRG3: 11-50% residual tumor cells; TRG4: > 50% residual tumor cells.

Table 2 — Prognostic factors for overall survival in 1017 patients with potentially curable carcinoma of the esophagus or esophagogastric junction, treated with surgery alone or neoadjuvant chemoradiotherapy (nCRT) plus surgery.

	Surgery alone (n=391) 1993 – 2009			nCRT plus surgery (n=626) 2001 – 2013					
	HR	95% CI	p	HR	95% CI	p	HR_{nCRT+S} / HR_S	95% CI	P_{int}
Age [per 10 years]	1.21	(1.05 – 1.40)	**0.009**	1.12	(0.98 – 1.28)	0.099	0.93	(0.76 – 1.13)	0.444
Gender									
Female	0.80	(0.57 – 1.13)	0.198	0.77	(0.55 – 1.08)	0.129	0.96	(0.59 – 1.56)	0.879
Male	1 (ref)	–	–	1 (ref)	–	–	1 (ref)	–	–
Weight loss (per kg)	1.01	(0.97 – 1.05)	0.617	1.01	(0.98 – 1.05)	0.462	1.00	(0.95 – 1.06)	0.872
Tumor histology									
Squamous cell carcinoma	1.93	(1.36 – 2.75)	<0.001	0.77	(0.53 – 1.12)	0.173	0.40	(0.24 – 0.67)	**0.001**
Adenocarcinoma	1 (ref)	–	–	1 (ref)	–	–	1 (ref)	–	–
Tumor location									
Cervical-to-middle third esophagus	1 (ref)	–	–	1 (ref)	–	–	1 (ref)	–	–
Lower third esophagus	1.05	(0.69 – 1.61)	0.807	0.93	(0.60 – 1.44)	0.740	0.88	(0.48 – 1.62)	0.681
Esophagogastric junction	0.79	(0.48 – 1.31)	0.359	0.72	(0.42 – 1.23)	0.232	0.92	(0.44 – 1.90)	0.812
Tumor length [per cm]	1.00	(0.95 – 1.05)	0.942	0.97	(0.92 – 1.04)	0.414	0.98	(0.90 – 1.06)	0.579
cT–stage									
cT1 – cT2	1 (ref)	–	–	1 (ref)	–	–	1 (ref)	–	–
cT3 – cT4	0.90	(0.63 – 1.29)	0.566	1.16	(0.81 – 1.67)	0.415	1.29	(0.78 – 2.14)	0.325
cN–stage									
cN0	1 (ref)	–	–	1 (ref)	–	–	1 (ref)	–	
cN1	0.94	(0.71 – 1.24)	0.653	1.46	(1.09 – 1.95)	**0.012**	1.55	(1.04 – 2.31)	**0.030**
Surgical approach									
Transhiatal approach	1 (ref)	–	–	1 (ref)	–	–	1 (ref)	–	–
Transthoracic approach or other	0.74	(0.55 – 0.98)	**0.036**	1.15	(0.87 – 1.52)	0.333	1.56	(1.04 – 2.33)	**0.030**
Radicality									
R_0	1 (ref)	–	–	1 (ref)	–	–	1 (ref)	–	–
R_1 – R_2	1.63	(1.25 – 2.13)	**<0.001**	1.35	(0.85 – 2.15)	0.202	0.83	(0.48 – 1.41)	0.486
Tumor grade									
Gx	–	–	–	1.94	(0.36 – 8.61)	0.385	–	–	–
G1	1 (ref)	–	–	1 (ref)	–	–	1 (ref)	–	–
G2	1.47	(0.72 – 3.00)	0.289	2.78	(0.58 – 13.25)	0.202	1.89	(0.36 – 9.93)	0.454
G3	1.66	(0.80 – 3.46)	0.176	2.80	(0.67 – 11.74)	0.159	1.69	(0.35 – 8.13)	0.514
pT-stage	–	–	–	0.76	(0.49 – 1.17)	0.318	–	–	–
pT0	1 (ref)	–	–	1 (ref)	–	–	1 (ref)	–	–
pT1	1.87	(0.87 – 4.03)	0.110	1.02	(0.64 – 1.61)	0.945	0.54	(0.22 – 1.33)	0.182
pT2	3.35	(1.64 – 6.85)	**0.001**	1.15	(0.76 – 1.73)	0.499	0.34	(0.15 – 0.78)	**0.011**
pT3 – pT4									
pN-stage	1 (ref)	–	–	1 (ref)	–	–	1 (ref)	–	–
pN0	2.07	(1.41 – 3.04)	**<0.001**	1.78	(1.32 – 2.39)	**<0.001**	0.86	(0.53 – 1.40)	0.538
pN1	2.99	(2.01 – 4.46)	**<0.001**	1.98	(1.29 – 3.02)	**<0.001**	0.66	(0.37 – 1.18)	0.161
pN2	4.57	(3.01 – 6.95)	**<0.001**	4.34	(2.38 – 7.93)	**<0.001**	0.95	(0.46 – 1.97)	0.890
pN3									
Tumor regression grade*	–	–	–	0.77	(0.52 – 1.12)	0.173	–	–	–
TRG1	–	–	–	1 (ref)	–	–	–	–	–
TRG2	–	–	–	1.21	(0.85 – 1.72)	0.302	–	–	–
TRG3	–	–	–	1.03	(0.69 – 1.54)	0.895	–	–	–
TRG4									

Legend table 2

HR: hazard ratio.

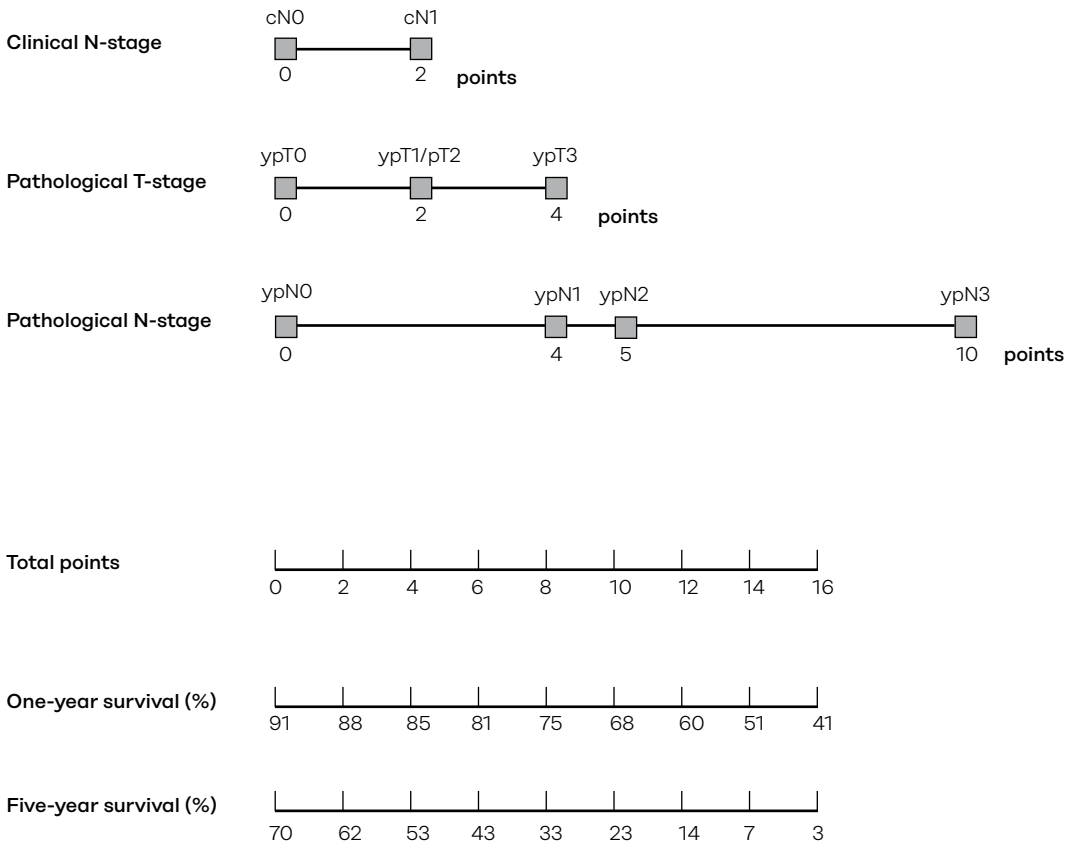
HR_{nCRT+S}/HR_S: HR associated with a characteristic among patients undergoing nCRT plus surgery divided by the HR for the same characteristic among patients undergoing surgery alone.

CI: confidence interval.

P_{int}: p-value for the treatment interaction (HR_{nCRT+S}/ HR_S).

* Also different groupings of TRG were tested: TRG1 *vs.* TRG2-4[39], TRG1 *vs.* TRG2-3 *vs.* TRG4[41] and TRG1-2 *vs.* TRG3-4[40] (data not shown). None of these groupings showed a significant independent effect.

Figure 1 — Nomogram for overall survival as developed in 626 patients with potentially curable carcinoma of the esophagus or esophagogastric junction, treated with neoadjuvant chemoradiotherapy (nCRT) plus surgery.



Legend figure 1

From the total points axis, a straight line down through the survival axes shows survival probabilities at one-and five years. Clinical N-stage according to UICC TNM Cancer Staging, 6th edition[30]; cN0: no clinical suspicion of pretreatment lymph node involvement, cN1: clinical suspicion of pretreatment lymph node involvement. Pathological T-stage according to UICC TNM Cancer Staging, 7th edition[31]; pT0: no residual tumor at the primary tumor site, pT1: (sub)mucosal involvement, pT2: proper muscle layer involvement, pT3: surrounding stroma involvement. Pathological N-stage according to UICC TNM Cancer Staging, 7th edition[31]; pN0: no lymph node positivity, pN1: 1-2 lymph nodes positive, pN2: 3-6 lymph nodes positive, pN3: ≥7 lymph nodes positive.

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Chapter 3

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Prognostic value of pretreatment pathological tumor extent in patients treated with neoadjuvant chemoradiotherapy plus surgery for esophageal or junctional cancer

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Abstract

Objective
We aimed to determine pretreatment pathological tumor extent in the resection specimen after neoadjuvant chemoradiotherapy (nCRT) and to assess its prognostic value in patients with esophageal cancer.

Methods
Patients with esophageal cancer, treated with nCRT plus surgery were included (2003 -2011). Pretreatment pathological T-stage (prepT-stage) and N-stage (prepN-stage) were estimated based on the extent of regressional changes and residual tumor cells in the resection specimen. Interobserver agreement was determined between three pathologists. The prognostic performance of prepT-stage and prepN-stage was scored using the difference in Akaike's information criterion (ΔAIC). PrepN-stage and posttreatment pathological N-stage (ypN-stage) were combined to determine the effect of nodal sterilization on prognosis.

Results
Overall concordance for prepT-stage and prepN-stage was 0.69 and 0.84, respectively. Prognostic strength of prepT-stage was similar to cT-stage and worse compared to ypT-stage (ΔAIC 1.3 *vs.* 2.0 and 8.9, resp.). In contrast, prognostic strength of prepN-stage was better than cN-stage and similar to ypN-stage (ΔAIC 17.9 *vs.* 6.2 and 17.2, resp.). PrepN+ patients who become ypN0 after nCRT have a worse survival compared to prepN0 patients, with a five year overall survival of 51% *vs.* 68%, p=0.019, respectively.

Conclusions
PrepT-stage and prepN-stage can be estimated reproducibly. Prognostic strength of prepT-stage is comparable to cT-stage, while prepN-stage is better than cN-stage. PrepN+ patients who become ypN0 after nCRT have a worse survival compared to prepN0 patients. Pretreatment pathological staging, should be considered useful as a new staging parameter for esophageal cancer and could also be of interest for other tumor types.

Introduction

An important indicator of prognosis in esophageal cancer is the TNM-stage.[1-3] The TNM-staging system consists of three categories which classify the depth of invasion of the primary tumor (T), the number of involved lymph nodes (N) and the presence of distant dissemination (M). The TNM-staging system has been validated extensively in literature for many tumor types treated with surgery alone, based on measurements in the surgical resection specimens, classified as the pathological TNM-staging (pTNM).[2,3]

In recent years, potentially curative treatment of esophageal cancer has shifted to include neoadjuvant therapy prior to surgical resection.[4-6] Unfortunately, in a post-neoadjuvant therapy setting, the pTNM-staging system, based on the residual disease in the resection specimen (ypTNM), largely loses its prognostic strength.[7-9] However, the percentage of residual viable tumor cells in the resection specimen after neoadjuvant therapy was found to be of prognostic value which resulted in several tumor regression grading (TRG) systems.[10-13]

Consequently, in patients undergoing neoadjuvant therapy, the conventional pretreatment stage can only be estimated using clinical evaluation criteria during initial clinical work-up. This pretreatment clinical TNM-staging (cTNM) relies on endoscopic ultrasonography (EUS), computed tomography (CT) and more recently positron emission tomography (PET) for the T- and N-stages and is known to be relatively inaccurate, especially for the N-stage.[14,15] Therefore, an updated TNM staging system is necessary, suited for the era of neoadjuvant treatment. More specifically, an improved estimation of the pretreatment stage is needed.

In the present study we introduce and validate a novel method of determining pretreatment pathological tumor extent, based on the extent of regressional changes (*e.g.* fibrosis, mucinous lakes, keratin pearls, and/or foreign body giant cell reactions) and on the presence of residual tumor cells in the resection specimen. We aim (I) to determine the interobserver reproducibility of this new pretreatment pathological staging system, (II) to compare this pretreatment pathological staging system with the pretreatment clinical staging system and (III) to determine the value of this new pretreatment pathological staging system for posttreatment prognostication.

Methods

Patient selection and clinical staging

Patients were included with potentially curable esophageal or junctional cancer, who were treated with neoadjuvant chemoradiotherapy (nCRT) plus surgery according to the CROSS regimen at the Erasmus MC – University Medical Center, Rotterdam or at the Academic Medical Center, Amsterdam, The Netherlands, between 2003 and 2011. Both squamous cell carcinoma (SCC) and adenocarcinoma (AC) tumor types were included. Patients who did not receive at least 80% of the planned dose of chemoradiotherapy, who received a different nCRT regimen or in whom surgical resection could not be completed, were excluded.

Clinical staging and treatment

In all patients, pretreatment work-up included endoscopy with histological biopsy, endoscopic ultrasonography (with fine needle aspiration when indicated), CT scan of the neck, chest and abdomen, external ultrasonography of the neck (with fine needle aspiration when indicated) and more recently FDG-PET-CT. Pretreatment clinical T-stage and N-stage were determined by endoscopic ultrasonography and CT-scanning and/or FDG-PET-CT scanning according to the Union for International Cancer Control (UICC) TNM Cancer Staging, 7th edition.[1] The neoadjuvant treatment regimen according to the CROSS regimen has been described before in detail.[6] Esophagectomy was performed either via a transthoracic approach (with two-field lymph node dissection) or a transhiatal approach. In both the transthoracic and the transhiatal approach, an upper abdominal lymphadenectomy was performed, including removal of nodes along the hepatic artery, splenic artery and origin of the left gastric artery.

Conventional pathological assessment

The resection specimens (primary tumor and all resected lymph nodes) were processed according to a standardized protocol.[16] Pathological T-stage and N-stage were scored according to the UICC TNM Cancer Staging, 7th edition[1], with N0: no nodes positive; N1: 1-2 lymph nodes positive; N2: 3-6 lymph nodes positive; N3: ≥7 lymph nodes positive. The tumor regression grade (TRG) was scored using the system as reported by Mandard *et al*.[10], TRG1: no residual tumor cells found; TRG2: 1-10% residual tumor cells; TRG3: 11-50% residual tumor cells; TRG4: >50% residual tumor cells; TRG5: no signs of tumor regression.

Pretreatment pathological assessment

The original tumor area —before nCRT— was estimated based on the extent of regressional changes (*e.g.* fibrosis, mucinous lakes, keratin pearls, and/or foreign body giant cell reactions) plus the presence of residual tumor cells in the resection specimen, as was previously described in patients with rectal cancer.[17-20] The evaluating pathologists (KB, GJAO, FJWtK, SLM) carefully examined all slides of all cases. The extent of regressional changes in the esophageal wall and peri-esophageal stroma was included in the interpretation of the 'pretreatment pathological T-stage' (prepT-stage), reflecting the estimated original invasion depth of the primary tumor. Pretreatment T4a tumors were categorized as prepT3, in order to prevent a staging category with very few patients, which could give unreliable results. The presence of such regressional changes in lymph nodes in addition to lymph nodes containing vital tumor cells was used for interpretation of the 'pretreatment pathological N-stage' (prepN-stage), reflecting the estimated number of originally involved lymph nodes.

Follow-up and data collection

Clinical characteristics were collected from prospectively maintained databases. Survival was determined using hospital records and municipal registers. All patients were regularly evaluated in the outpatient clinic during the first five postoperative years. Overall survival was calculated from the day of surgery and was limited at five years to reduce the effect of death by other causes.

Data analysis

Data were described as medians with an interquartile range (IQR) in case of continuous variables and frequencies with percentages in case of categorical variables. Grouped data were compared using Student's t-test and Pearson's chi-squared test. A value of $p<0.05$ (two-sided) was considered statistically significant. Statistical analysis was performed with SPSS 21 for Windows (SPSS, Chicago, IL, USA).

Interobserver agreement

In order to determine the reproducibility of prepT-staging and prepN-staging, the interobserver agreement was quantified in a subgroup of 90 consecutive patients from the Erasmus MC, Rotterdam. The interobserver agreement was determined between three independently scoring upper-GI pathologists (KB, GJAO, FJWtK) for prepT-stage, ypT-stage, prepN-stage, ypN-stage and TRG using the weighted kappa statistic (κ_w)[21,22] per pair of scorers and using the intraclass correlation coefficient (ICC)[23] to quantify the overall concordance. In case of disagreement, the median score was used for further analyses. A κ_w or ICC of 0.0 or less was considered to represent poor agreement, 0.01–0.20 slight, 0.21–0.40 fair, 0.41–0.60 moderate, 0.61–0.80 substantial and 0.81–1.00 near-perfect agreement.[24] PrepN-stage and ypN-stage were correlated as both ordinal variables and continuous variables.

Comparison of staging systems
Survival was calculated by the Kaplan–Meier method and differences between groups were assessed by the log-rank test. Prognostic strength of a model was measured by the likelihood ratio chi-squared statistic (LR χ^2) of the corresponding Cox proportional hazards model minus two times the degrees of freedom (df). This corresponds to the difference between Akaike's information criterion (AIC) of the model and the null-model (ΔAIC).[25] A higher ΔAIC value indicates better prognostic ability, adjusted for the statistical complexity of the model fit. PrepN-stage and ypN-stage were analyzed as ordinal variables and as continuous variables. For models with continuous variables, restricted cubic splines with three knots (corresponding with two degrees of freedom) were used.

Combining pretreatment and posttreatment N-staging systems
Pretreatment pathological N-stage (prepN-stage) was considered to represent the estimated lymph node involvement before neoadjuvant chemoradiotherapy, based on the presence of residual tumor plus regressional changes in resected lymph nodes. Nodes that showed regressional changes, without the presence of residual tumor were considered to have been sterilized by neoadjuvant chemoradiotherapy. Using both the pretreatment pathological N-stage (prepN) and posttreatment pathological N-stage (ypN), patients were categorized into three groups: I) patients who never had nodal involvement, II) patients who had nodal involvement pretreatment, but became node-negative after nCRT and III) patients who remained node positive after nCRT.

Results

Clinical and histopathological characteristics
Some 206 patients were evaluated, of whom 24 were excluded because they did not undergo surgical resection and two additional patients were excluded because they received less than 80% of the planned nCRT regimen. In total, 180 patients were included. Median age at diagnosis was 61 years (table 1). Most patients were male (76%), had an adenocarcinoma (77%), most often clinically staged as cT3 (75%) and cN+ (68%). Patients were most often staged as prepT3 (77%) and prepN+ (56%) (table 2). The median (IQR) number of resected lymph nodes was 18 (13-23).

Interobserver agreement
In 90 of 180 patients, the resection specimens were independently scored by three upper-GI pathologists. We found high concordance with intraclass correlation coefficients (ICC) between 0.7 to 0.9 (table 2).

Comparison of prepT-stage to cT-stage and ypT-stage
Some 43 of 180 patients (24%) had non-concordant pretreatment T-stages (table 4a). Of these 43 patients, 21 had a more advanced and 22 had a less advanced prepT-stage compared to the cT-stage. The overall survival curves according to cT-stage, prepT-stage and ypT-stage are shown in figure 1. Prognostic strength of prepT-stage was comparable to cT-stage and worse compared to ypT-stage (ΔAIC 1.3 *vs.* 2.0 and 8.9, resp., table 3).

Comparison of prepN-stage to cN-stage and ypN-stage
Some 96 of of 180 patients (53%) had non-concordant pretreatment N-stages (table 4b). Of these 96 patients, 39 had a more advanced and 57 had a less advanced prepN-stage compared to cN-stage, More specifically, out of 57 clinically node negative patients, 22 patients (39%) showed pathological signs of pretreatment nodal involvement. Conversely, out of 123 clinically node positive patients, 45 patients (37%) showed no pathological signs of pretreatment nodal involvement in the resection specimen. The overall survival curves according to cN-stage, prepN-stage and ypN-stage are shown in figure 2. Prognostic strength of prepN-stage was better than cN-stage and similar to ypN-stage (ΔAIC 17.9 *vs.* 6.2 and 17.2, resp., table 3). Counting of involved lymph nodes further improved prognostic strength of prepN-stage, but not for ypN-stage (ΔAIC 22.2 and 13.1, resp.).

Combining prepN-stage and ypN-stage
As shown in figure 3, the group of patients who never had nodal involvement (*i.e.* no residual tumor and no regressional changes) had a better survival compared to patients who had nodal involvement

pretreatment, but became node-negative after nCRT and compared to patients who remained node positive after nCRT. The five year overall survival was 68% *vs.* 51%, p=0.019 and 68% *vs.* 36%, p<0.001, respectively. Patients who had nodal involvement pretreatment, but became node-negative after nCRT had a (statistically not significant) better five year overall survival compared to patients who remained node positive after nCRT (51% *vs.* 36% p=0.282). Finally, combining prepN-stage and ypN-stage in a multivariable model did not improve the prognostic strength of the univariable prepN-stage model (ΔAIC 18.8 , table 3).

Discussion

We found that the pathological estimations of pretreatment primary tumor extent (prepT) and especially pretreatment nodal involvement (prepN) in the resection specimen were highly reproducible in patients with esophageal or junctional cancer, treated with neoadjuvant chemoradiotherapy (nCRT) followed by surgery.

Clinical T-stage, based on EUS and CT, had comparable prognostic strength to prepT-stage. This might be explained by the relatively high accuracy of EUS for determining depth of tumor invasion, which is well above 80%[26] and increases to over 90% for locally more advanced tumors[27] (*i.e.* tumors invading the peri-esophageal stroma; pT3). The comparable strength of cT-stage to prepT-stage might be further explained by the distorting effect of the desmoplastic reaction (*i.e.* tumor-stroma interaction), which is frequently present at the invasive front and cannot easily be distinguished from therapy-induced fibrosis. Thereby, possibly, overestimating the percentage of tumors invading into the peri-esophageal stroma. Prognostic strength of prepN-stage was better than for cN-stage. Meaning, prepN-stage gave a more reliable estimation of nodal involvement. Estimation of nodal involvement by EUS and CT is known to be unreliable, with reported accuracies ranging from 70% to 80% and from 59% to 75%, respectively.[14,15]

Interestingly, prognostic strength of prepN-stage was also better than that of ypN-stage. Thus, indicating that prepN-stage was better at separating patients with good prognosis from patients with worse prognosis than ypN-stage. Several previous studies have emphasized the importance of continued node positivity after neoadjuvant chemoradiotherapy.[7,28-31] Also, the present study confirms the importance of ypN-stage (as expressed by its high ΔAIC). However, it appears that an accurate pathological estimation of pretreatment nodal involvement is even more informative than posttreatment pathological nodal involvement. Conceptually this is understandable, since most patients die from distant

disease recurrence after nCRT plus surgery and the risk of systemic dissemination is expected to be correlated more with pretreatment than with posttreatment nodal positivity.[32,33] This also explains that patients who had no residual disease in the resected lymph nodes (ypN0), but who did have pretreatment nodal involvement (prepN+) had a worse prognosis, as compared to patients who did not have any pretreatment nodal involvement (prepN0). Whether improvement in survival after nCRT is caused by the actual sterilization of lymph nodes, or whether a good nodal response is simply a marker of more favorable tumor biology cannot be concluded from the present data.

Finally, the kappa values found for prepT-stage (κ_w range: 0.59–0.78) and prepN-stage (κ_w range: 0.84–0.84) were comparable to the kappa values found for tumor regression grade (TRG; κ_w range: 0.84–0.93) and were generally higher than normally found for other diagnostic modalities, such as endoscopic ultrasonography (EUS; T-stage, κ_w range: 0.29–0.69 and N-stage, κ_w range: 0.49–0.56)[34] and computed tomography (CT; T-stage, κ_w range: 0.15–0.68 and N-stage, κ_w range: 0.03–0.45)[35]. This suggests that these new histopathological parameters could indeed have clinical applicability.

Currently, we can only speculate on additional treatment options for patients with (pretreatment) nodal involvement. Primarily, a need exists for an effective systemic treatment, which could be given adjuvantly (based on prepN-staging in the resection specimen) with the purpose of sterilizing distant (micro)metastases. The benefit of such additional treatment should then be studied for different prepN-stages.

In patients undergoing neoadjuvant therapy, the conventional pretreatment stage can only be estimated using clinical evaluation criteria during initial clinical work-up. However, clinical staging and especially clinical N-staging, is known to be relatively inaccurate. The relevance of the proposed prepN staging system is that this method allows for the exact histopathological correlation with ypN stage at the level of individual lymph nodes. In this study it has allowed us to address the biological significance of pretreatment node positivity in patients treated with nCRT plus surgery. This novel pretreatment pathological staging, which has also been recently described by Nieman *et al*.[36] should be validated in a larger, independent group of patients. If proven valid, pretreatment pathological staging, and especially pretreatment pathological N-staging, should be considered a useful new staging parameter for esophageal and junctional cancers and could also be of interest for other tumor types that are treated by neoadjuvant therapy followed by surgical resection.

Conclusions

The pretreatment pathological T-stage (prepT-stage) and pretreatment pathological N-stage (prepN-stage) can be reproducibly estimated in the resection specimen. Prognostic strength of prepT-stage is comparable to pretreatment clinical T-stage (cT-stage), while prognostic strength of prepN-stage is better than pretreatment clinical N-stage (cN-stage). Furthermore, prepN-stage better predicts overall survival than posttreatment pathological N-stage (ypN-stage). Patients who have pretreatment nodal involvement, but become node-negative after nCRT have a worse survival compared to patients without pretreatment nodal involvement. If proven valid, pretreatment pathological staging, and especially pretreatment pathological N-staging, should be considered a useful new staging parameter for esophageal and junctional cancers and could also be of interest for other tumor types that are treated by neoadjuvant therapy followed by surgical resection.

Table 1 — Clinical and histopathological characteristics in 180 patients with esophageal or junctional cancer treated with neoadjuvant chemoradiotherapy plus surgical resection.

Complete cohort					
	(n=180) n (%)*			(n=180) n (%)*	
Age [years]			**Number of nodes resected**		
median (p25 – p75)	61 (55 – 67)		median (p25 – p75)	18 (13 – 23)	
Gender					
female	43	(24)	**ypT-stage**[Δ]		
male	137	(76)	ypT0	59	(33)
			ypT1	32	(18)
Tumor type			ypT2	29	(16)
squamous cell carcinoma	42	(23)	ypT3	60	(33)
adenocarcinoma	138	(77)			
			ypN-stage[Δ]		
cT-stage[○]			ypN0	123	(68)
cT1	7	(4)	ypN1	40	(22)
cT2	38	(21)	ypN2	13	(7)
cT3	135	(75)	ypN3	4	(2)
cN-stage[○]			**Tumor regression grade**[¥] **(TRG)**		
cN0	57	(32)	TRG1	58	(32)
cN1	74	(41)	TRG2	49	(27)
cN2	46	(26)	TRG3	41	(23)
cN3	3	(2)	TRG4	32	(18)
			TRG5	-	-
prepT-stage[◊]					
prepT1	12	(7)			
prepT2	30	(17)			
prepT3	138	(77)			
prepN-stage[◊]					
prepN0	80	(44)			
prepN1	63	(35)			
prepN2	28	(16)			
prepN3	9	(5)			

Legend table 1

* Data presented as median (interquartile range) or number (%). Percentages may not add up to 100 due to rounding.

○ Clinical T-stage and N-stage were determined by endoscopic ultrasonography and/or CT-scanning and/or FDG-PET-scanning according to the Union for International Cancer Control (UICC) TNM Cancer Staging, 7th edition[1].

◇ Pretreatment pathological T-stage (prepT-stage) and N-stage (prepN-stage) represent the estimated extent of tumor before neoadjuvant chemoradiotherapy, based on the location of residual tumor plus regressional changes in the esophageal wall and resected lymph nodes. These regressional changes include fibrosis, mucous lakes, keratin pearls and foreign body giant cell reactions. Both prepT and prepN categories were staged according to the pT- and pN-stages as defined by the 7th edition of the TNM staging manual[1].

Δ Posttreatment pathological T-stage (ypT-stage) and N-stage (ypN-stage), as measured in the resection specimen were (re)scored according to UICC TNM Cancer Staging, 7th edition[1]; pT1: (sub)mucosal involvement, pT2: proper muscle layer involvement, pT3: surrounding stroma involvement; N0: no lymph node positivity, N1: 1-2 lymph nodes positive, N2: 3-6 lymph nodes positive, N3: ≥7 lymph nodes positive.

¥ The tumor regression grade (TRG) was scored using the system as reported by Mandard *et al.*[10]; TRG1: no residual tumor cells found, TRG2: 1-10% residual tumor cells, TRG3: 11-50% residual tumor cells, TRG4: > 50% residual tumor cells, TRG5: no signs of tumor regression.

Table 2 — Interobserver agreement between three pathologists for pretreatment T-stage (prepT-stage), pretreatment N-stage (prepN-stage), posttreatment T-stage (ypT-stage), posttreatment N-stage (ypN-stage) and tumor regression grade (TRG) in the resection specimen of 90 patients with esophageal or junctional cancer treated with neoadjuvant chemoradiotherapy plus surgical resection.

		1 to 2	1 to 3	2 to 3	Overall
	Data type	κ_w	κ_w	κ_w	ICC
prepT-stage◇	ordinal	0.78	0.59	0.71	0.69
ypT-stageΔ	ordinal	0.92	0.89	0.93	0.92
prepN-stage◇	ordinal	0.84	0.84	0.84	0.84
ypN-stageΔ	ordinal	0.95	0.92	0.93	0.93
TRG¥	ordinal	0.84	0.86	0.93	0.88

Legend table 2

◇ Pretreatment pathological T-stage (prepT-stage) and N-stage (prepN-stage) represent the estimated extent of tumor before neoadjuvant chemoradiotherapy, based on the location of residual tumor plus regressional changes in the esophageal wall and resected lymph nodes. These regressional changes include fibrosis, mucous lakes, keratin pearls and foreign body giant cell reactions. Both prepT and prepN categories were staged according to the pT- and pN-stages as defined by the 7th edition of the TNM staging manual[1].

Δ Posttreatment pathological T-stage (ypT-stage) and N-stage (ypN-stage), as measured in the resection specimen were (re)scored according to UICC TNM Cancer Staging, 7th edition[1]; pT1: (sub)mucosal involvement, pT2: proper muscle layer involvement, pT3: surrounding stroma involvement; N0: no lymph node positivity, N1: 1-2 lymph nodes positive, N2: 3-6 lymph nodes positive, N3: ≥7 lymph nodes positive.

Table 3 — Prognostic stratification of 180 patients with esophageal or junctional cancer treated with neoadjuvant chemoradiotherapy plus surgical resection based on pretreatment clinical T-stage (cT-stage), pretreatment pathological T-stage (prepT-stage), and post-treatment pathological T-stage (ypT-stage), and pretreatment clinical N-stage (cN-stage), pretreatment pathological N-stage (prepN-stage) and posttreatment pathological N-stage (ypN-stage).

T-staging

	Data type	LR χ^2	df	ΔAIC
cT-stage^{O}	ordinal	6.0	2	2.0
prepT-stage$^{\diamond}$	ordinal	5.3	2	1.3
ypT-stage$^{\Delta}$	ordinal	14.9	3	8.9

N-staging

	Data type	LR χ^2	df	ΔAIC
cN-stage^{O}	ordinal	12.2	3	6.2
prepN-stage$^{\diamond}$	ordinal	23.9	3	17.9
ypN-stage$^{\Delta}$	ordinal	23.2	3	17.2
prepN-stage$^{\diamond}$	continuous	26.2	2	22.2
ypN-stage$^{\Delta}$	continuous	17.1	2	13.1
prepN-stage$^{\diamond +}$ **ypN**$^{\Delta}$	continuous	26.8	4	18.8

Legend table 3

O Pretreatment clinical T-stage (cT-stage) and N-stage (cN-stage) were determined by endoscopic ultrasonography and/or CT-scanning and/or FDG-PET-scanning and (re) scored according to the Union for International Cancer Control (UICC) TNM Cancer Staging, 7th edition[1].

◇ Pretreatment pathological T-stage (prepT-stage) and N-stage (prepN-stage) represent the estimated extent of tumor before neoadjuvant chemoradiotherapy, based on the location of residual tumor plus regressional changes in the esophageal wall and resected lymph nodes. These regressional changes include fibrosis, mucous lakes, keratin pearls and foreign body giant cell reactions. Both prepT and prepN categories were staged according to the pT- and pN-stages as defined by the 7th edition of the TNM staging manual[1].

Δ Posttreatment pathological T-stage (ypT-stage) and N-stage (ypN-stage), as measured in the resection specimen were (re)scored according to UICC TNM Cancer Staging, 7th edition[1]; pT1: (sub)mucosal involvement, pT2: proper muscle layer involvement, pT3: surrounding stroma involvement; N0: no lymph node positivity, N1: 1-2 lymph nodes positive, N2: 3-6 lymph nodes positive, N3: ≥7 lymph nodes positive.

LR χ^2: likelihood ratio chi-squared statistic

df: degrees of freedom

ΔAIC: difference between Akaike's information criterion[25] of the model and the null-model. This measure represents the prognostic strength of a model and is calculated by the likelihood ratio chi-squared statistic (LR χ^2) of the corresponding Cox proportional hazards model minus two times the degrees of freedom (df). A higher ΔAIC value indicates better prognostic ability, adjusted for the statistical complexity of the model fit.

Table 4 — Interobserver agreement between three pathologists for pretreatment T-stage (prepT-stage), pretreatment N-stage (prepN-stage), posttreatment T-stage (ypT-stage), posttreatment N-stage (ypN-stage) and tumor regression grade (TRG) in the resection specimen of 90 patients with esophageal or junctional cancer treated with neoadjuvant chemoradiotherapy plus surgical resection.

A

prepT - stage$^{\diamond}$

		1	2	3	Total
cT - stage^{O}	**1**	2	3	2	7
	2	7	15	16	38
	3	3	12	120	135
Total		12	30	138	180

B

prepN - stage$^{\diamond}$

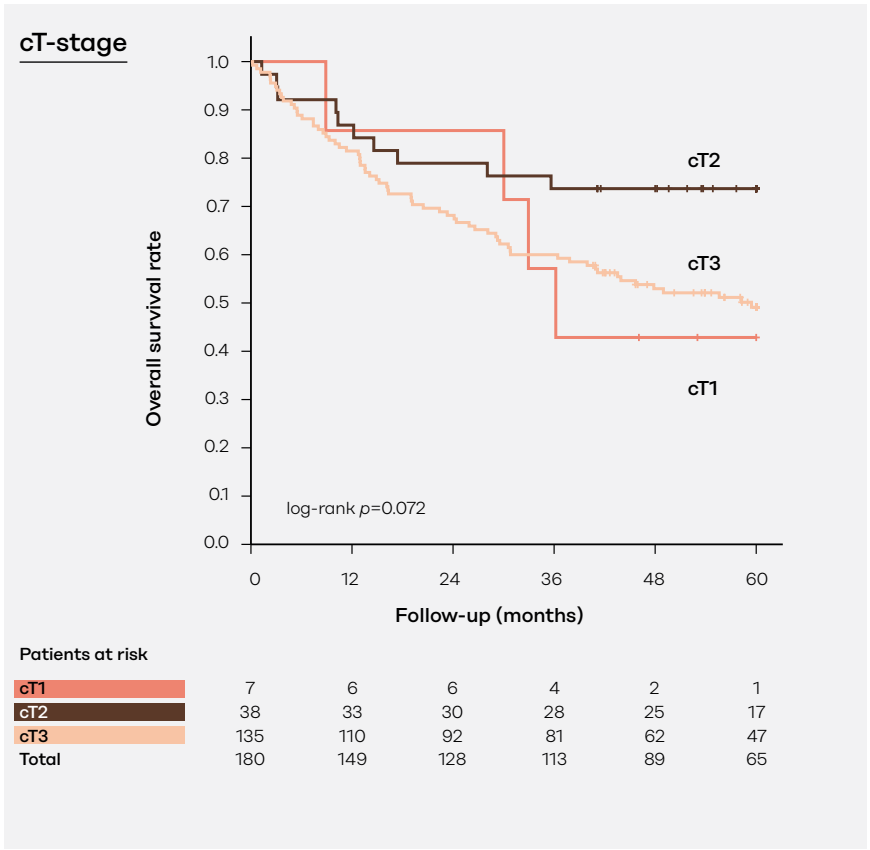
		0	1	2	3	Total
cT - stage^{O}	**0**	35	15	7	0	57
	1	26	36	9	3	74
	2	19	10	12	5	46
	3	0	2	0	1	3
Total		80	63	28	9	180

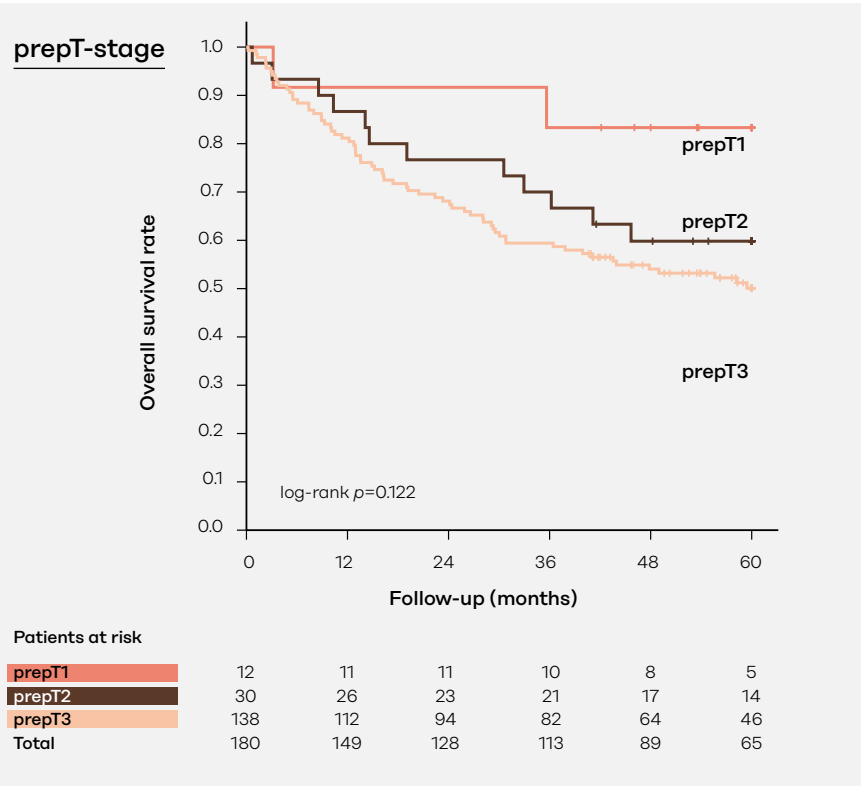
Legend table 4

O Pretreatment clinical T-stage (cT-stage) and N-stage (cN-stage) were determined by endoscopic ultrasonography and/or CT-scanning and/or FDG-PET-scanning and (re) scored according to the Union for International Cancer Control (UICC) TNM Cancer Staging, 7th edition[1].

◇ Pretreatment pathological T-stage (prepT-stage) and N-stage (prepN-stage) represent the estimated extent of tumor before neoadjuvant chemoradiotherapy, based on the location of residual tumor plus regressional changes in the esophageal wall and resected lymph nodes. These regressional changes include fibrosis, mucous lakes, keratin pearls and foreign body giant cell reactions. Both prepT and prepN categories were staged according to the pT- and pN-stages as defined by the 7th edition of the TNM staging manual[1].

Figure 1 — Overall survival according to pretreatment clinical T-stage (cT-stage), pretreatment pathological T-stage (prepT-stage) and posttreatment pathological T-stage (ypT-stage).





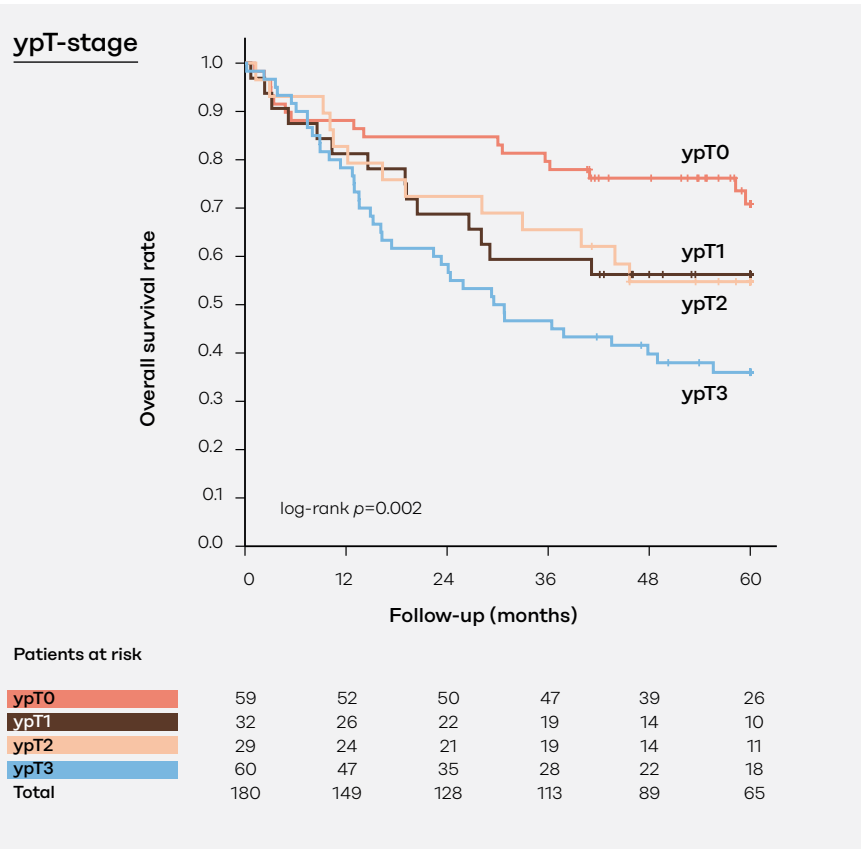
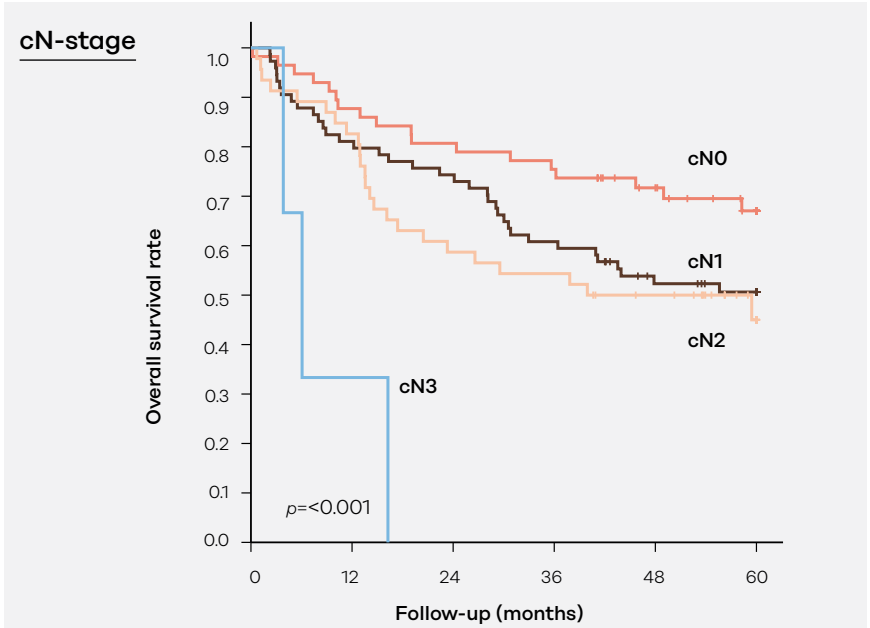
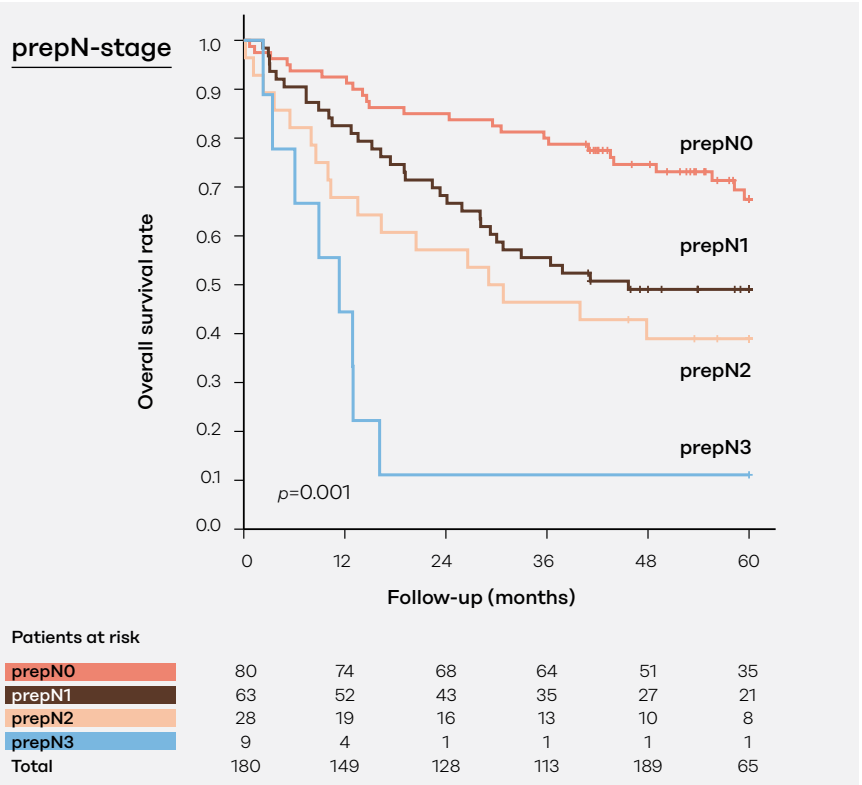


Figure 2 — Overall survival according to pretreatment clinical N-stage (cN-stage), pretreatment pathological N-stage (prep-N-stage) and posttreatment pathological N-stage (ypN-stage).



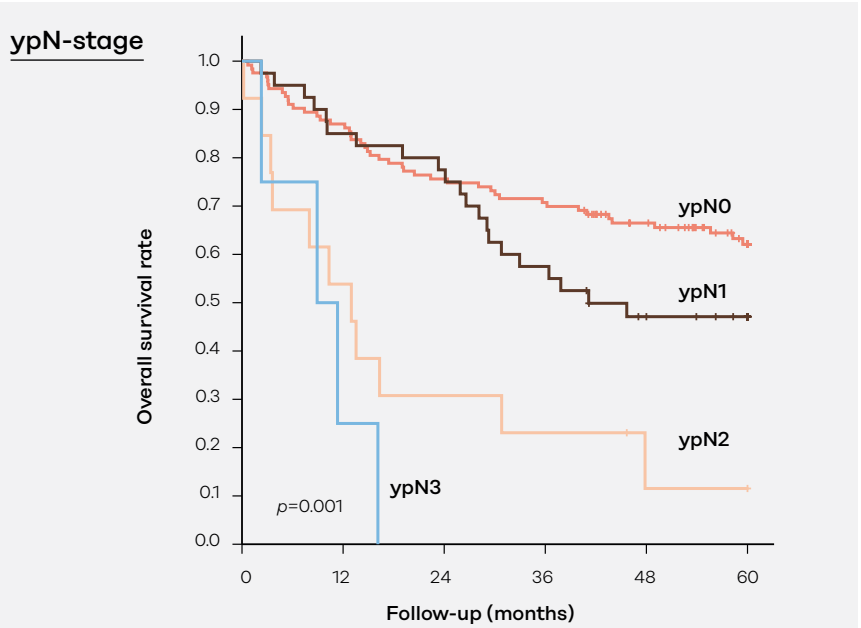
Patients at risk

cN0	57	50	46	43	35	26
cN1	74	60	55	45	34	30
cN2	46	38	27	25	20	9
cN3	3	1	-	-	-	-
Total	180	149	128	113	89	65



Patients at risk

prepN0	80	74	68	64	51	35
prepN1	63	52	43	35	27	21
prepN2	28	19	16	13	10	8
prepN3	9	4	1	1	1	1
Total	180	149	128	113	189	65



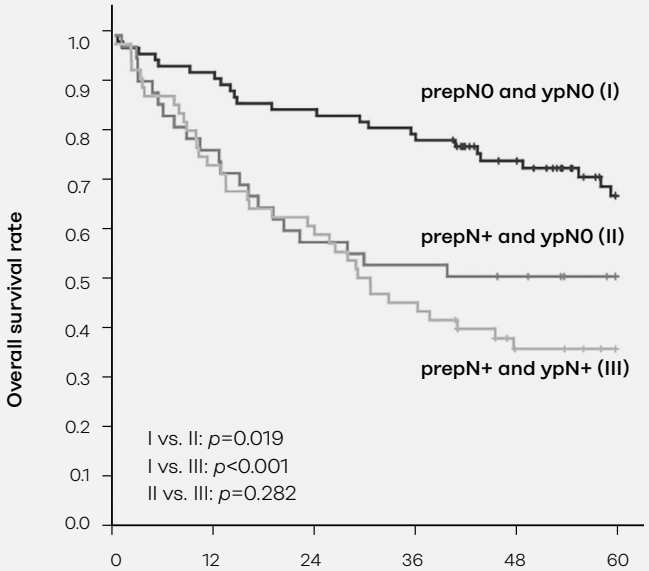
Patients at risk

ypN0	123	107	93	87	72	52
ypN1	40	34	31	23	16	12
ypN2	13	7	4	3	1	1
ypN3	4	1	-	-	-	-
Total	180	149	128	113	89	65

Figure 3 — Overall survival according to combined scoring of pre-treatment pathological N-stage (prepN) and posttreatment pathological N-stage (ypN).

prepN0: no lymph node positivity pretreatment, prepN+: ≥1 lymph node(s) positive pretreatment.

ypN0: no lymph node positivity posttreatment, ypN+: ≥1 lymph node(s) positive posttreatment.



Patients at risk						
prepN0 and ypN0	80	74	68	64	51	35
prepN+ and ypN0	43	33	25	25	21	17
prepN+ and ypN+	57	42	35	35	17	13
Total	180	149	128	113	89	65

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Chapter 4

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Lymph node retrieval during esophagectomy with and without neoadjuvant chemoradiotherapy; prognostic and therapeutic impact on survival

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Abstract

Objectives
We aimed to examine the association between total number of resected nodes and survival in patients after esophagectomy with and without neoadjuvant chemoradiotherapy (nCRT).

Background Data
Most studies concerning the potentially positive effect of extended lymphadenectomy on survival have been performed in patients who underwent surgery alone. As nCRT is known to frequently 'sterilize' regional nodes, it is unclear whether extended lymphadenectomy after nCRT is still useful.

Methods
Patients from the randomized CROSS-trial who completed the entire protocol (*i.e.* surgery alone or chemoradiotherapy plus surgery) were included. With Cox regression models we compared the impact of number of resected nodes as well as resected positive nodes on survival in both groups.

Results
161 patients underwent surgery alone and 159 patients received multimodality treatment. Median (interquartile range) number of resected nodes was 18(12-27) and 14(9-21), with 2(1-6) and 0(0-1) resected positive nodes respectively. Persistent lymph node positivity after nCRT had a greater negative prognostic impact on survival as compared to lymph node positivity after surgery alone. Total number of resected nodes was significantly associated with survival for patients in the surgery alone arm (hazard ratio (HR) per 10 additionally resected nodes, 0.76; p=0.007), but not in the multimodality arm (HR 1.00; p=0.98).

Conclusions
The number of resected nodes had a prognostic impact on survival in patients after surgery alone, but its therapeutic value is still controversial. After nCRT, number of resected nodes was not associated with survival. These data question the indication for maximization of lymphadenectomy after nCRT.

Introduction

Esophageal cancer is associated with early and chaotic lymphatic dissemination to both the neck, chest and abdomen[1, 2]. The lymphadenectomy accompanying esophagectomy is the main oncological factor that can be influenced by the surgeon, besides a complete resection of the primary tumor. Many investigators have previously attempted to explore the potential benefits of extended lymphadenectomy which include more accurate disease staging, better locoregional disease control, and perhaps even improved long-term survival. For staging purposes a more extended lymphadenectomy is intuitively superior to a more limited nodal dissection[3, 4]. The therapeutic impact of extended lymphadenectomy in esophageal cancer surgery, however, has remained controversial. Some authors state that surgery has reached its maximum therapeutic impact with limited lymphadenectomy, while others believe that the course of the disease can be influenced favorably by aggressive surgery with a more extended lymphadenectomy[5,6]. Although most studies have concluded that lymph node retrieval is associated with improved survival, the majority of these studies have been performed in patients undergoing surgery alone, which has led to recommendations regarding the optimal extent of lymphadenectomy ranging from 6-30 nodes[7,8]. Other studies investigated designated fields of dissection[3,4]. Prospective trials have been performed comparing survival after transhiatal and transthoracic esophagectomy[9], but a recent meta-analysis did not show any difference in survival between limited transhiatal and extended transthoracic operations[10].

Especially after publication of the randomized controlled CROSS trial[11], neoadjuvant chemoradiotherapy (nCRT) has become standard of care for esophageal cancer patients in many countries. As nCRT is known to frequently 'sterilize' regional nodes, it is unclear whether extended lymphadenectomy after nCRT is still indicated for prognostic and therapeutic reasons. The aim of the present study was, therefore, to examine the association between the total number of resected nodes and survival in patients with esophageal cancer undergoing surgical resection with and without nCRT.

Methods

Study population and follow-up

The study population consisted of patients who participated in the randomized CROSS-trial from March 2004 through December 2008[11]. Patients with histologically confirmed, potentially curable carcinoma of the esophagus or esophagogastric junction were randomly assigned to receive surgery alone or neoadjuvant chemoradiotherapy followed by surgery. The randomization process was stratified for histological tumor type, center and clinical N-stage. Patients were excluded who underwent exploratory thoracotomy or laparotomy only. Follow-up took place at regular intervals with a minimal follow-up of 24 months.

Clinical and pathological staging

Pretreatment clinical staging included endoscopy (and ultrasonography) with biopsy and CT of the neck, chest, and upper abdomen; and external ultrasonography of the neck, with fine-needle aspiration of suspected cervical lymph nodes. The surgical resection specimen was processed according to a standardized protocol. The clinical and pathological staging were based on the 6th and 7th edition of the TNM staging system respectively[12.] Tumor regression after nCRT was classified in the resection specimen as major response: ≤10% viable tumor cells and minor response: >10% viable tumor cells.

Neoadjuvant treatment and surgical approach

Patients randomized to neoadjuvant treatment underwent weekly administration of carboplatin (doses titrated to achieve an area under the curve of 2 mg/ml/min) and paclitaxel (50 mg/m^2) for 5 weeks and concurrent radiotherapy (41.4 Gy in 23 fractions, 5 days/week), followed by surgery.

For esophageal carcinomas at or above the level of the carina a transthoracic esophagectomy (TTE) with two-field lymph node dissection was performed. For carcinomas located well below the level of the carina, either a TTE with two-field lymph node dissection or a transhiatal esophagectomy(THE) was performed. THE encompassed *en bloc* dissection of the primary tumor and its adjacent lymph nodes under direct vision through the widened diaphragmatic hiatus up to the level of the inferior pulmonary vein. Dissected lymph nodes in the upper abdomen included the paracardial, lesser curvature, left gastric artery, celiac trunk, common hepatic artery, and splenic artery nodes. TTE included *en bloc* dissection of the azygos vein, thoracic duct, ipsilateral pleura, and all peri-esophageal tissue in the posterior mediastinum. Compared to THE, the resection specimen after TTE additionally included the middle mediastinal, subcarinal, paratracheal and aortopulmonary window lymph nodes. In the present study, 'extended' lymphadenectomy was defined in terms of numbers of lymph nodes retrieved.

Statistical analysis

Descriptive statistics included median and interquartile range for continuous variables and percentages for categorical variables. Mann-Whitney, Chi-square, and log-rank tests were used to assess statistical significance (p<0.05, two-sided). Overall survival was defined as the time interval between day of randomization and day of censoring or death and analysed with Kaplan-Meier and Cox regression analysis. Scatter plots of number of resected nodes versus number of resected positive nodes were constructed separately for both randomization arms. In these scatter plots, lines were fitted representing equal probabilities of death as calculated with Cox regression models. All analyses were performed using SPSS version 19.0 (SPSS Inc, Chicago, IL, USA) and R (version 2.14, R foundation for statistical computing, Vienna, Austria).

Results

Patient and tumor characteristics

Of 368 patients enrolled in the original CROSS trial, 180 were randomly assigned to nCRT+surgery, and 188 to surgery alone. In the nCRT+surgery group 161 patients actually underwent resection, of whom two patients were excluded from the present analysis because of missing values on the exact number of resected nodes. In the surgery alone group 161 actually underwent resection. In both groups, two out of three patients had signs of lymph node involvement during pretreatment investigations (table 1). Both groups were similar in the surgical approaches that were chosen. nCRT resulted in clear downstaging; in almost forty percent of patients no vital tumor cells were identified in the esophageal wall after nCRT (ypT0). R_0 resection rate increased from 69% in the surgery alone group to 93% in the nCRT+surgery group (p<0.01).

Impact of nCRT on number of resected nodes and number of resected positive nodes

The distribution of the number of resected nodes for both randomization groups is presented in figure 1, showing a leftward shift (*i.e.* fewer resected nodes) in the nCRT+surgery group. Median number (interquartile range) of resected nodes was 18(12-27) for the surgery alone group and 14(9-21) for the nCRT+surgery group (table 1). Mean difference in number of resected nodes between the surgery alone and nCRT+surgery group was 4.3 (p<0.001). Number of resected nodes was not associated with radicality of resection in both groups (data not shown).

Median number (interquartile range) of resected positive nodes for the surgery alone and nCRT +surgery group was 2(1-6) and 0(0-1) respectively (table 1), resulting in a leftward shift in the 7th TNM N-stage distribution of the nCRT+surgery group (Supplementary figure 1). Fewer positive nodes (mean difference, 3.4 nodes; $p<0.001$), but a comparable number of negative nodes (mean difference, 1.0 nodes; $p=0.37$) were resected in the nCRT+surgery group as compared to the surgery alone group (Supplementary figure 2).

Impact of number of resected nodes on number of resected positive nodes

In the surgery alone group a positive association was identified between number of resected nodes and number of resected positive nodes. This association was absent in the nCRT+surgery group (figure 2). The mean number of resected positive nodes in patients who underwent surgery alone ranged from 2.4 in patients with 0-10 resected nodes to 5.9 in patients ≥25 resected nodes.

Impact of number of resected (positive) nodes on survival

For surviving patients, the median follow-up was 48.7 months (range 25.5-80.9). The overall survival rate at 5 year was 44%, with 37% in the surgery alone group as compared to 50% in the nCRT+surgery group ($p=0.004$).

At univariable analysis, age, ypT-stage, resection margin involvement and number of resected positive nodes tended to be associated with survival in both groups (table 2). In multivariable Cox regression analysis, the number of resected nodes was significantly associated with survival (HR 0.76 per every 10 additionally resected nodes; $p<0.01$) in patients who underwent surgery alone. However, in the nCRT+surgery group, number of resected nodes was not associated with survival (HR 1.00, $p=0.87$), nor was it associated with survival within ypN0, ypN1 or ypN1-ypN3 patients (data not shown). The number of resected positive nodes was associated with survival in both groups, but lymph node positivity after nCRT was associated with a more negative impact on survival compared to lymph node positivity after surgery alone (HR 1.18 vs HR 1.12 per every additionally resected positive node, respectively), especially in combination with a minor pathological response to nCRT (HR 1.38, $p<0.05$; data not shown). Additionally, a stratified analysis for histological tumor type showed that the significant impact of number of resected nodes observed in adenocarcinoma patients treated by surgery alone (every 10 additionally resected nodes HR=0.71; $p<0.05$) disappeared after nCRT (HR=1.06; n.s.). In the group of squamous cell carcinoma patients there was a similar (smaller) effect after nCRT, but sample sizes were probably too small to reach significance (surgery alone: HR=0.73; n.s. *vs.* nCRT+surgery: HR=0.84; n.s.).

In figure 3 scatter plots are shown that depict the same correlation between number of resected nodes and number of resected positive nodes as is visualized in figure 2, but now for all individual patients. At a given number of resected positive nodes, the probability of death in the surgery alone group will become lower when the number of resected nodes increases (figure 3A), but will remain unchanged and will even tend to become higher in the nCRT+surgery group (figure 3B).

Discussion

After nCRT, the number of resected nodes and number of resected positive nodes were significantly decreased, as compared to the surgery alone group. Also, the positive correlation between number of resected nodes and number of resected positive nodes, which was significant in the surgery alone group, was not present in the nCRT+surgery group. The number of resected nodes was an independent prognostic factor for survival in patients who underwent surgery alone, but not in patients treated with nCRT followed by surgery. The addition of nCRT to surgery resulted in a significantly reduced number of resected positive nodes, but after this multimodality treatment node positivity was more strongly inversely associated with survival than after surgery alone.

Prognostic implications of number of resected nodes

Identifying positive nodes is informative for a patients prognosis. In the present study, the decreased number of nodes retrieved in the nCRT+surgery group resulted exclusively from a reduction in number of resected positive nodes, while the number of resected negative nodes was similar in both groups (Supplementary figure 2). This might be because many positive nodes are sterilized by nCRT[13]. Therefore, many initially positive nodes will contribute to the node negative category in the resection specimen after nCRT. The overall decrease in nodes resected after nCRT might therefore be compensated in the node negative category by the addition of formerly positive (*i.e.* sterilized) nodes. Interestingly, not only did the number of resected nodes and number of resected positive nodes decrease upon addition of nCRT to surgery, also the 'upstaging' effect of number of resected nodes on number of resected positive nodes disappeared (figure 2). This (absent) correlation suggests that the number of resected positive nodes found after nCRT is less dependent on sampling compared to resected positive nodes found after surgery alone.

In patients treated with surgery alone, the number of resected nodes was not correlated with overall survival in univariable analysis. However, in multivariable analysis, after correction for the number of

resected positive nodes, the number of resected nodes did show an independent association with overall survival (table 2). The difference in association from univariable to multivariable analysis is most likely caused by the dominant and confounding effect of resected positive nodes. Thus, after correction for the number of resected positive nodes, the smaller but significant prognostic effect of number of resected nodes is revealed.

For patients undergoing nCRT plus surgery, however, neither in univariable analysis, nor in multivariable analysis an association was found between the number of resected nodes and overall survival. Apparently, the prognostic value of the total number of resected nodes for survival is lost in patients treated with nCRT + surgery, even after correction for the number of resected positive nodes.

In the CROSS trial, the favorable effect of nCRT on lymph node positivity has been clearly shown: in the surgery alone group 76% of patients were pathologically node positive, versus 32% in the nCRT+surgery group. However, lymph node positivity in the nCRT+surgery group in itself tended to have a stronger negative prognostic impact on survival as compared to that in the surgery alone group. Apparently, persistent lymph node positivity after nCRT reflects a biologically unfavorable tumor biology, which is in line with previous publications[14-17].

Therapeutic considerations

After correction for the number of resected positive nodes, the number of resected nodes was significantly associated with survival in the surgery alone group (table 2). Removal of negative nodes might hence have not only a prognostic impact, but also a therapeutic impact in this group. The most important hypothesis supporting such genuine survival benefit of an extended lymphadenectomy is the clearance of micrometastases that can be present in up to 50% of histology-negative nodes and are associated with a poor outcome[18-20].

Some previous studies have shown that increasing the number of resected nodes is still relevant after nCRT[21-23], while other studies have concluded that it is not.[16, 24-26] In the present data, within the nCRT+-surgery group, no such prognostic impact of the number of resected nodes could be identified, l*et al*one any therapeutic impact on survival. This could possibly be explained by the sterilization of micrometastases after chemoradiotherapy.[27]

Some authors question any therapeutic impact of extended lymphadenectomy. In their view, lymph node metastases are simply markers of systemic disease and removal of the primary lesion plus the easily accessible peritumoral nodes alone will yield a similar survival[28]. Their

alternative explanation is that the suggested therapeutic effect is based on stage migration. Stage migration occurs when positive nodes in the extended part of the dissection change N-stage to a higher category (surgery alone group in figure 2), but at the same time have a more favorable prognosis than patients with a similar number of positive nodes from a more limited dissection (the so-called 'Will Rogers phenomenon'[29]). This 'stage purification' leads to unreliable stage-by-stage comparisons of survival.

In the present study, 'extended lymphadenectomy' was defined in terms of numbers of lymph nodes retrieved, which is a more reliable variable to study compared to surgical approach, which is not always synonymous with extent of lymph node stations sampling. Unfortunately, data on the exact location of lymph node stations from which individual lymph nodes were retrieved were not available. The strength of the present study is that patients were randomized. Therefore, the described difference in impact of the number of resected nodes on survival between both arms can be attributed to neoadjuvant chemoradiotherapy specifically. The multicenter design is both a strength (because of great variability and therefore generalizability) and a limitation (since there was no strict protocol for surgical approach nor for extent of lymph node stations sampling). To properly address the impact of surgical approach on lymph node retrieval and survival, a new randomized trial should be performed comparing a transhiatal and transthoracic approach after nCRT. Finally, the relatively small number of patients per randomization arm limited the statistical power.

In conclusion, lymph node positivity, especially if persistent after nCRT, is a strong negative prognostic factor for overall survival. The number of resected lymph nodes has an independent prognostic impact on survival in patients who undergo surgery alone. The therapeutic value of extended lymphadenectomy, however, remains questionable in this group. After nCRT, the number of resected nodes is not associated with survival. These data question the indication for maximization of lymph node dissection after nCRT for staging purposes as well as for therapeutic reasons.

Table 1 — Clinical and tumor characteristics of 320 patients with esophageal or junctional cancer who underwent surgical resection with or without neoadjuvant chemoradiotherapy (nCRT) in the CROSS trial[11].

	Surgery alone		nCRT + Surgery	
	(n=161)		(n=159)	
Age – median [years] (interq. range)	60	(54-66)	60	(55-67)
Male sex – no. (%)	129	(80.1)	121	(76.1)
Tumor type –no. (%)				
Squamous cell carcinoma	37	(23.0)	37	(23.3)
Adenocarcinoma	121	(75.2)	119	(74.8)
Other	3	(1.9)	3	(1.9)
Tumor location –no. (%)				
Proximal third esophagus	3	(1.9)	2	(1.3)
Middle third esophagus	16	(9.9)	24	(15.1)
Distal third esophagus	123	(76.4)	111	(69.8)
Esophago-gastric junction	18	(11.2)	22	(13.8)
Not specified	1	(0.6)	-	
Differentiation grade in biopsy –no. (%)				
Well differentiated	11	(6.8)	5	(3.1)
Moderately differentiated	73	(45.3)	50	(31.4)
Poorly differentiated	69	(42.2)	68	(42.8)
Not specified	8	(5.0)	36	(22.6)
Clinical N-stage (TNM6)* –no. (%)				
cN0	53	(32.9)	56	(35.2)
cN1	100	(62.1)	101	(63.5)
Not specified	8	(5.0)	2	(1.3)
Operative approach‡ –no. (%)				
TTE	87	(54.0)	88	(55.3)
THE	72	(44.7)	71	(44.7)
Other	2	(1.2)		
Complete pathological response –no. (%)	n/a		47	(29.6)
(y)pT stage (TNM7†)				
0/is	-		62	(39.0)
1	13	(8.1)	15	(9.4)
2	19	(11.8)	32	(20.1)
3	126	(78.3)	48	(30.2)
4	3	(1.9)	1	(0.6)
Not specified			1	(0.6)
Resection margin involvement∞ –no. (%)	111	(68.9)	147	(92.5)
R_0	49	(30.4)	12	(7.5)
R_1	1	(0.6)		
Not specified				
(y)pN stage (TNM7°) –no. (%)	39	(24.2)	108	(67.9)
0	43	(26.7)	35	(22.0)
1	41	(25.5)	11	(6.9)
2	38	(23.6)	5	(3.1)
3				
Number of resected nodes – median no. (interq. range)	18	(12-27)	14	(9-21)
Number of resected positive nodes – median no. (interq. range)	2	(1-6)	0	(0-1)

Legend table 5

* Clinical lymph-node (N) stage was assessed by means of endoscopic ultrasonography, CT, or 18F-fluorodeoxyglucose positron-emission tomography and was classified according to 6th edition of the UICC TNM classification; ‡TTE=transthoracic esophagectomy; THE=transhiatal esophagectomy; †Pathologic TNM stage was classified according to the 7th edition of the UICC TNM staging system; ∞R_0=resection margin microscopically tumor-free, ≥ 1mm; R_1=resection margin macroscopically tumor-free, but microscopically <1mm; °Pathologic node category according to 7th TNM-staging system: N0 (no positive nodes), N1 (1-2 positive nodes), N2 (3-6 positive nodes) and N3 (>6 positive nodes).

Table 2 — Hazard ratios (HR) for overall survival from univariable and multivariable Cox-regression analysis in 320 esophageal or junctional cancer patients who underwent surgical resection with or without neoadjuvant chemoradiotherapy (nCRT) in the CROSS trial [11].

		Univariable analysis (HR (95% CI))	
	Category	**Surgery alone**	**nCRT+surgery**
Age	Every 10 additional years	1.28 (1.03-1.60)	1.16 (0.90-1.51)
(y)pT stage	0*/in situ*	n/a	0.48 (0.29-0.81)
	ypT1	0.12 (0.03-0.50)	0.64 (0.28-1.44)
	ypT2	0.56 (0.30-1.06)	0.55 (0.31-1.01)
	ypT3	1 (ref)	-
	ypT4	0.28 (0.04-2.04)	7.11 (0.92-54.84)
Resection margin involvement	R_0	1 (ref)	-
	R_1	1.34 (0.90-2.00)	1.62 (0.78-3.38)
Number of resected nodes	Every 10 additionally resected nodes	0.95 (0.79-1.14)	1.02 (0.84-1.25)
Number of resected positive nodes	Every additionally resected positive node	1.11 (1.08-1.15)	1.15 (1.06-1.25)

		Multivariable analysis (HR (95% CI))	
	Category	**Surgery alone**	**nCRT+surgery**
Age	Every 10 additional years	1.20 (0.94-1.52)	1.26 (0.93-1.70)
(y)pT stage	0*/in situ*	n/a	0.55 (0.32-0.95)
	ypT1	0.14 (0.03-0.59)	0.64 (0.28-1.51)
	ypT2	0.80 (0.42-1.54)	0.44 (0.23-0.85)
	ypT3	-	-
	ypT4	0.25 (0.03-1.69)	5.44 (0.62-47.74)
Resection margin involvement	R_0	-	-
	R_1	1.42 (0.93-2.10)	1.20 (0.53-2.73)
Number of resected nodes	Every 10 additionally resected nodes	0.76 (0.61-0.95)	1.00 (0.84-1.25)
Number of resected positive nodes	Every additionally resected positive node	1.12 (1.08-1.16)	1.18 (1.07-1.29)

Figure 1 — Distribution of number of resected lymph nodes as assessed in the resection specimen of patients who underwent surgery alone (n=161) or neoadjuvant chemoradiotherapy (nCRT) followed by surgery (n=159). Compared to the surgery alone group, a leftward shift (*i.e.* fewer resected nodes) was observed in the nCRT+surgery group.

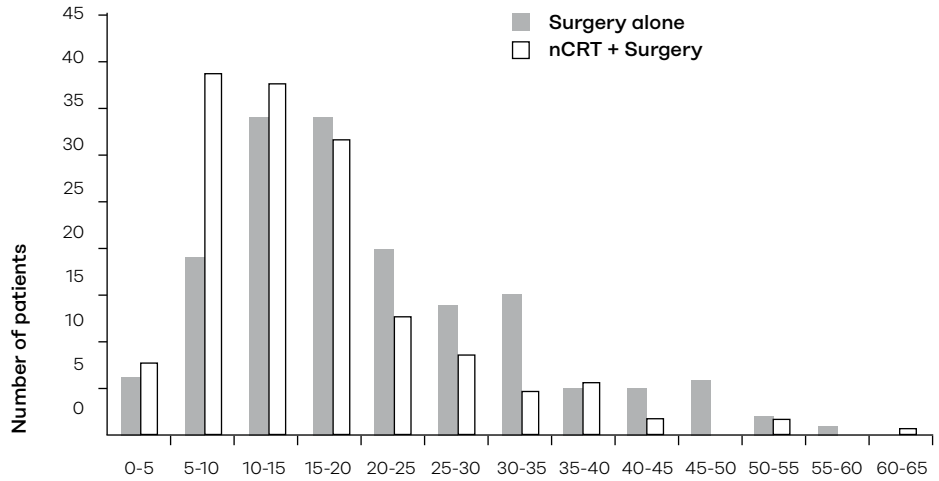


Figure 2 — Correlation between number of resected nodes (quartiles) and mean number (95% confidence interval) of resected positive nodes in patients who underwent surgery alone (n=161) or chemoradiotherapy (nCRT) followed by surgery (n=159).

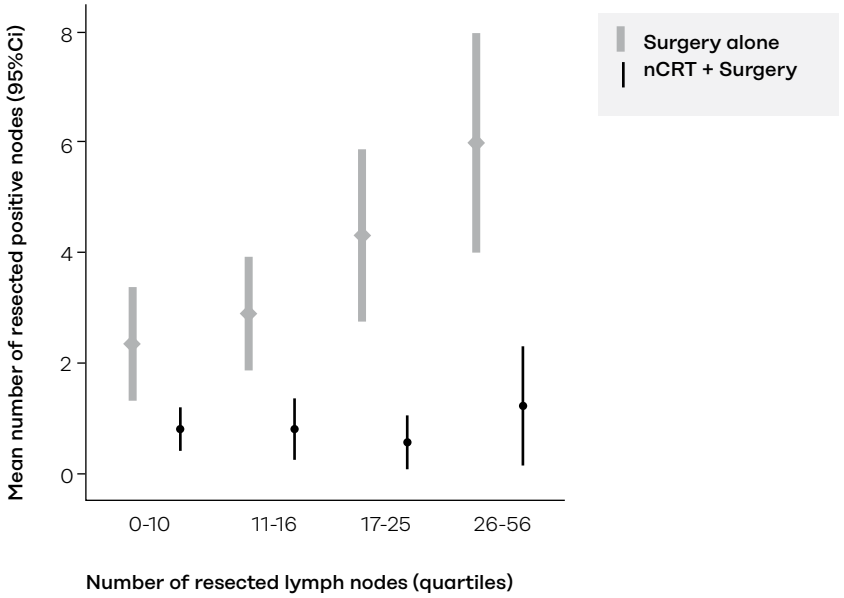
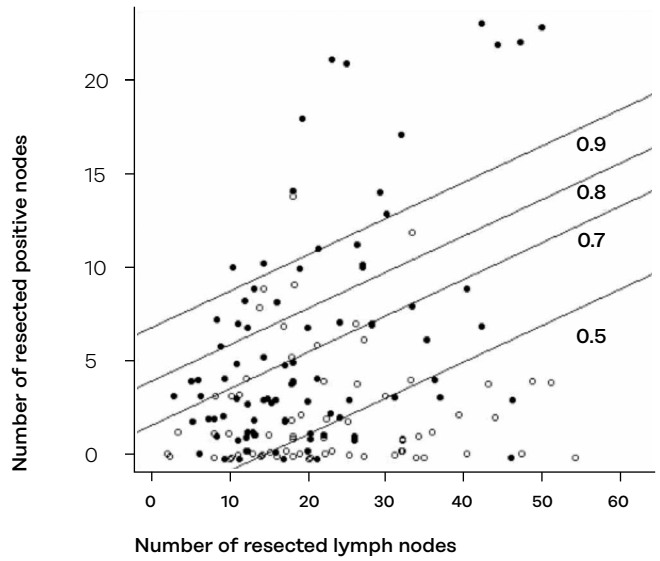
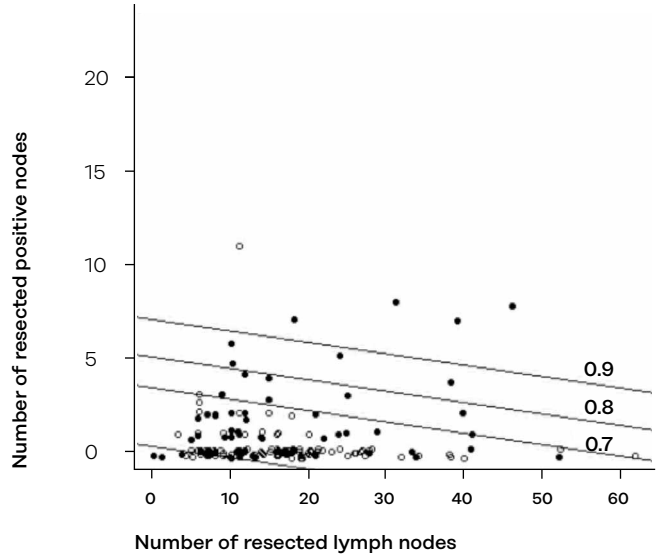


Figure 3 — Correlation between number of resected nodes and number of resected positive nodes in individual patients who underwent surgery alone (A: n=161) or neoadjuvant chemoradiotherapy (nCRT) followed by surgery (B: n=159). Open circles indicate patients who were alive at end of follow-up; closed circles indicate patients who had died at end of follow-up.

A



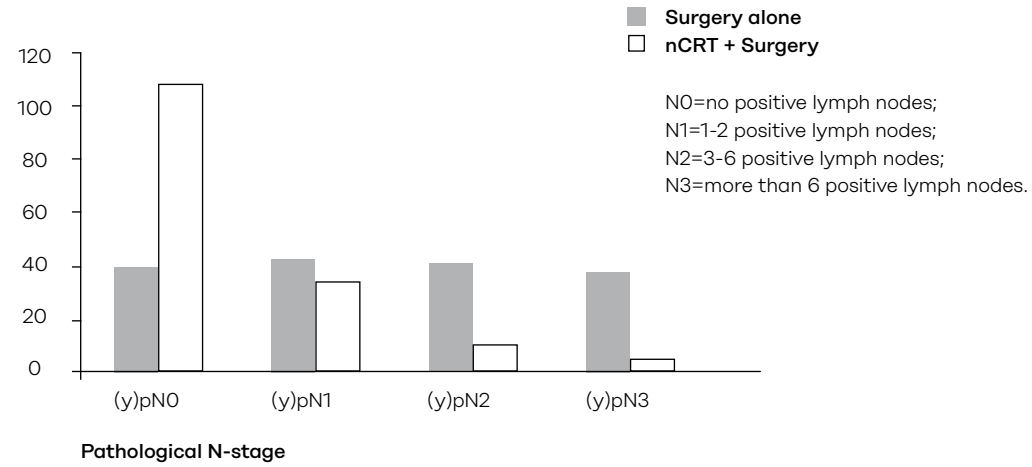
B



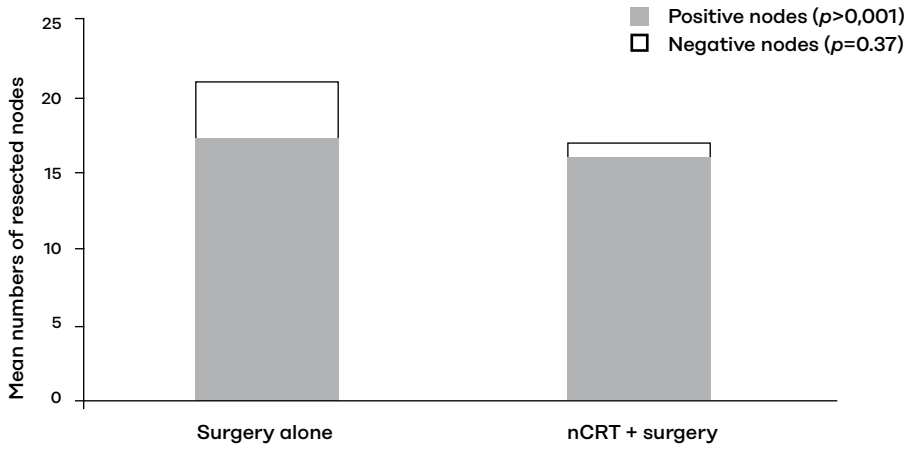
Legend figure 3

Lines represent equal probabilities of death as can be calculated by the proportion of closed (dead) and open (alive) circles. In both groups (A and B), an increase in the number of resected positive nodes results in a higher probability of death. In the patients who underwent surgery alone, lines are sloped i.e. at a given number of resected positive nodes more resected nodes in the specimen are associated with a decreased probability of death (A). In patients in the nCRT+surgery group, the probability lines have a more horizontal course, i.e. at a given number of resected positive nodes more resected nodes are not associated (and even tend to be positively associated) with probability of death (B).

Supplementary figure 1 — Pathological N-stage according to 7th edition of TNM staging system as assessed in patients who underwent surgery alone (n=161) or neoadjuvant chemoradiotherapy (nCRT) followed by surgery (n=159). Data indicate a leftward shift (*i.e.* fewer resected positive nodes) in the nCRT+surgery group.



Supplementary figure 2 — Comparison of mean number of positive and negative lymph nodes as assessed in the resection specimen of patients who underwent surgery alone (n=161)or neoadjuvant chemoradiotherapy (nCRT) followed by surgery(n=159).



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Extra discussions

Discussants 1

T. R. Demeester (Los Angeles, CA):
To Dr Talsma and the members of the CROSS trial, I want to congratulate you on a very carefully done and impeccably analyzed study. You have shown us that lymph nodes containing metastatic tumors have a prognostic impact on survival and can be destroyed through neoadjuvant chemoradiotherapy. You have also shown us that as the surgical therapy increases the number of nodes removed, survival also increases. But, this does not hold true for surgery after neoadjuvant chemoradiotherapy. And you have shown us that involved lymph nodes not destroyed by neoadjuvant chemoradiotherapy have a greater negative impact on survival than unexposed involved nodes. Based on these observations, you question, in your manuscript, the benefit of an en bloc esophagectomy to maximize lymph node resection to improve survival after neoadjuvant chemoradiotherapy. Your question is reasonable if the 2 assumptions are correct. The first assumption is that the surgical resections done in the study, transhiatal and transthoracic esohagectomy, were similar to the number of lymph nodes removed. The second assumption is that the stratification of involved lymph nodes was equal in the 2 arms of the study.

I have 3 questions for you: First, were the operations performed, whether transhiatal or transthoracic esophagectomies, similar in the number of nodes removed? If so, what was the basis for this conclusion, and if not, how did you adjust this factor?

Second, randomization is designed to be evenly distributed between the 2 arms of the study, as unknown factors affect survival. Factors known to affect survival, such as metastatic lymph nodes, need to be stratified. In your study, you stratified tumor histology, the center in which the operation was performed and the number of metastatic lymph nodes based on clinical staging. It would be difficult to stratify for metastatic lymph nodes by a clinical staging, when it had, according to my calculations, a 73% error rate, compared to the pathologic staging in the surgical alone arm of the study. Would you agree with this, and if not, do you have any way of confirming that there was an even distribution of metastatic lymph nodes in the 2 arms of the study, when using a clinical staging process with a 73% error rate?

Third, your question of the benefits of the en bloc esophagectomy to maximize lymph node resection after neoadjuvant chemoradiotherapy is in conflict with the experiences of others, of whom I am one, who have reported that en bloc esophagectomy provides a significantly better survival rate than THE for patients who have a complete response or who have had residual disease after neoadjuvant chemoradiotherapy. Is there any way you can reconcile these positive findings against your rather negative suggestions?

Response From K. Talsma, J. van Lanschot (Rotterdam, The Netherlands):
Thank you, Professor Demeester, for your questions. I will start with your first question, in which you refer to the surgical approach, which is heterogeneous in our population. At the end of the day, I think that what the reader would perhaps like to know is whether a more limited surgical approach after chemoradiotherapy would be acceptable. But, for us, it is impossible to answer this question with the present data. I agree that there were no strict instructions for surgical approach or extended lymphadenectomy. There were no instructions, in terms of the locations of nodes, so that they could be sampled or removed. But, we think that these specific surgical center preferences will be equally distributed in both randomization arms because this was stratified for each surgical center, and therefore, for surgical approach, and perhaps, surgical effort or aggressiveness. We are aware that defining extended lymphadenectomy only in terms of the number of nodes is a simplification. Extended lymphadenectomy is also about location, of course, and approach. However, we decided to use numbers because we think that they are more reliable and robust than surgical approach alone, as a transthoracic approach might in some cases even be less aggressive than a transhiatal approach; however, it is not synonymous with extended lymphadenectomy. In response to your question of whether a more limited approach is acceptable after chemoradiotherapy, thiswould really need a new HIVEX-type of randomized trial in the era of chemoradiation.

Your second question refers to the randomization process, which is used to stratify for unknown confounders. I fully agree that you should not correct things, which you are actually really interested in, such as the clinical stage you mentioned. Up until now, it is still very disappointing how poorly we can predict what the N stage actually is before surgery and neoadjuvant treatment. You mentioned that the error rate is also very high in the surgical alone group, if you compare the clinical N stage with the pathological data on lymph node involvement. Actually, we did a stratified analysis for clinical N stage and there was no difference. This is probably because it is just determined by the flip of a coin, or the chance that lymph nodes are involved during clinical staging. The chance that, at baseline, the actual number of positive nodes was different between the 2 randomization arms is as high as it is for the other confounders to be different, and we know that all of these were symmetrically distributed between the 2 groups. The third question refers to the other studies published. I am aware of the paper you mentioned. In patients with a complete pathological response, it was shown that there was still an increased survival for extended lymphadenectomy. But, I think that it was also mentioned in the paper that patients who really benefited from this extended surgical approach were nonresponders in the T-stage and had persistent nodal disease. So, these are the patients who depend more on the surgical part of the treatment than others. It still corroborates our hypothesis that there is a local clearance effect on the surgical field because of chemoradiation, and patients with persistent nodal disease depend more on surgical treatment.

Discussants 2

R. van Hillegersberg (Utrecht, The Netherlands):
Thank you very much for your interesting results. You mentioned that you think that the number of nodes is lower in the chemoradiotherapy group, due to the fact that the positive nodes cannot be identified anymore; they have been destroyed by the chemoradiotherapy. How did you conclude this, as I think that you did not show any evidence for this?

Another comment is that you also showed that there are still positive nodes, even in patients with complete tumor response to chemoradiotherapy. So, I think it would be too early to conclude that we could omit the lymphadenectomy in esophageal resection. There is no reliable diagnostic tool available to preoperatively identify patients with positive nodes after chemoradiation. So, even if you performed a restaging with biopsies, which did not show a viable tumor in the esophagus, I think we cannot omit an extended lymphadenectomy based on the results of this study.

Response From K. Talsma, J. van Lanschot (Rotterdam, The Netherlands):
We do not actually think that there is preferential sterilization of positive nodes. However, it has been shown that there is lymphocytic stroma fibrosis after radiation. Some lymph nodes really disappeared, and both positive and negative lymph nodes will disappear. However, the reduction in negative nodes will have been compensated by positive nodes, which became negative. Actually, if you imagine that this is a glass of Guinness (see Supplemental figure 2 in the manuscript), the foam will go down into the beer a little bit. So, in both groups, there is not a preferential sterilization with either positive or negative nodes, but the negative nodes were just compensated by the nodes that were positive before. Finally, I also agree that it is too early to omit an extended lymphadenectomy after this study.

Discussants 3

J. Reynolds (Dublin, Ireland):
Can I ask you whether, based on your data, the proponents of the value of lymph node ratio should reconsider this position, particularly in patients who have had neodjuvant chemoradiation? It really is all about the number of involved nodes; this numerator is key as long as the denominator is a large enough sample size. This is certainly my opinion—the number of positive nodes outweighs a ratio, which is impacted by neoadjuvant therapy. What is your view on this?

Response From K. Talsma, J. van Lanschot
(Rotterdam, The Netherlands): We deliberately decided to use positive and negative nodes, and not the lymph node ratio, as this would be difficult to use. If 68% of patients will become lymph node negative, then the nominator will be, for the biggest part, 0. We also think that it is easier to interpret the data if you just stick to positive and negative nodes.

Chapter 5

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Three-Gene Immunohistochemical Panel Adds to Clinical Staging Algorithms to Predict Prognosis for Patients With Esophageal Adenocarcinoma

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Abstract

Purpose

Esophageal adenocarcinoma (EAC) is a highly aggressive disease with poor long-term survival. Despite growing knowledge of its biology, no molecular biomarkers are currently used in routine clinical practice to determine prognosis or aid clinical decision making. Hence, this study set out to identify and validate a small, clinically applicable immunohistochemistry (IHC) panel for prognostication in patients with EAC.

Patients and Methods

We recently identified eight molecular prognostic biomarkers using two different genomic platforms. IHC scores of these biomarkers from a UK multicenter cohort (n=374) were used in univariate Cox regression analysis to determine the smallest biomarker panel with the greatest prognostic power with potential therapeutic relevance. This new panel was validated in two independent cohorts of patients with EAC who had undergone curative esophagectomy from the United States and Europe (n=666).

Results

Three of the eight previously identified prognostic molecular biomarkers (epidermal growth factor receptor [EGFR], tripartite motif-containing 44 [TRIM44], and sirtuin 2 [SIRT2]) had the strongest correlation with long-term survival in patients with EAC. Applying these three biomarkers as an IHC panel to the validation cohort segregated patients into two different prognostic groups (p=0.01). Adjusting for known survival covariates, including clinical staging criteria, the IHC panel remained an independent predictor, with incremental adverse overall survival (OS) for each positive biomarker (hazard ratio, 1.20; 95% CI, 1.03 to 1.40 per biomarker; p=0.02).

Conclusion

We identified and validated a clinically applicable IHC biomarker panel, consisting of EGFR, TRIM44, and SIRT2, that is independently associated with OS and provides additional prognostic information to current survival predictors such as stage.

Introduction

Esophageal adenocarcinoma (EAC) is a highly lethal cancer with a rapidly increasing incidence in the western world.[1] Despite advances in clinical care, the prognosis for EAC remains dismal with less than 20% of patients surviving 5 years.[2] Currently, standard staging algorithms based on tumor depth (T-stage), the presence and number of regional nodes with metastatic disease (N-stage), and the presence or absence of distant metastasis (M-stage) are used to predict survival for these patients.[3] This approach, however, does not take into account the biology and molecular features of each individual tumor, which may explain the widely varying 5-year overall survival, ranging from 11 to 41%, within groups of patients who otherwise appear similar by these standard staging algorithms.[4] It is increasingly evident that tremendous heterogeneity between patients exists in the biology underlying esophageal adenocarcinoma; and hence the ideal staging system would take into account the biology and molecular features of each individual tumor and correlate prognosis with patient-specific tumor biomarkers.[5,6] Importantly, advancing knowledge of the molecular characteristics of the tumor would also enable the application of targeted therapies to improve selective killing of cancer cells.[7-9]

Our group has previously described two independent methods to identify molecular prognostic markers in EAC using gene expression analysis and array-comparative genomic hybridization arrays.[10,11] These two independent studies identified eight biologically relevant molecular targets (TRIM44, SIRT2, EGFR, PAPPS2, NEIL2, WT1, MTMR9, DCK) which could be screened via immunohistochemistry (IHC) to help prognosticate patients with EAC. However, testing for multiple biomarkers via IHC would decrease clinical applicability and also, not all identified targets appear to have equal prognostic or therapeutic value. Therefore, to facilitate clinical utility, we aimed to create a small optimized panel of IHC markers selected from these eight potential molecular targets that can be used to segregate patients into different prognostic groups.

Methods

Study population used in generation and validation of best prognostic targets

Our original study cohort from six tertiary centers (OCCAMS study group) in the United Kingdom used to identify the eight molecular prognostic markers in EAC has previously been described.[10,11] Briefly, this cohort consisted of 374 patients with esophageal and gastroesophageal junction adenocarcinomas who underwent potentially curative surgery at one of the six OCCAMS centers.

For independent validation of the refined IHC biomarker panel, two independent retrospective cohorts of EAC patients (where paraffin material is available) from the University of Pittsburgh Medical Centre (UPMC) and the Erasmus MC, University Medical Center Rotterdam (EMC) were used. Following approval by the relevant institutional review boards, the clinical data from both centers were combined into a single database and reviewed for consistency. All patients had pathologically confirmed adenocarcinoma of the esophagus or gastroesophageal junction (GEJ), underwent esophagectomy with curative intent, and were followed up at their respective centers. In total, 363 patients from University of Pittsburgh Medical Center (UPMC; 1996-2009) in Pittsburgh, Pennsylvania and 314 patients from Erasmus Medical Center (EMC; 1995-2006) in Rotterdam were included as a combined validation cohort (n=677).

Clinical characteristics of patients from both validation centers are detailed in table 1. As expected, most patients who underwent surgery in both centers were male. However, there are some notable differences in clinical characteristics between the 2 centers. Patients from Pittsburgh were slightly older (67.0 versus 64.7 years old $p<0.001$) and had a shorter follow up time (24.5 versus 24.2 months $p=0.039$). More patients in the Pittsburgh cohort had an R_0 resection (94.4 versus 70.1 % $p<0.001$); accompanied by a lower rate of recurrence (36.8 versus 64.0 per cent $p<0.001$). More patients from the Pittsburgh cohort also had earlier T-stage and correspondingly fewer patients from Pittsburgh had neoadjuvant chemotherapy compared to patients from Rotterdam (2.5 versus 14.8 % $p<0.001$).

Generation and validation of a biomarker panel to prognosticate EAC patients

To generate a new IHC biomarker panel from the previously identified eight molecular prognostic targets, a Cox proportional hazards model was used to evaluate the hazard ratio for each molecular target in order to rank their prognostic importance. Molecular targets with the highest hazard ratios were selected and brought forward for validation in the above mentioned cohorts of EAC patients.

For validation of the three selected molecular targets as a prognostic IHC panel, archival slides from each tumor specimen from UPMC and EMC were reviewed by an expert pathologist who marked out areas representative of the tumor, accounting for tumor heterogeneity. Cores (0.6 mm) from 3 areas were then removed from paraffin blocks and TMAs constructed. IHC was performed on a Bond System (Leica Microsystems (UK) Ltd, Milton Keynes, UK) according to manufacturer's recommendations. Antibody sources, conditions used for IHC and scoring criteria are detailed in supplementary materials and methods.

Clinical End Points and Statistical analysis

The primary clinical end point in the validation study is overall survival (OS), defined as the time from surgery to death resulting from any cause. Patients who died within one month of surgery were deemed to have post-operative mortality and were excluded from further analysis. In total, 666 patients were included in the survival analysis. Death beyond 5 years was censored. To compare differences in demographic and clinical factors between the two validation cohorts, Student's t-test or Mann-Whitney test was used for continuous variables and the chi-square test was used for categorical variables. Kaplan-Meier curves were plotted to compare the five-year survival by the number of dysregulated molecular targets in the IHC panel. A multivariate Cox proportional hazards model was used to estimate the independent association between the IHC panel and prognosis after adjusting for demographic and clinical factors. A p-value of < 0.05 was considered statistically significant. All analyses were done using SPSS version 19.0 (SPSS Company, Chicago, IL).

Results

Generation of the revised prognostic biomarker panel

Using univariate Cox regression, IHC scores for each of the eight molecular prognostic targets were analyzed together for the first time in the original OCCAMS cohort (n=374). A significantly increased hazard for death was identified with dysregulation of EGFR, TRIM44 and SIRT2. (Supplementary table 1) Dysregulation of PAPPS2, NEIL2 and MTMR9 were associated with a non-significant increased hazard ratio for death, whereas WT1 and DCK were associated with a non-significant decreased hazard ratio for death. The biomarkers that were statistically significantly associated with differential hazard for death (TRIMM44, SIRT2 and EGFR) were then selected for inclusion into a combined biomarker panel for validation.

Validation of the revised prognostic biomarker panel

Validation of the new biomarker panel comprising TRIMM44, SIRT2 and EGFR began with internal validation using the OCCAMS study group. (Supplementary figure 2) In the OCCAMS study patients, median survival time for patients with 0, 1, 2 and 3 dysregulated molecular markers is 44.7, 18.2, 14.0, and 7.0 months respectively ($p=0.002$). For every one additional dysregulated molecular marker, the HR increases by 1.44 (95% CI 1.18 to 1.75). After adjusting for age, sex, T stage, N stage, neoadjuvant chemotherapy, and tumor differentiation (grade), the hazard for death for each additional dysregulated molecular biomarker increased by 1.35 (95%CI 1.09 to 1.68).

External validation of the IHC 3-biomarker panel was then performed on the cohort of patients from UPMC and EMC (table 2). When patients in the validation cohorts were grouped according to the total number of dysregulated markers, there was no difference in the overall survival of patients with 0 or 1 dysregulated markers (median survival time=38.8 months versus 29.8 months respectively, p=0.48). These two groups of patients were hence combined into one prognostic group. Due to the small number of patients with 3 dysregulated markers, these patients were also combined with patients with 2 dysregulated markers to facilitate clinical utility of the IHC panel. Patients with 2 or 3 dysregulated markers had a much poorer prognosis compared with patients with 0 or 1 dysregulated marker (Median survival 22.0 months versus 31.4 months respectively, p=0.004) (figure 1). The relative hazard ratios with dysregulation for each individual target are shown in Supplementary table 2. Representative examples of dysregulated and non-dysregulated IHC expression of TRIMM44, SIRT2 and EGFR performed on the validation cohorts from Pittsburgh and Rotterdam are shown in figure 2. The results of the IHC panel for each of the individual validation cohorts are shown in Supplementary figure 3.

Determining the independent prognostic value of the 3-biomarker IHC panel

Having demonstrated that the 3-biomarker panel had significant and additive predictive value for long-term survival, multivariate Cox regression analysis was performed to determine whether the IHC panel provided additional prognostic information independent of clinco-pathological features known to affect prognosis. Adjusting for centre, pathological T stage, N stage, age, sex, resection margin status, tumor differentiation and treatment with neoadjuvant chemotherapy, the results of the IHC panel remain significant in the multivariate cox regression model. (table 3) As seen from table 3, the hazard ratio for death increases by 1.20 (95% CI 1.03 to 1.40) for each increase on the IHC panel. The prognostic value of combined pathologic stage and increasing number of molecular markers was then assessed. Stratifying patients with both stage and number of molecular markers showed a clear separation of the entire cohorts into distinct prognostic groups in the validation cohorts (figure 3, p<0.001). The breakdown of all patients in various stages of the disease and number of molecular marker dysregulated are detailed in Supplementary table 3. Patients in stage I disease cannot be further stratified with the IHC panel. However, patients in stage II and III disease can be prognosticated based on the number of markers dysregulated. Patients with stage II disease have an increased hazard ratio for death of 1.376 (95% CI 1.018 to 1.860) for each increase on the IHC panel while patients with stage III disease similarly have an increased risk of death for every increase in number of dysregulated marker (HR 1.215, 95% CI 1.031 to 1.433).

Discussion

This present study demonstrates that a IHC panel consisting of a combination of three molecular markers generated and validated on 1040 EAC patients can be used to aid prognosis prediction in EAC patients. Furthermore, this IHC panel is independent of clinical features known to influence prognosis and can serve as an adjunct to current staging systems.

Reducing mortality from advanced esophageal adenocarcinoma remains the greatest challenge in the field.[12,13] To achieve this aim, accurate prognostication, development of better surgical care and chemo-radiotherapeutic regimes as well as identification of novel therapeutics are key areas to address.[5,6,12,14-16]

Previous studies have attempted to better determine prognosis for EAC patients using nomograms based on clinical features.[17-19] There have also been a large number of studies correlating molecular markers, identified by either a candidate gene approach or by a gene expression microarray approach, to prognosis in this disease.[5,20,21] However, none of these molecular panels have reached clinical utility, largely because these biomarkers or prognostic signatures are generated in underpowered cohorts. To best determine prognosis, we envision that a combination of clinical features and well-validated prognostic markers will be required to stratify EAC patients into clinically meaningful prognostic subgroups. Importantly, the molecular markers used must be independent of clinical features and ideally should account for the biology of these tumors rather than being purely molecular 'passengers'. This strategy formed the basis of this study to identify an independently prognostic IHC signature to be used in conjunction with current staging modalities.

The primary goal of this study was to identify a panel of biomarkers that could be readily implemented into clinical practice and provided improved prognostication for patients with this disease. This alone is of value to patients and their providers as it will facilitate more accurate discussions about long-term survival when compared to the discussion using current clinico-pathologic features alone. The ultimate goal, however, would be to use these molecular biomarkers to guide therapeutic interventions. Unlike other cancer types such as breast cancer, colorectal cancer and non-small cell lung cancer where targeted therapies are already in clinical use,[22] targeted therapy for EAC is still in its infancy. Biomarker targets with prognostic significance and biologic importance in tumor development would be the ideal clinical panel. Based on our own work and that of other investigators, the 3 biomarkers in our panel may provide both prognostic significance and

aid in identification of patient subgroups in whom targeted therapy may be useful. For example, EGFR is currently being evaluated as a potential target for therapy in this disease.[23,24] SIRT2 is a gene with known tumour suppressor and oncogenic functions in different tumour subtypes. In EAC, our data strongly suggest SIRT2 to be a tumour suppressor. A recent report highlighted SIRT2 to be a tumour suppressor in hepatocellular carcinoma and breast cancers by regulating mitosis and genome integrity[25]. Importantly, the authors report that SIRT2 deficiency causes increased levels of Aurora-A and Aurora-B (mitotic regulators) which results in promotion of tumour growth. Inhibitors targeting Aurora-A and Aurora-B are already being evaluated in clinical trials with promising results[26-28]. The next step would be to evaluate if SIRT2 deficient EAC would respond to targeted therapy with Aurora inhibitors in clinical studies. Lastly, TRIM44 belongs to a large family of TRIM proteins and recent scientific advances have identified this class of proteins to have the potential for pharmacological inhibition in cancer.[29,30] Although TRIM44 has previously unknown functions, recent data from our laboratory have demonstrated that EAC and breast tumours with high TRIM44 levels are highly susceptible to mTOR inhibition in vitro and in vivo, highlighting the fact that TRIM44 expression levels could serve as a biomarker for sensitivity to mTOR inhibitors.[31]

A major strength of our study is the use of a simple IHC panel consisting of three molecular markers as a tool to help prognosticate EAC patients. These 3 molecular targets were identified as the most prognostic targets out of the 8 previously identified targets from our previous publications.[10,11] Consistently, the combined panel of these 3 marker could be used to predict prognosis in EAC patients independently of current staging algorithms. Existing pathology services can easily adopt the IHC panel as a routine test to determine the status of these three molecular prognostic targets. The determination of whether a tumor is dysregulated or not for these markers is also straightforward and can be done in the same setting when a pathologist reviews the slides for staging. This is in contrast to using other more sophisticated platforms that are often not available or appropriately optimized in many clinical settings. A prime example of a molecular target being utilized in the clinic due to ease of detection with IHC is HER2/NEU. HER2/NEU is a molecular target screened in breast cancers with IHC and fluorescent *in-situ* hybridization (FISH); patients with tumors overexpressing HER2/NEU have been shown to benefit from treatment with trastuzumab.[32] Because our biomarkers have been optimized in IHC using paraffin-embedded tissues, we were able to capitalize on the large stores of EAC specimens in pathology repositories at two high volume centers to perform our validation. This provided a large number of patients from each center and made the validation process significantly more robust; importantly, this allowed for multivariate Cox regression to dissect the role of various known prognostic factors. In addition, the fact that the biomarker panel worked in both cohorts of patients provided evidence that this IHC biomarker panel was generally applicable to patients with EAC independent of the differences in clinical practices. Lastly, the IHC panel could potentially be applied to preoperative biopsies in order to prognosticate patients pre-operatively. This will circumvent problems from using other techniques where the availability of sufficient tissue for extraction of DNA, RNA or proteins can be problematic.

As with all other studies, there are limitations to our current study, including the retrospective design. However, we rationalized that in order for a prospective study to successfully validate any molecular signature, it must be robust and easily applicable. This led us to streamline our panel of eight molecular prognostic targets, identified from our previous studies, to three of our most promising targets for further work. In addition, we wanted to evaluate whether our IHC panel could prognosticate patients from different centers with different patient populations, different treatment regimens and different surgical approaches. A prospective trial to evaluate our three-gene IHC panel, similar to the MINDACT trial for the Mammaprint®, will be the next step.[33] The second limitation of this study is that the IHC panel cannot supercede the TNM staging and should be used in conjunction with TNM preoperatively to inform clinical decisions. Our IHC panel hence provides a useful and objective adjunct to current staging criteria that incorporates the heterogeneity that exists in the biology of EAC We have shown that combining TNM staging criteria with our IHC panel allows segregation of stage II and III patients into distinct prognostic groups. There is minimal effect in applying the IHC panel in stage I patients as these patients are diagnosed early and are very likely to have a good prognosis regardless (68.0% 5 year survival). In addition, surgery performed for these patients are also likely to be curative and complete removal of a biologically aggressive tumour which has not metastasized is probably sufficient to confer a good prognosis. The patients with no abnormal markers in the Cambridge cohort had a better prognosis compared to those with 1 marker dysregulated. This was not the case in the validation cohorts largely due to the small number of patients in these subgroups and individuals with dysregulation of 1 molecular marker had differences in early disease stages between the geographical cohorts. The third limitation to this study is that although IHC is easily applicable to standard clinical pathology laboratories the scoring of each target using IHC is subjective; however this problem can be minimized with standard staining intensity pictures to allow for accurate classification of the staining pattern.

In conclusion, our study confirms that a simple IHC panel of three molecular biomarkers can provide prognostic information in EAC patients independently of clinical prognostic variables and is applicable to patient cohorts from different continents. These data support the notion that distinct molecular features govern the clinical phenotypes of this disease. Using the IHC panel as an adjunct to current staging systems could be of particular relevance in the pre-operative setting where staging data is less accurate or in selected populations of patients for which the optimal therapeutic approach could be influenced by molecular prognostic information. Although identification of patients in a very poor prognosis group would not necessarily dictate withdrawal of chemotherapy or the choice of curative surgery, this study has also identified novel molecular targets which should be investigated to determine whether they can offer more tailored therapeutic options to EAC patients.

Table 1 — Clinical demographics of patients in the two validation cohorts.

	Pittsburgh	Rotterdam	p
Number of patients	**356**	**310**	
Age at surgery	67.0 (23-91)	64.7 (33-90)	<0.01
Follow up time	24.5 (0.49-156.50)	24.2 (1.08-191.41)	0.04
Gender			
Male	297 (83.4%)	268 (86.5%)	0.28
Female	59 (16.6%)	42 (13.5%)	
Recurrence	131 (36.8%)	183 (64.0%)	<0.01
Radicality			<0.01
R_0	335 (94.4%)	216 (70.1%)	
R_1	20 (5.6%)	92 (29.9%)	
Histology grade			0.20
Well	30 (8.5%)	15 (4.9%)	
Moderately	150 (42.4%)	132 (43.4%)	
Poorly	174 (49.2%)	157 (51.6%)	
Pathological T stage			<0.01
T1	155 (43.5%)	45 (14.6%)	
T2	37 (10.4%)	54 (17.5%)	
T3	159 (44.7%)	207 (67.0%)	
T4	5 (1.4%)	3 (1.0%)	
Pathological N stage			<0.01
N0	152 (42.8%)	102 (33.0%)	
N1	201 (56.6%)	167 (54.0%)	
N2	2 (0.6%)	40 (12.9%)	
Pathological M stage			<0.01
M0	342 (96.1%)	250 (80.9%)	
M1	14 (3.9%)	59 (19.1%)	
Chemotherapy			<0.01
Yes	9 (2.5%)	46 (14.8%)	
No	347 (97.5%)	264 (85.2%)	
Alive	161 (45.2%)	90 (29.0%)	<0.01

*Data shown reflect median (range), or number (percentage); Sum of numbers may not add up to the size of patients in cohort due to missing data

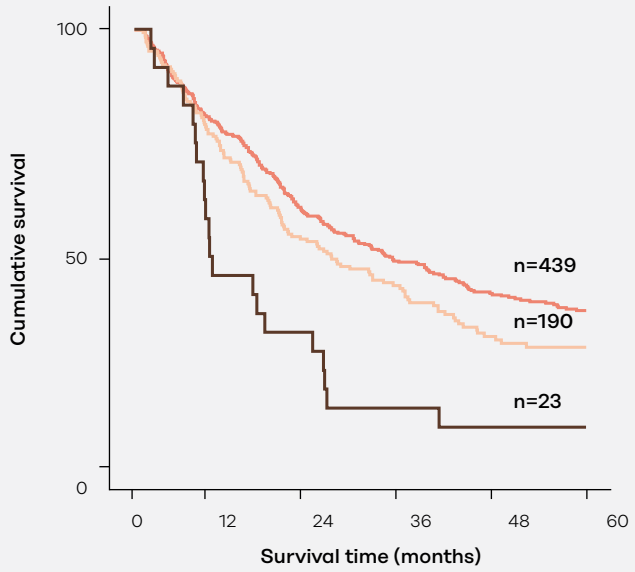
Table 2 — IHC panel gene dysregulation frequency in the validation cohorts.

	Pittsburgh		Rotterdam		p
Number of dysregulated molecular markers					
0	47	(13.5%)	20	(6.6%)	<0.01
1	203	(58.5%)	169	(55.4%)	
2	89	(25.6%)	101	(33.1%)	
3	8	(2.3%)	15	(4.9%)	
TRIM44					
Non-dysregulated	133	(38.0%)	80	(26.2%)	<0.01
Dysregulated	217	(62.0%)	225	(73.8%)	
EGFR					
Non-dysregulated	323	(91.5%)	240	(77.4%)	<0.01
Dysregulated	30	(8.5%)	70	(22.6%)	
SIRT2					
Non-dysregulated	187	(53.0%)	186	(60.0%)	0.07
Dysregulated	166	(47.0%)	124	(40.0%)	

Table 3 — Univariate and multivariate survival analysis in the combined validation cohorts.

	Univariate			Multivariate		
	HR	95%CI	p	HR	95%CI	p
N stage						
N0	Ref			Ref		
N1	3.14	2.48 to 3.97	<0.01	1.79	1.36 to 2.37	<0.01
N2	3.66	2.50 to 5.36	<0.01	1.99	1.29 to 3.06	<0.01
T stage						
T1	Ref			Ref		
T2	1.45	0.99 to 2.11	0.06	1.02	0. 68 to 1.54	0.92
T3	3.60	2.77 to 4.68	<0.01	1.93	1.39 to 2.69	<0.01
T4	9.04	4.31 to 18.94	<0.01	7.01	2.96 to 16.56	<0.01
Age (per year increase)	1.01	1.00 to 1.02	0.03	1.02	1.01 to 1.03	<0.01
Gender						
Female	Ref			Ref		
Male	1.15	0.87 to 1.52	0.31	1.02	0.77 to 1.37	0.87
Radicality						
R_0	Ref			Ref		
R_1	2.75	2.19 to 3.46	<0.01	1.64	1.26 to 2.14	<0.01
Histology grade						
Well-differentiated	Ref			Ref		
Moderately-differentiated	3.01	1.59 to 5.71	<0.01	1.73	0.89 to 3.35	0.10
Poorly-differentiated	5.67	3.01 to 10.68	<0.01	2.80	1.45 to 5.43	<0.01
Chemotherapy						
No	Ref			Ref		
Yes	0.64	0.43 to 0.94	0.02	0.63	0.41 to 0.98	0.04
Study Centre						
Pittsburgh	Ref			Ref		
Rotterdam	1.22	1.00 to 1.48	0.05	0.93	0.73 to 1.18	0.56
IHC panel (per score increase)	1.30	1.12 to 1.50	<0.01	1.20	1.03 to 1.40	0.02

Figure 1 — The IHC panel is highly prognostic. Application of the IHC panel to all validation cohort patients segregated patients into two main prognostic groups (p=0.004).



Patients at risk						
Dysregulation of 0 or 1 molecular markers	439	327	230	164	126	100
Dysregulation of 2 molecular markers	190	130	92	60	37	34
Dysregulation of 3 molecular markers	23	10	6	3	2	2

Figure 2 — Representative examples of dysregulated and non-dysregulated IHC expression of EGFR, SIRT2 and TRIM44 on the tissue microarrays. Overexpression of EGFR and TRIM44 constitutes dysregulation while loss of SIRT2 constitutes dysregulation based on their effect on prognosis and known biologic role.

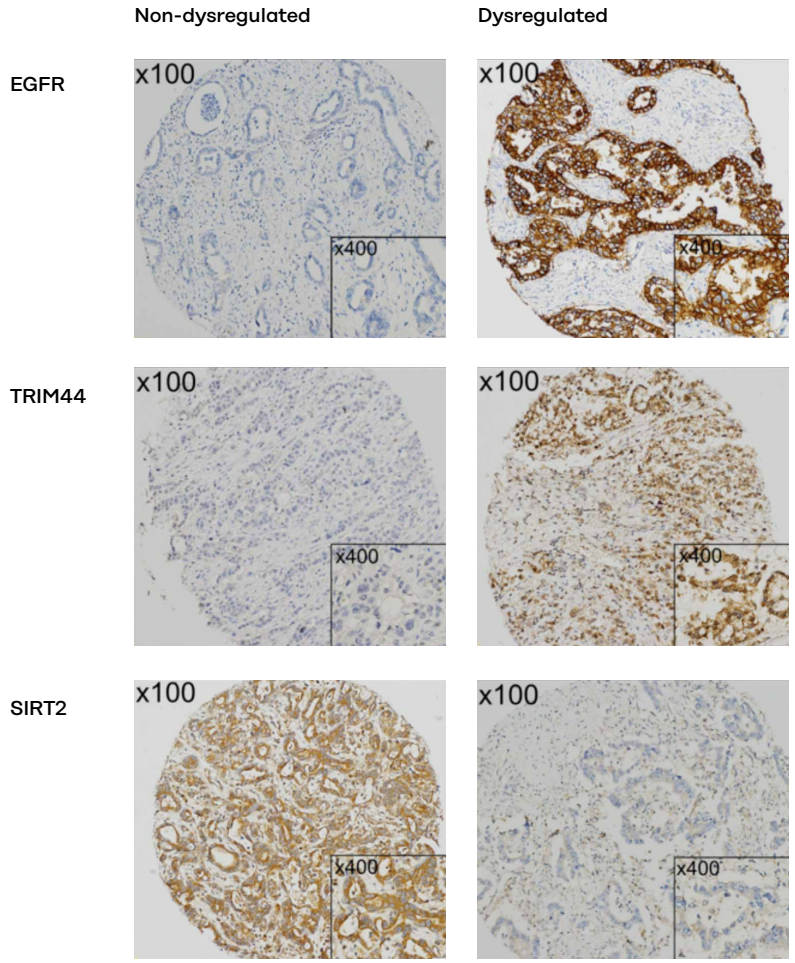
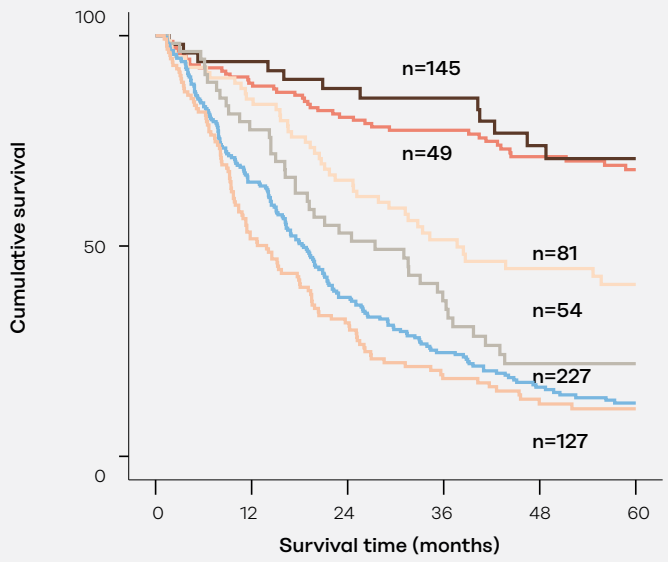


Figure 3 — The IHC panel adds to current staging criteria in prognosticating patients. Combination of stage and the IHC panel separated patients into distinct prognostic groups (p<0.001).



Patients at risk						
Stage I & 0-1 marker	143	145	124	104	90	62
Stage I & 2-3 markers	43	49	44	41	34	23
Stage II & 0-1 marker	77	81	67	50	31	22
Stage II & 2-3 markers	50	54	41	28	18	9
Stage III & 0-1 marker	218	227	145	80	47	19
Stage IIII & 2-3 markers	119	127	64	37	19	10

Supplemental Materials and Methods

Scoring criteria

Scoring for each of the selected targets was based on intensity of staining in tumor epithelial cells. For each marker a core was scored on a scale from 0 (no staining) to 3 (strong staining) if at least 10% of tumor epithelial cells stained positive. For SIRT2 and TRIM44 positive staining was cytoplasmic and for EGFR positive staining was membranous. For scoring of SIRT2, each core was scored independently by two scorers blinded to clinical outcomes and the scores were averaged. For scoring of EGFR and TRIM44 staining, the maximum score for each individual case (comprising of 3 cores) was used. All scores differing by more than 1 were reviewed and a consensus was reached. The rationale of doing so is that we noted that staining of the 3 targets on whole paraffin sections of EAC demonstrated that TRIM44 and EGFR can have heterogenous staining while decreased exoression or loss of SIRT2 staining tends to be homogenous. This is also to simplify scoring from a clinical perspective as the maximum intensity of staining for EGFR and TRIM44 on whole tissue sections should be used to determine the status of the staining.

The determination of whether a marker is dysregulated is based on the known biology of the molecular targets based on our previous study. SIRT2 is a known tumor suppressor and EGFR and TRIM44 have been shown to confer an oncogenic effect when overexpressed. Hence, a score of 0 or 1 is considered dysregulated while a score of 2 or 3 is considered non-dysregulated in the case of SIRT2. Conversely, a score of 0 or 1 is considered non-dysregulated while a score of 2 or 3 is considered dysregulated in the case of EGFR and TRIM44. The final scores were then checked by an expert GI pathologist for 12.5% (1/8) of all cores for each validation target to confirm that the consensus score was accurate.

Supplementary Table 1 — Hazard ratios of each molecular marker derived via univariate cox regression analysis in the original OCCAMS cohort.

	95% CI	p
TRIM44	1.31 (1.01-1.70)	0.04
SIRT2	1.31 (1.03-1.67)	0.03
EGFR	1.52 (1.03-2.26)	0.04
PAPPS2	1.24 (0.96-1.61)	0.10
NEIL2	1.12 (0.87-1.43)	0.39
WT1	0.71 (0.39-1.30)	0.27
MTMR9	1.14 (0.87-1.51)	0.34
DCK	0.98 (0.75-1.28)	0.86

Supplementary table 2 — Hazard ratios of each dysregulated molecular marker in the validation cohorts.

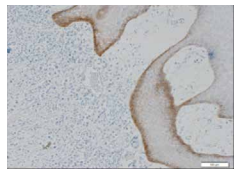
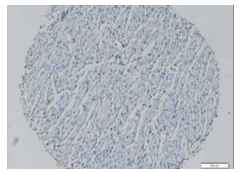
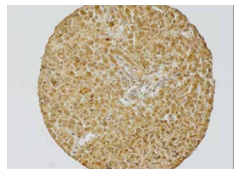
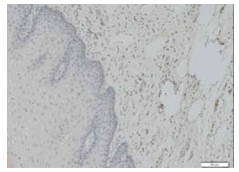
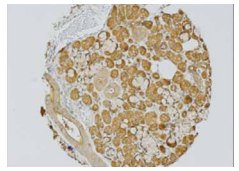
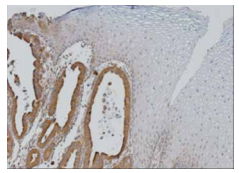
Molecular marker	Intensity Score	HR	95% CI		p
TRIM44	0	Ref			
	1	1.46	0.89	2.44	0.14
	2	1.59	0.96	2.63	0.07
	3	1.94	1.09	3.44	0.02
SIRT2	3	Ref			
	2	1.69	1.10	2.60	0.02
	1	1.81	1.24	2.64	<0.01
	0	1.37	0.96	1.97	0.08
EGFR	0	Ref			
	1	0.83	0.66	1.04	0.10
	2	1.41	1.05	1.91	0.02
	3	0.94	0.58	1.52	0.80

Supplementary Table 3 — Prognostic effect of the IHC panel in different stages of patients in all three cohorts.

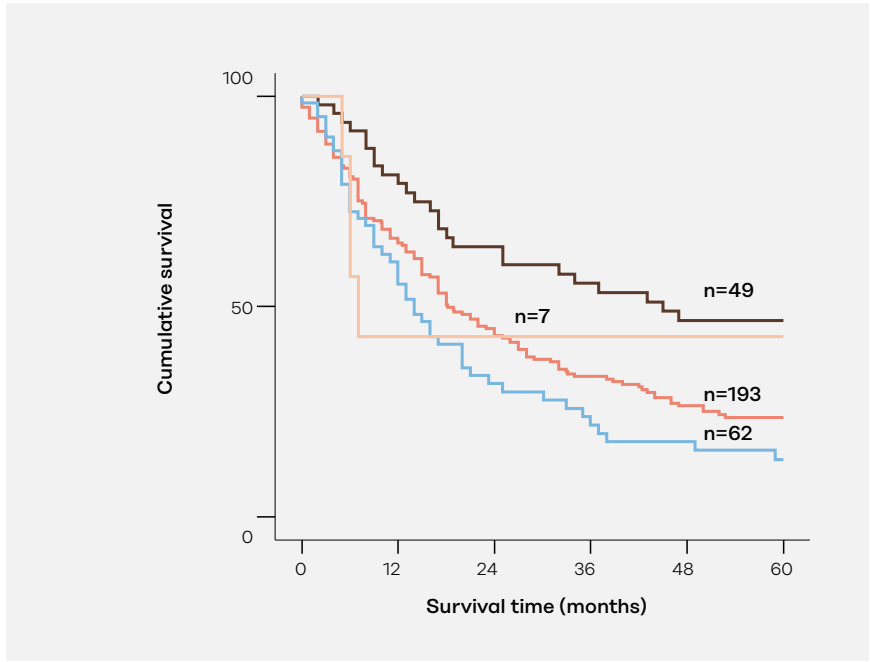
			Univariate analysis			Multivariate analysis*			
		No of MM dysregulated							
stage	n		HR	95% CI	p	HR	95% CI		p
I	241	0	Ref			Ref			
		1	0.69	0.32 1.47	0.33	0.94	0.43 2.06		0.88
		2	0.97	0.41 2.28	0.94	1.01	0.40 2.54		0.98
		3	1.92	0.41 9.03	0.41	4.37	0.77 24.91		0.10
		Per 1 MM	1.17	0.77 1.79	0.46	1.15	0.75 1.78		0.52
II	214	0	Ref			Ref			
		1	1.84	1.05 3.22	0.03	1.85	0.92 3.74		0.09
		2	1.99	1.10 3.61	0.02	1.97	0.94 4.15		0.07
		3	2.59	0.75 8.96	0.13	6.30	1.55 25.54		0.01
		Per 1 MM	1.31	1.03 1.67	0.03	1.38	1.02 1.86		0.04
III	537	0	Ref			Ref			
		1	1.66	1.15 2.41	<0.01	1.38	0.92 2.06		0.12
		2	1.77	1.20 2.62	<0.01	1.43	0.93 2.21		0.10
		3	2.78	1.56 4.97	<0.01	2.73	1.44 5.18		<0.01
		Per 1 MM	1.26	1.09 1.45	<0.01	1.22	1.03 1.43		0.02

*Adjusted for age, sex, study centre, N stage, T stage, radicality, tumour grade, and chemo therapy.

Supplementary figure 1 — antibody sources and conditions used for IHC.

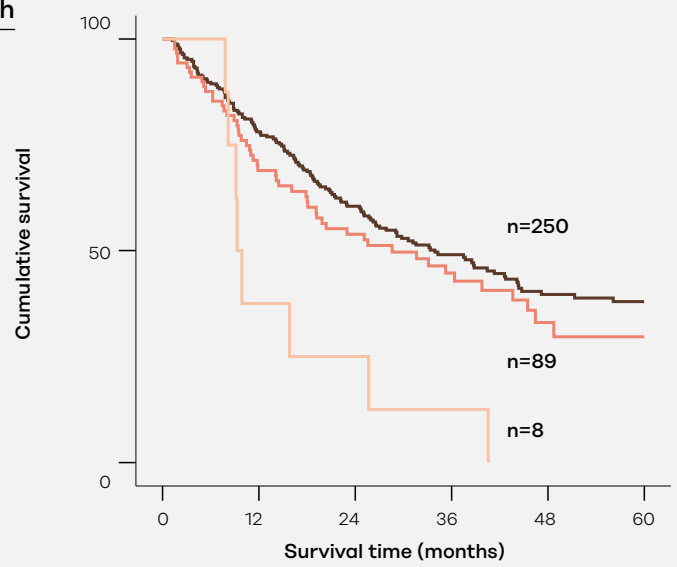
Target	Product number	Antibody source	IHC conditions	Normal control tissues
EGFR	NCL-L-EGFR	Novocastra, Leica, Milton Keynes, UK	1/10 dilution IHC protocol F HIER Epitope Retrieval Solution 1 for 30 min	Positive - Normal squamous  Negative - Heart tissue 
TRIM44	ab69307	Abcam Plc, Cambridge, UK	1/100 dilution IHC protocol F HIER Epitope Retrieval Solution 2 for 30 min	Positive control - Liver  Negative - Normal squamous 
SIRT2	HPA011165	Atlas Antibodies AB, Stockholm, Sweden	1/100 Dilution IHC protocol F HIER Epitope Retrieval Solution 2 for 30 minutes	Positive - Parotid  Negative - Normal squamous *Note positively staining glands next to negative control tissue 

Supplementary figure 2 — Internal validation of TRIM44, SIRT2 and EGFR as a combined IHC panel to prognosticate EAC patients in the original OCCAMS cohort.



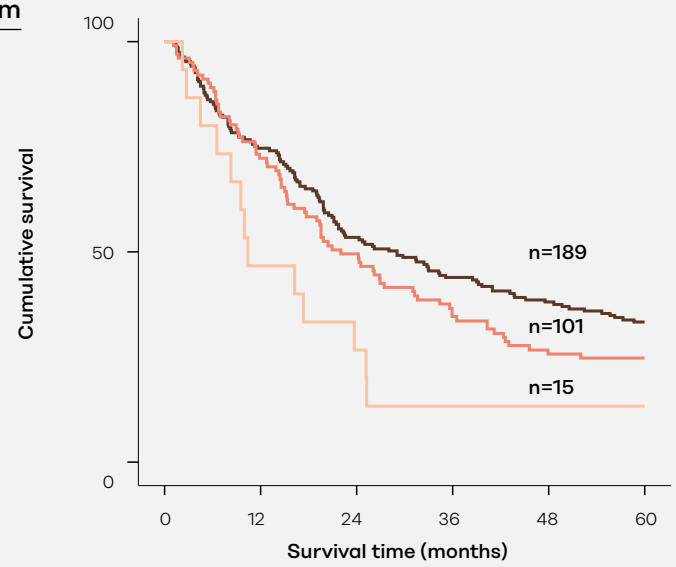
Supplementary figure 3 — Application of the IHC panel to all patients segregated patients into three prognostic groups in both centers (Pittsburgh: p=0.003; Rotterdam: p=0.021).

Pittsburgh



Patients at risk						
Dysregulation of 0 or 1 molecular markers	250	186	129	81	54	37
Dysregulation of 2 molecular markers	89	57	42	25	11	9
Dysregulation of 3 molecular markers	8	3	2	1	0	0

Rotterdam



Patients at risk						
Dysregulation of 0 or 1 molecular markers	189	141	101	83	72	63
Dysregulation of 2 molecular markers	101	73	50	35	26	25
Dysregulation of 3 molecular markers	15	7	4	2	2	2

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Chapter 6

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Long-term results of a randomised controlled trial comparing neoadjuvant chemoradiotherapy plus surgery with surgery alone for oesophageal or junctional cancer (CROSS trial)

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Abstract

Background
The CROSS trial compared neoadjuvant chemoradiotherapy (nCRT) plus surgery to surgery alone in squamous cell carcinoma and adenocarcinoma of the oesophagus or oesophago-gastric junction. Initial results, after a median follow-up 45 months, showed an absolute increase in five-year overall survival of 13% in favour of the nCRT plus surgery group. Here, we report long-term results after a minimal follow-up of five years.

Methods
Patients with clinically resectable oesophageal or junctional cancer (cT-1N1M0 or cT2-3N0-1M0, TNM 6th ed.) were randomly assigned to receive weekly administration of carboplatin (AUC=2 mg/ml per min) and paclitaxel (50 mg/m^2 of body-surface area) for 5 weeks with concurrent radiotherapy (41.4 Gy given in 23 fractions, 5 days per week), followed by surgery, or surgery alone. The primary endpoint was overall survival and analyses were performed on an *intention-to-treat* basis. Randomisation was performed centrally, according to computer-generated randomisation lists for each stratum, with random block sizes of 4 or 6. This trial is registered with the Netherlands Trial Register, number NTR487.

Findings
Between March 30, 2004 and December 2, 2008, 368 patients were enrolled in this study, of whom 366 patients were analysed, 178 in the nCRT plus surgery group and 188 in the surgery alone group. The nCRT regimen was completed by 95% (162/171) of patients who received any nCRT in the multimodality group. Some 8% (13/171) and 11% (18/171) of patients experienced grade 3 or higher haematological- and non-haematological toxicity, respectively. After a median follow-up for surviving patients of 84.1 months (range, 61.1–116.8), median survival was 49 months in the nCRT plus surgery group and 24 months in the surgery alone group. Five-year overall and progression-free survival rates were 47% and 44% in the nCRT plus surgery group and 33% and 27% in the surgery alone group, respectively. Five-year overall and progression-free survival were significantly higher in the nCRT plus surgery group in both squamous cell carcinoma (61% *vs.* 30%, p=0.008 and 58% *vs.* 28%, p=0.006, respectively) and adenocarcinoma (43% *vs.* 33%, p=0.038 and 41% *vs.* 27%, p=0.010, respectively). Patients in the nCRT plus surgery group developed significantly less locoregional and distant progression, which was already observed within the first year after randomisation, as compared to patients in the surgery alone group (HR 0.45 95%CI 0.30–0.66 and HR 0.63 95%CI 0.46–0.86, respectively).

Interpretation
Long-term follow-up confirms the overall and progression-free survival benefits for neoadjuvant chemoradiotherapy when added to surgery in resectable oesophageal or junctional cancer patients. This improvement is statistically significant and clinically relevant for both squamous cell carcinoma and adenocarcinoma subtypes. Therefore, neoadjuvant chemoradiotherapy according to CROSS followed by surgical resection should be considered a standard of care for resectable locally advanced oesophageal or junctional cancer patients.

Funding
Funded by the Dutch Cancer Foundation (KWF Kankerbestrijding).

Introduction

Oesophageal cancer is an aggressive disease, characterized by substantial percentages of locoregional and distant recurrence after primary surgical resection and a poor five-year overall survival rate that rarely exceeds 40%.[1-3] Much effort has been put into improving tumour resectability, long-term locoregional control and overall survival, through addition of chemo- and/or radiotherapy to surgery, in a neoadjuvant and/or adjuvant setting.[2-5] However, many studies have failed to show significant long-term survival benefit.[6,7]

The randomised CROSS (ChemoRadiotherapy for Oesophageal cancer followed by Surgery Study) trial[8] compared neoadjuvant chemoradiotherapy (nCRT) plus surgery to surgery alone. The trial enrolled 368 patients between March 2004 and December 2008 from eight Dutch participating centres, randomly assigning patients to the nCRT plus surgery group or to the surgery alone group. Initial results were published in 2012 after a minimal follow-up of 24 months (median follow-up 45 months). We observed an absolute 13% benefit in five year overall survival in favour of the multimodality group. The nCRT regimen was completed by 94% of patients who recveived any nCRT, with a relatively low frequency of high-grade toxic effects. Furthermore, a microscopically radical resection (*i.e.* no vital tumour present at 1 mm or less from the proximal, distal, or circumferential resection margins) was achieved in 92% of patients in the multimodality group, compared to 69% in the surgery alone group (p<0.001).

Here, we aim to investigate the consistency of longer-term results with our earlier findings and to analyse secondary end-points, such as progression-free survival and recurrence patterns.

Methods

Study design and participants

Full details of patients' eligibility criteria and procedures of this trial have been reported previously.[8,9] Briefly, patients were eligible if aged 75 years or younger, had adequate haematological, renal, hepatic and pulmonary function and a WHO-performance score of 2 or better, without a past or current history of other malignancy. Only patients with locally advanced (cT1N1M0 or cT2-3N0-1M0, according to the Union for International Cancer Control (UICC) TNM Cancer Staging, 6th edition[10]), histologically proven and potentially curable squamous cell carcinoma or adenocarcinoma of the oesophagus or oesophago-gastric junction (*i.e.* tumours involving both the cardia and the oesophagus on endoscopy) were eligible for inclusion. All patients provided written informed consent. The institutional review board at each participating centre approved the study protocol.

Randomisation

Patients were stratified according to histological tumour type, treatment centre, clinical N-status and WHO-performance score. Randomisation was performed centrally, using computer generated randomisation lists for each stratum, with random block sizes of 4 or 6.

Procedures

All patients underwent pretreatment staging, including upper gastrointestinal endoscopy with histological biopsy and endoscopic ultrasonography (EUS), CT scanning of the neck, chest and upper abdomen, and external ultrasonography of the neck, with fine needle aspiration of suspected lymph nodes on indication.

Carboplatin (AUC=2 mg/ml per min) and paclitaxel (50 mg/m^2 of body-surface area) were administered intravenously in five cycles, starting on days 1, 8, 15, 22 and 29. A total concurrent radiation dose of 41.4 Gy was given in 23 fractions of 1.8 Gy, five fractions per week, starting on the first day of the first chemotherapy cycle. Total duration of neoadjuvant treatment was 23 days. If on days 8, 15, 22, or 29 the white blood cell count was below 1.0x10^9/L or the platelet count was below 50x10^9/L administration of nCRT was delayed by one week until recovery above these thresholds. Also, in case of mucositis with oral ulcers or protracted vomiting despite antiemetic premedication, nCRT was delayed by one week. Further chemotherapy was withheld in case febrile neutropenia (defined as a neutrophil count below 0.5x10^9/L and a temperature above 38.5°C), persistent creatinine clearance of less than 50% of the pretreatment level, symptomatic cardiac arrhythmia or atrioventricular block (with the exception of first-degree atrioventricular block), or other major organ toxicity, grade 3 or higher (with the exception of oesophagitis). During nCRT, laboratory tests (including complete blood cell counts and creatinine) were performed on a weekly basis, while radiological evaluations were performed only on indication. All patients in the nCRT plus surgery group were included into the '*intention-to-treat*' analysis, irrespective of total dose of nCRT received.

Patients in the surgery alone group were operated on as soon as possible, while patients in the nCRT plus surgery group were preferably operated on within 4-6 weeks after completion of nCRT. For carcinomas at or above the level of the carina a transthoracic oesophageal resection with two-field lymph node dissection was performed. For carcinomas located well below the level of the carina, either a transthoracic approach with two-field lymph node dissection or a transhiatal approach was performed, depending on both patient characteristics and local preferences. For carcinomas involving the OGJ, a transhiatal oesophageal resection was favoured. In both approaches, an upper abdominal lymphadenectomy, including resection of nodes along the hepatic artery, splenic artery and left gastric artery, was performed.

For TNM-classification, tumour grading and stage grouping, the sixth edition of the UICC TNM Cancer Staging was used.[10] Proximal, distal and circumferential resection margins were evaluated. Microscopically radical resection (R_0) was defined as a tumour-free resection margin ≥1 mm.

During the first year after treatment completion, patients were seen every three months. In the second year, follow-up took place every six months, and subsequently yearly until five years after treatment. Additional interim visits were scheduled if complaints arose before the next scheduled visit. Diagnostic investigations were only performed on indication during follow up.

Outcomes

The primary endpoint was overall survival. Overall survival was calculated from the date of randomisation to the date of all-cause death or to the last day of follow-up. Last day of follow-up was 31st December 2013, guaranteeing a minimal potential follow-up of 60 months for all included patients. Secondary endpoints included progression-free survival and progression-free interval. Progression-free survival was defined as the interval between randomisation and the earliest occurrence of disease progression resulting in primary (or peroperative) irresectability of disease, locoregional recurrence (after completion of therapy), distant dissemination (during therapy or after completion of therapy) or death from any cause. This definition for progression-free survival was taken from the modified STEEP criteria for neoadjuvant

treatment trials.[12,13] Last day of follow-up for progression-free survival varied, depending on the last (scheduled) contact with the patient. Progression-free interval was similar to progression-free survival, with the difference that treatment related deaths and non-oesophageal-cancer-related deaths were not counted as events. Locoregional progression was defined as either progression of locoregional disease during therapy (resulting in irresectability) or as locoregional recurrence after completion of therapy. Locoregional sites included the mediastinum, the supraclavicular region and the celiac trunk region. Distant progression was defined as occurrence of disseminated disease, either during therapy or after completion of therapy. Distant disease included cervical and (para-aortic) lymph node dissemination below the level of the pancreas, malignant pleural effusions, peritoneal carcinomatosis and further haematogenous (organ) dissemination. Furthermore, no additional adverse event data were gathered for this current update.

Statistical analysis

Data were analysed according to the '*intention-to-treat*' principle. In order to detect a difference of six months in median overall survival (22 months in the nCRT plus surgery group versus 16 months in the surgery alone group; two-sided test; alpha level, 0.05; beta level, 0.80), it was calculated that 175 patients were needed per group. Statistical significance was set to 0.05.

The Kaplan-Meier method was used for estimating overall and progression-free survival with the log-rank test for determining significance. Univariable and multivariable Cox proportional-hazards models were used to determine the effect of neoadjuvant chemoradiotherapy in subgroups, adjusting for baseline covariates.[8] Univariable Cox regression modelling was used to analyse differences in progression-free interval between treatment groups, expressed as hazard ratios (HRs). Follow-up time was divided to study the temporal distribution of disease progression. Three separate analyses were performed, including follow-up until 6 months, 12 months and 24 months after randomisation. Progression was defined as locoregional or distant. Patients in whom both types of disease progression occurred had events scored in both categories. In the scoring of disease progression in one category, disease progression in the other category and death without progression were censored. For each time-point the number of events was compared between treatment groups, before the cut-off time-point and after. Statistical analysis was performed by JS and EWS using Statistical Package for Social Sciences version 21.0 (SPSS Inc., Chicago, IL, USA). This trial is registered with the Netherlands Trial Register, number NTR487.

Role of funding source

This trial was financially supported by the Dutch Cancer Foundation (KWF Kankerbestrijding). The funder had no role in the study design, data collection, data analysis, data interpretation or writing of the manuscript. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between March 30, 2004 and December 2, 2008, 368 patients were enrolled in the study; 180 patients were randomised to the nCRT plus surgery group (of whom 2 patients later withdrew consent) and 188 patients to the surgery alone group (figure 1). Baseline characteristics were well balanced between the two treatment groups (table 1). A single patient in the surgery alone group was originally misclassified as not having received a resection. However, in the current update it was found that this patient had undergone a resection abroad. This misclassification bears no influence on current or previous analyses, due to the '*intention-to-treat*' principle. Also, a patient in the nCRT plus surgery group moved abroad and was therefore lost to follow-up 73 months after randomisation. The nCRT regimen was completed by 95% (162/171) of patients who received any nCRT in the multimodality group. Some 8% (13/171) and 11% (18/171) of patients experienced grade 3 or higher haematological- and non-haematological toxicity, respectively. The most common grade 3 or higher toxicities were leukopenia in 6% (11/171), anorexia in 5% (9/171) and fatigue in 3% (5/171) of patients. In the nCRT plus surgery group, 90% (161/178) of patients underwent resection, as compared to 86% (162/188) of patients in the surgery alone group. The percentage of transhiatal resections was comparable between both treatment groups, 45% (72/161) in the nCRT plus surgery group as compared to 44% (72/162) in the surgery alone group (χ^2 0.01, p=0.96).

Overall survival

At the time of this analysis, median follow-up for surviving patients was 84.1 months (range, 61.1–116.8). Of the 366 analysed patients, 126 patients were still alive at final analysis, 73 of 178 patients in the nCRT plus surgery group and 53 of 188 patients in the surgery alone group. This corresponds with 19 (22%) and 21 (18%) additional events since the last follow-up of the original publication.[8] Median overall survival was 48.6 months (95%CI 32.1–65.1) in the nCRT plus surgery group and 24.0 months (95%CI 14.2–33.7) in the surgery alone group. Median overall survival for squamous cell carcinomas was 81.6 months (95%CI 47.2–116.0) in the nCRT plus surgery group and 21.1 months

(95%CI 15.4–26.7) in the surgery alone group. Median overall survival for adenocarcinomas was 43.2 months (95%CI 24.9–61.4) in the nCRT plus surgery group and 27.1 months (95%CI 13.0–41.2) in the surgery alone group. The overall survival rates at one, two, three and five years were 81% (145/178), 67% (119/178), 58% (103/178) and 47% (83/178), respectively, in the nCRT plus surgery group, as compared to 70% (131/188), 50% (94/188), 44% (83/188) and 33% (62/188) in the surgery alone group. During follow-up, 16 patients died from treatment related causes, *i.e.* during nCRT or during postoperative hospital stay (9 patients in the nCRT plus surgery group and 7 patients in the surgery alone group) and 23 patients died from non-disease-related causes beyond the first 90 postoperative days (13 patients in the nCRT plus surgery group and 10 patients in the surgery alone group).

Overall survival was significantly improved in the nCRT plus surgery group as compared to the surgery alone group (log-rank p=0.002; figure 2a). This significant difference was observed both in patients with squamous cell carcinoma as well as in patients with adenocarcinoma (log-rank p=0.008 and p=0.038, respectively; figure 2b). The estimated number of patients who need to be treated to prevent one additional death at five year was 7.1 (95%CI 4.6–13.2).[14] The overall survival benefit of nCRT plus surgery was generally confirmed across subgroups (table 2). The concordance of the multivariable model for overall survival in all patients was 0.584.[15] The proportionality of hazards assumption for the main analysis was not violated (χ^2 0.77, p=0.38).[16]

Progression-free survival

Of the 366 analysed patients, 116 patients were alive and disease free (eventually without evidence of recurrent disease) at final analysis, 69 of 178 patients in the nCRT plus surgery group and 47 of 188 patients in the surgery alone group. Median progression-free survival was 37.7 months (95%CI 23.7–51.8) in the nCRT plus surgery group and 16.2 months (95%CI 10.7–21.7) in the surgery alone group. Median progression-free survival for squamous cell carcinomas was 74.7 months (95%CI 55.1–94.4) in the nCRT plus surgery group and 11.6 months (95%CI 4.4–18.8) in the surgery alone group. Median progression-free survival for adenocarcinomas was 29.9 months (95%CI 15.9–43.9) in the nCRT plus surgery group and 17.7 months (95%CI 11.9–23.5) in the surgery alone group. The progression-free survival rates at one, two, three and five years were 71% (127/178), 60% (106/178), 51% (90/178) and 44% (74/178), respectively, in the nCRT plus surgery group, as compared to 54% (102/188), 41% (77/188), 35% (66/188) and 27% (50/188) in the surgery alone group.

Progression-free survival was significantly improved in the nCRT plus surgery group as compared to the surgery alone group (log-rank p<0.001; figure 3a). This significant difference was observed both in patients with squamous cell carcinoma as well as in patients with adenocarcinoma (log-rank p=0.006 and p=0.010, respectively; figure 3b). The estimated number of patients who need to be treated to prevent one additional disease progression at five year was 6.1 (95%CI 4.2–10.0).[14] The progression-free survival benefit of nCRT plus surgery was generally confirmed across subgroups (supplementary table 1).

Locoregional and distant progression-free interval

We studied the progression-free intervals, as opposed to progression-free survival, to focus in more detail on recurrence patterns in both treatment groups. From randomisation, 211 patients showed disease progression (table 3). In the nCRT plus surgery group, 87 patients had disease progression, of whom 39 had locoregional progression and 70 had distant progression (22 patients had both locoregional and distant progression). In the surgery alone group, 124 patients had disease progression, of whom 72 had locoregional progression and 90 had distant progression (38 patients had both locoregional and distant progression). Disease progression during therapy (causing adjustment from curative to palliative treatment intent) occurred in 17 patients in the nCRT plus surgery group and in 26 patients in the surgery alone group.

Patients in the nCRT plus surgery group developed significantly less locoregional progression and significantly less distant progression, as compared to patients in the surgery alone group (HR 0.45 95%CI 0.30–0.66, p<0.001 and HR 0.63 95%CI 0.46–0.87, p=0.004, respectively; table 3). The reduction in locoregional progression was already apparent during the first six months of follow-up and remained significant in the period after the first 24 months of follow-up (HR 0.50 95%CI 0.31–0.79, p=0.003 and HR 0.39 95%CI 0.17–0.89, p=0.025, respectively; supplementary table 2). This indicates that the effect of reduction in locoregional progression continued throughout an extended period after randomisation. The reduction in distant progression was also already found during the first six months of follow-up (HR 0.38 95%CI 0.18–0.78, p=0.009). The effect remained significant during the first 24 months of follow-up, but not thereafter (HR 0.57 95%CI 0.40–0.81, p=0.002 and HR 0.94 95%CI 0.48–1.83, p=0.845, respectively). Thereby, indicating that the reduction in distant progression occurred primarily within the first 24 months after randomisation.

Discussion

These long-term results, after a median follow-up for surviving patients of 84 months, confirm the initially observed survival benefit for nCRT plus surgery as compared to surgery alone (median overall survival 49 months *vs.* 24 months and five-year overall survival rate 47% *vs.* 33%, respectively). The improvement in distant disease control occurred within the first two years after start of treatment, while the improvement in locoregional control continued for more years. These results further support the clinical value of this multimodality treatment strategy.

The overall survival benefit and the progression-free survival benefit were confirmed for both histological subtypes and for other clinically relevant subgroups. While unvariable and multivariable hazard ratios for individual subgroups were reported for informative purposes, no significant interactions in treatment effect were identified for any of the subgroups. This means that differences in treatment effect between subgroups could well have arisen by chance, and the overall treatment effect should be considered valid for all considered subgroups. In other words, there is no clear evidence to assume that the adjusted overall treatment effect of nCRT does not also apply to adenocarcinoma patients. We, therefore, conclude that both squamous cell carcinoma patients and adenocarcinoma patients benefit significantly from the CROSS regimen.

By adding nCRT to primary surgery, locoregional disease control was significantly improved (table 3). Interestingly, the largest reported trials with neoadjuvant chemotherapy (nCT) only showed limited improvement in R_0 resection rates, in pathologically complete response (pCR) rates and in locoregional recurrence rates. Furthermore, two small randomised trials[17,18] comparing nCT plus surgery to nCRT plus surgery both found similar R_0 resection rates between treatment groups, but significantly higher pCR rates and lower locoregional recurrence rates in the nCRT plus surgery groups. Therefore, results from these nCT trials point towards neoadjuvant radiotherapy combined with sensitizing chemotherapy rather than nCT alone as the likely cause of improved locoregional control, as achieved in the CROSS trial.

In the CROSS trial, not only locoregional control, but also distant disease control improved significantly in the nCRT plus surgery group (table 3). Theoretically, this improved distant disease control may be explained in several ways. First, if fewer locoregional recurrences occur, then possibly less distant dissemination develops from these locoregional recurrences. Secondly, it has been described in some cancer types that effective treatment of the primary tumour in the presence of disseminated disease can prolong survival. Therefore, a mechanism by which improved locoregional control might improve distant disease control could simply be control of the primary tumour itself, thereby removing a currently unknown stimulus for disseminated tumour outgrowth. A third explanation is that improved distant disease control could be caused by a direct systemic effect of chemotherapy. In the current study, we found a significant reduction in distant disease progression already within the first six months after randomisation (supplementary table 2). Such an early reduction in distant disease progression, without evidence of reduction beyond the first 24 months, supports a direct systemic effect of this neoadjuvant chemotherapy regimen. The reduction in distant disease progression achieved by this neoadjuvant chemoradiotherapy regimen is comparable to reductions achieved with more protracted (and more toxic) perioperative chemotherapy regimens.[4,5]

Results from this trial may not be readily extrapolated to patients with poorer performance status, patients with more advanced age or patients with tumour located in the proximal or middle oesophagus, due to the relative scarcity of occurrences in these categories. The value of this treatment regimen will need to be confirmed for these patients in future follow-up studies.

Despite recent advances in curative treatment of oesophageal or junctional cancers, benefit of (neo)adjuvant treatment is generally quite limited and a definitive statement on the optimal perioperative treatment in terms of survival is still lacking. A recent meta-analysis suggested a (non-significant) advantage of neoadjuvant chemoradiotherapy over neoadjuvant chemotherapy alone in both the direct and the indirect comparisons (HR 0.77 95%CI 0.53–1.12 and HR 0.88 95%CI 0.76–1.01, respectively).[7] Probably the ongoing Japanese randomised NExT trial (JCOG1109)[19] and the Irish randomised Neo-AEGIS trial (ICORG 10-14)[20] will provide more definitive evidence on the optimal perioperative treatment for squamous cell carcinoma and adenocarcinoma, respectively. Unless convincing, contrary results become available, strong evidence from the CROSS trial continues to support neoadjuvant chemoradiotherapy as a standard of care for both squamous cell carcinoma and adenocarcinoma of the oesophagus or oesophago-gastric junction.

Conclusions

Neoadjuvant chemoradiotherapy according to the CROSS regimen improves long term overall and progression-free survival in oesophageal and junctional cancer patients. This improvement is statistically significant and clinically relevant for both squamous cell carcinoma and adenocarcinoma subtypes and for both locoregional and distant control. The improvement in distant control occurs early after start of treatment, suggesting a direct systemic effect of chemotherapy, while the improvement in locoregional control continues longer. Neoadjuvant chemoradiotherapy according to CROSS followed by surgical resection should be considered a standard of care for resectable locally advanced oesophageal or junctional cancer patients.

Research in context

Evidence before this study

Based on the extensive meta-analysis by Sjoquist *et al.*[7], at the initiation of the CROSS trial in 2004, results from four previous randomised trials[21-24] comparing neoadjuvant concurrent chemoradiotherapy plus surgery to surgery alone had been reported. Chemotherapy in these trials consisted of cisplatin and 5-fluorouracil (and also vinblastine in a single trial), with a total concurrent radiation dose ranging from 40 to 45 Gy. However, these trials included only small numbers of patients and showed opposing results. Our previous non-randomised phase II feasibility trial[11], tested a regimen of weekly administrations of carboplatin and paclitaxel with 41.4 Gy concurrent radiotherapy and showed a radical resection percentage of 100%, with low treatment related toxicity. These promising short-term results provided the rationale for testing this CROSS neoadjuvant chemoradiotherapy regimen in a subsequent randomised phase III trial.

Added value of this study

At long-term follow-up, the CROSS trial has now shown that treatment of locally advanced oesophageal or junctional cancer with carboplatin, paclitaxel and concurrent radiotherapy followed by surgery significantly improves 5-year overall and progression-free survival, as compared to treatment with surgery alone.

Implications of all the available evidence

Despite the favourable results of the initial CROSS trial[8], pre- or peri-operative chemotherapy is still considered standard of care in some countries for oesophageal and junctional cancer patients. Mainly because of the important results of the MAGIC trial[4], which compared perioperative chemotherapy, consisting of epirubicin, cisplatin, and infused fluorouracil plus surgery to surgery alone. However, only a minority of included patients had distal oesophageal cancers (14%) or junctional cancers (12%). Thus questioning the applicability of these results for oesophageal and junctional cancers. Furthermore, the MAGIC trial, which was published in 2006 after a minimum follow-up of less than two years, has not yet reported its long-term results. This makes it unclear whether the initially reported survival benefit of perioperative chemotherapy is sustained at long-term follow-up. Probably, the ongoing Japanese randomised NExT trial (JCOG1109)[19] and the Irish randomised Neo-AEGIS trial (ICORG 10-14)[20] will provide more definitive evidence on the current optimal pre- or peri-operative treatment for oesophageal squamous cell carcinoma and adenocarcinoma, respectively. Future research should focus on more individualised treatment strategies, such as 'watchful waiting' protocols after neoadjuvant therapy, where surgery is offered only to those patients in whom locoregional disease is detected (in the absence of signs of distant dissemination). Also, newer, more effective combinations of systemic agents need more study, such as adding targeted therapy to existing chemoradiotherapeutic treatment regimens.

Table 1 — Baseline characteristics of 366 analysed patients, according to treatment group.

	nCRT plus surgery	Surgery alone
Characteristics	**(n=178)**	**(n=188)**
Age [years]	60 (55 – 67)	60 (53 – 66)
Gender		
Women	44 (25)	36 (19)
Men	134 (75)	152 (81)
Tumour histology		
Squamous cell carcinoma	41 (23)	43 (23)
Adenocarcinoma	134 (75)	141 (75)
Could not be determined	3 (2)	4 (2)
Tumour length [cm]	4 (3 – 6)	4 (3 – 6)
Tumour location		
Proximal third oesophagus	4 (2)	4 (2)
Middle third oesophagus	25 (14)	24 (13)
Distal third oesophagus	104 (58)	107 (57)
Oesophagogastric-junction	39 (22)	49 (26)
Missing data	6 (3)	4 (2)
Clinical T-stage		
cT1	1 (1)	1 (1)
cT2	26 (15)	35 (19)
cT3	150 (84)	147 (78)
cT4	-	1 (1)
Could not be determined	1 (1)	4 (2)
Clinical N-stage		
cN0	59 (33)	58 (31)
cN1	116 (65)	120 (64)
Could not be determined	3 (2)	10 (5)
WHO-performance score		
0	144 (81)	163 (87)
1	34 (19)	25 (13)

Legend table 1

Baseline characteristics of 366 analysed patients, according to treatment group
Data presented as median (25^{th}-75^{th} percentile) or number (%).

Table 2 — Univariable and multivariable hazard ratios for all-cause mortality in 366 patients according to subgroup characteristics.

Subgroup	nCRT plus surgery	Surgery alone	Inter-action	Univariable			Multivariable		
	(n=178)	(n=188)	p	HR	95% CI	p	aHR	95% CI	p
	number of events (%)	number of events (%)							
All patients	105 (59)	135 (72)	0.078	0.68	(0.53 – 0.88)	**0.003**	0.69	(0.53 – 0.89)	**0.004**
Gender			0.451						
Women	25 (14)	24 (13)		0.83	(0.47 – 1.45)	0.502	0.85	(0.48 – 1.50)	0.570
Men	80 (45)	111 (59)		0.65	(0.49 – 0.86)	**0.003**	0.66	(0.49 – 0.88)	**0.004**
Tumour histology			0.207						
Squamous cell carcinoma	21 (12)	32 (17)		0.48	(0.28 – 0.83)	**0.009**	0.46	(0.26 – 0.79)	**0.005**
Adenocarcinoma	81 (46)	101 (54)		0.73	(0.55 – 0.98)	**0.037**	0.75	(0.56 – 1.01)	0.059
Clinical N-stage			0.170						
cN0	27 (15)	42 (22)		0.50	(0.31 – 0.80)	**0.004**	0.49	(0.30 – 0.80)	**0.004**
cN1	77 (43)	85 (45)		0.81	(0.59 – 1.10)	0.176	0.83	(0.61 – 1.13)	0.237
WHO-performance score			0.729						
0	84 (47)	117 (62)		0.66	(0.50 – 0.88)	**0.004**	0.67	(0.51 – 0.90)	**0.006**
1	21 (12)	18 (10)		0.75	(0.40 – 1.41)	0.367	0.79	(0.41 – 1.51)	0.473

Legend table 2

Univariable and multivariable hazard ratios for all-cause mortality in 366 patients according to subgroup characteristics.

HR: hazard ratio (nCRT plus surgery *vs.* surgery alone); aHR: adjusted hazard ratio; CI: confidence interval.

Multivariable analysis included the following baseline characteristics: gender, tumour histology, clinical N-stage and WHO-performance score.

Clinical lymph-node (N) stage was based on endoscopic ultrasonography, computed tomography, or 18F-fluorodeoxyglucose positron-emission tomography; cN0: no nodes suspected or positive, cN1: at least one node suspected or positive.

World Health Organisation (WHO) performance status[25]; grade 0: able to carry out all normal activity without restrictions, grade 1: restricted in physically strenuous activity but ambulatory and able to carry out light work.

Table 3 — Number of patients with locoregional or distant progression and comparison of locoregional and distant progression between treatment groups.

	nCRT plus surgery	Surgery alone	HR	95% CI	P
	(n=178)	(n=188)			
	number of events (%)	number of events (%)			
Locoregional progression	39 (22)	72 (38)	0.45	(0.30 – 0.66)	**<0.001**
Distant progression	70 (39)	90 (48)	0.63	(0.46 – 0.87)	**0.004**
Overall progression	87 (49)	124 (66)	0.58	(0.44 – 0.76)	**<0.001**

Legend table 3

Number of patients with locoregional or distant progression and comparison of locoregional and distant progression between treatment groups.

HR: hazard ratio (nCRT plus surgery *vs.* surgery alone); CI: confidence interval. Comparison between treatment groups was based on univariable cause-specific Cox regression modelling of progression-free intervals. Deaths from non-disease-related causes were censored.

Overall progression was defined as either locoregional progression or distant progression. Patients with both locoregional disease progression and distant disease progression (n=22 in the nCRT plus surgery group and n=38 in the surgery alone group) were counted in both locoregional progression and distant progression categories.

Supplementary table 1 — Univariable and multivariable hazard ratios for overall disease progression in 366 patients according to subgroup characteristics.

Subgroup	nCRT plus surgery	Surgery alone	Inter-action	Univariable			Multivariable		
	(n=178)	(n=188)	p	HR	95% CI	p	aHR	95% CI	p
	number of events (%)	number of events (%)							
All patients	109 (61)	141 (75)	0.168	0.64	(0.49 – 0.82)	**0.001**	0.64	(0.50 – 0.83)	**0.001**
Gender			0.673						
Women	27 (15)	26 (14)		0.71	(0.41 – 1.21)	0.205	0.73	(0.42 – 1.25)	0.248
Men	82 (46)	115 (61)		0.62	(0.47 – 0.82)	**0.001**	0.63	(0.47 – 0.84)	**0.001**
Tumour histology			0.459						
Squamous cell carcinoma	22 (12)	33 (18)		0.48	(0.28 – 0.82)	**0.007**	0.46	(0.27 – 0.80)	**0.005**
Adenocarcinoma	84 (47)	105 (56)		0.69	(0.52 – 0.92)	**0.010**	0.70	(0.53 – 0.94)	**0.017**
Clinical N-stage			0.138						
cN0	28 (16)	43 (23)		0.49	(0.30 – 0.78)	**0.003**	0.47	(0.29 – 0.76)	**0.002**
cN1	80 (45)	89 (47)		0.76	(0.56 – 1.03)	0.072	0.78	(0.58 – 1.06)	0.108
WHO-performance score			0.933						
0	88 (49)	121 (64)		0.63	(0.48 – 0.83)	**0.001**	0.65	(0.49 – 0.85)	**0.002**
1	21 (12)	20 (11)		0.62	(0.33 – 1.14)	0.129	0.67	(0.36 – 1.25)	0.207

Legend supplementary table 1

Univariable and multivariable hazard ratios for overall disease progression in 366 patients according to subgroup characteristics.

HR: hazard ratio (nCRT plus surgery *vs.* surgery alone); aHR: adjusted hazard ratio; CI: confidence interval.

Multivariable analyses included the following baseline characteristics: gender, tumour histology, clinical N-stage and WHO-performance score.

Clinical lymph-node (N) stage was based on endoscopic ultrasonography, computed tomography, or 18F-fluorodeoxyglucose positron-emission tomography; cN0: no nodes suspected or positive, cN1: at least one node suspected or positive.

World Health Organisation (WHO) performance score[25]; grade 0: able to carry out all normal activity without restrictions, grade 1: restricted in physically strenuous activity but ambulatory and able to carry out light work.

Supplementary table 2 — Number of patients with locoregional or distant progression and comparison of locoregional and distant progression between treatment groups, during and after the first 6, 12 and 24 months of follow-up.

	nCRT plus surgery		Surgery alone		HR	95% CI	p
	(n=178)		(n=188)				
	number of events (%)						
Locoregional progression							
During first 6 months	11	(6)	22	(12)	0.50	(0.31 – 0.79)	**0.003**
After first 6 months	28	(16)	50	(27)	0.43	(0.27 – 0.68)	**<0.001**
During first 12 months	21	(12)	43	(23)	0.45	(0.29 – 0.72)	**0.001**
After first 12 months	18	(10)	29	(15)	0.45	(0.25 – 0.81)	**0.008**
During first 24 months	30	(17)	56	(30)	0.44	(0.27 – 0.69)	**<0.001**
After first 24 months	9	(5)	16	(9)	0.39	(0.17 – 0.89)	**0.025**
Distant progression							
During first 6 months	10	(6)	26	(14)	0.38	(0.18 – 0.78)	**0.009**
After first 6 months	60	(34)	64	(34)	0.72	(0.51 – 1.03)	0.073
During first 12 months	30	(17)	55	(29)	0.49	(0.31 – 0.76)	**0.002**
After first 12 months	40	(22)	35	(19)	0.84	(0.53 – 1.32)	0.450
During first 24 months	50	(28)	75	(40)	0.57	(0.40 – 0.81)	**0.002**
After first 24 months	20	(11)	15	(8)	0.94	(0.48 – 1.83)	0.845
Overall progression							
During first 6 months	19	(11)	43	(23)	0.44	(0.26 – 0.75)	**0.003**
After first 6 months	68	(38)	81	(43)	0.64	(0.47 – 0.89)	**0.008**
During first 12 months	42	(24)	78	(41)	0.49	(0.34 – 0.71)	**<0.001**
After first 12 months	45	(25)	46	(24)	0.71	(0.47 – 1.07)	0.106
During first 24 months	63	(35)	100	(53)	0.55	(0.40 – 0.75)	**<0.001**
After first 24 months	24	(13)	24	(13)	0.70	(0.40 – 1.23)	0.212

Legend supplementary table 2

Number of patients with locoregional or distant progression and comparison of locoregional and distant progression between treatment groups, during and after the first 6, 12 and 24 months of follow-up.

HR: hazard ratio (nCRT plus surgery *vs.* surgery alone); CI: confidence interval. Comparison between treatment groups was based on univariable Cox regression modelling of progression-free intervals.

Overall progression was defined as either locoregional progression or distant progression. Patients with both locoregional disease progression and distant disease progression (n=22 in the nCRT plus surgery group and n=38 in the surgery alone group) were counted in both locoregional progression and distant progression categories.

Figure 1 — CONSORT diagram for patient enrolment in the randomised CROSS trial.

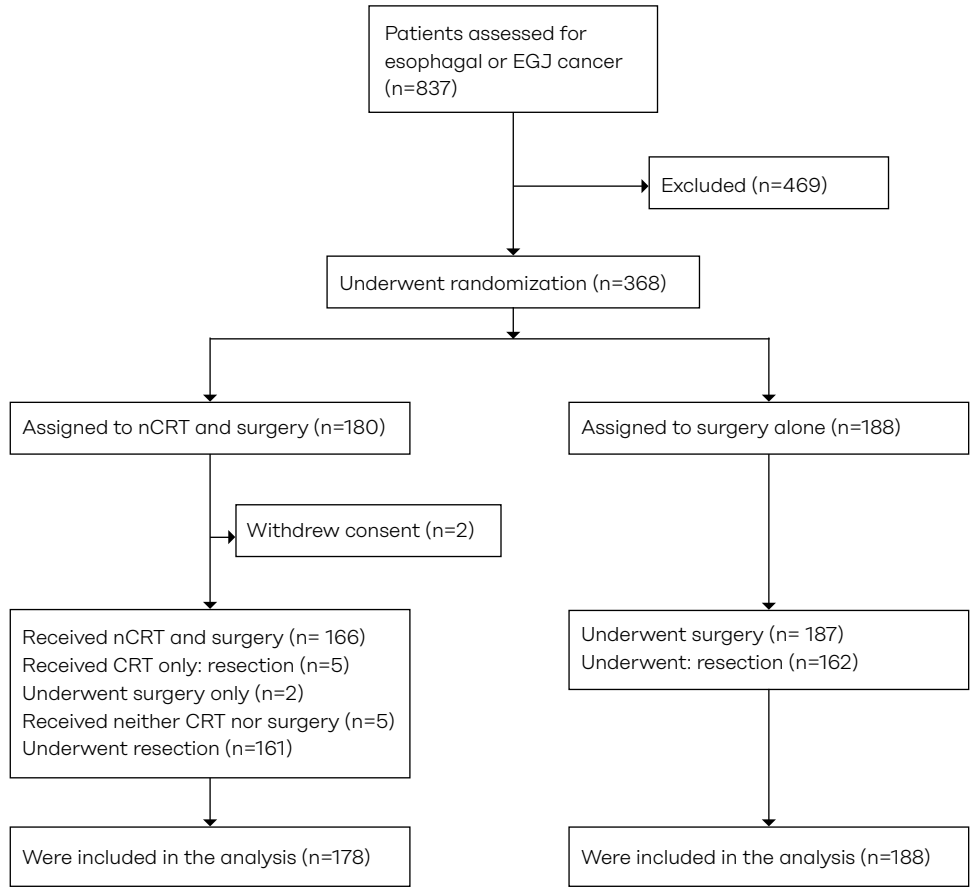
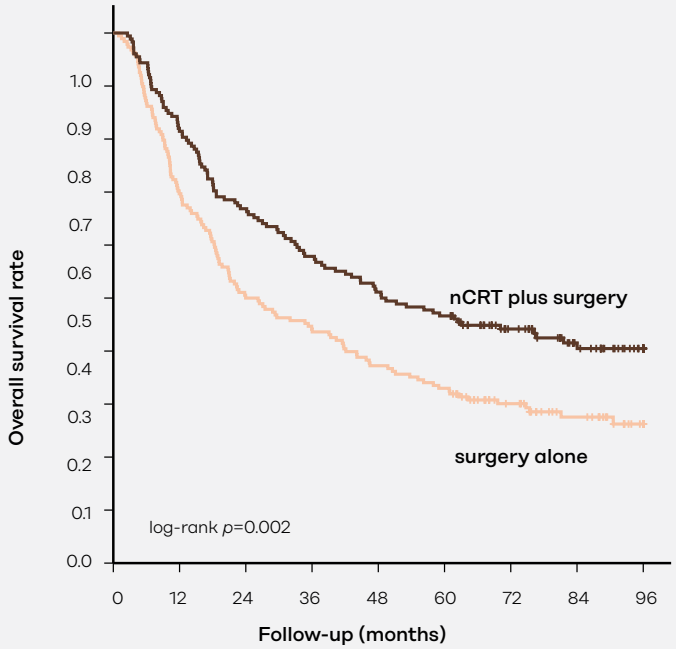


Figure 2a — Overall survival, according to treatment group.

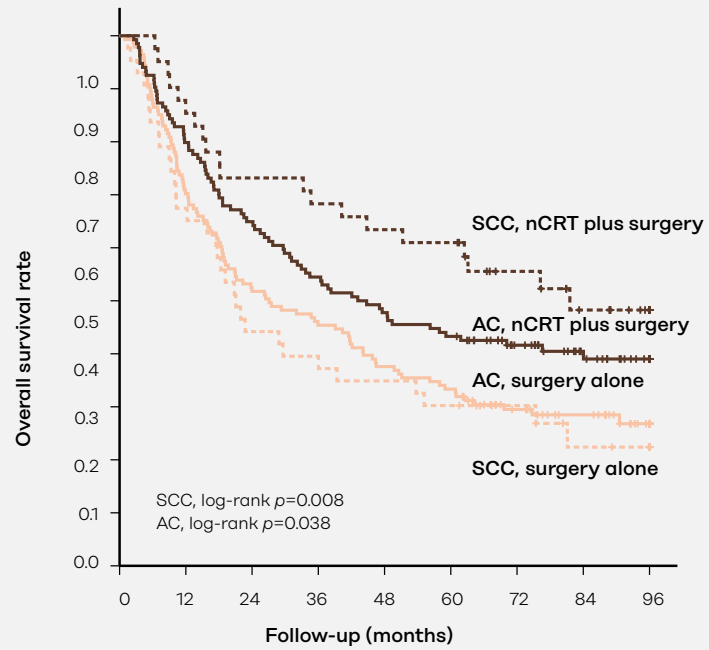


Patients at risk

nCRT plus surgery	178	145	119	103	91	83	59	40	22
surgery alone	188	131	94	83	70	62	42	28	14
Total	366	276	213	186	161	145	101	68	36

Comparison based on log-rank test, nCRT plus surgery vs. surgery alone, χ^2 9.8, degree of freedom (df) 1, p=0.002.

Figure 2b — Overall survival, according to treatment group and histological tumour type.



Patients at risk

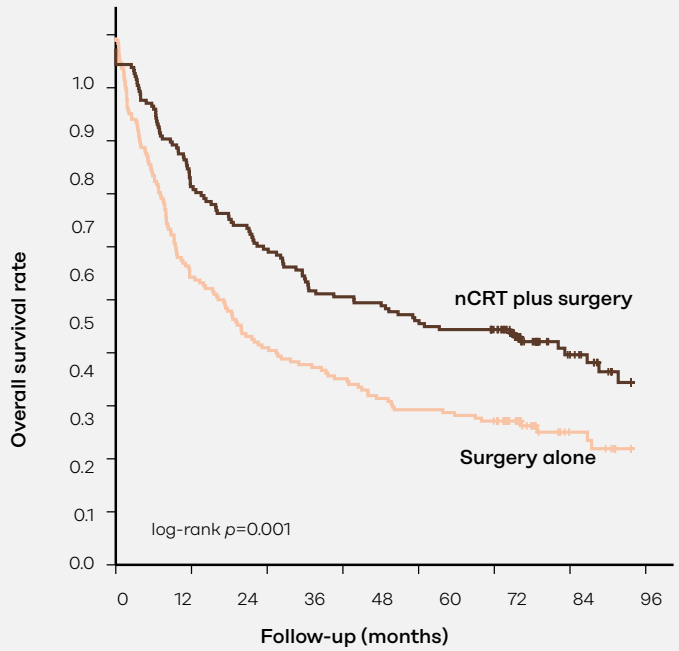
SCC, nCRT plus surgery	41	35	30	28	26	25	17	11	6
SCC, surgery alone	43	29	19	17	15	13	9	5	4
AC, nCRT plus surgery	134	107	87	73	64	58	42	29	16
AC, surgery alone	141	99	73	64	53	47	32	23	10
Total	359	270	209	182	158	143	100	68	36

Overall survival, according to treatment group and histological tumour type
SCC: squamous cell carcinoma, AC: adenocarcinoma.

Comparison based on log-rank test, SCC nCRT plus surgery vs. surgery alone, χ^2 7.0, df 1, p=0.008; AC nCRT plus surgery vs. surgery alone, χ^2 4.3, df 1, p=0.008.

Seven patients were excluded from this analysis because histological tumour type could not be determined.

Figure 3a — Progression-free survival, according to treatment group.



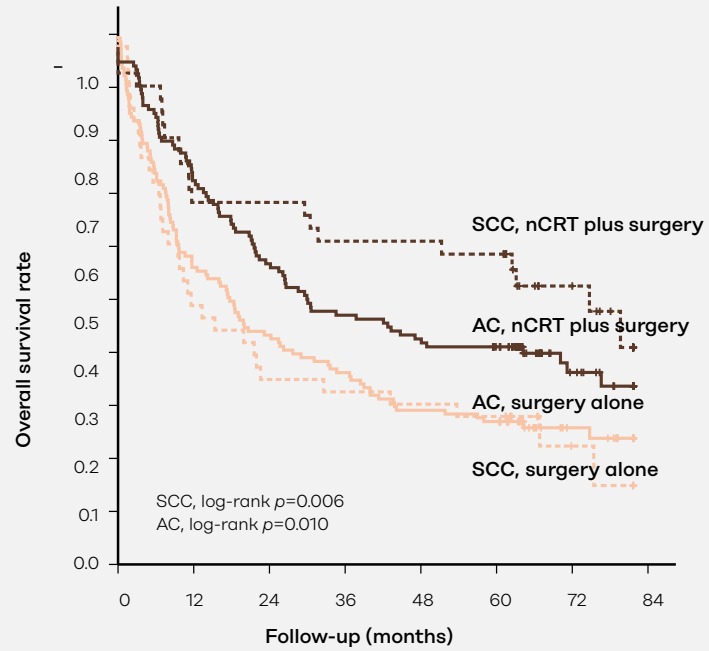
Patients at risk

nCRT plus surgery	178	127	106	90	82	74	31	14
surgery alone	188	102	77	66	55	50	16	6
Total	366	229	183	156	137	124	47	20

Progression-free survival, according to treatment group.

Comparison based on log-rank test, nCRT plus surgery *vs.* surgery alone, χ^2 13.7, degree of freedom (df) 1, p<0.001.

Figure 3b — Progression-free survival, according to treatment group and histological tumour type.



Patients at risk

SCC, nCRT plus surgery	41	28	28	25	25	24	12	5
SCC, surgery alone	43	21	15	14	13	12	3	1
AC, nCRT plus surgery	134	97	76	63	57	50	19	9
AC, surgery alone	141	79	61	51	41	38	13	5
Total	359	225	180	153	136	124	47	20

Progression-free survival, according to treatment group and histological tumour type
SCC: squamous cell carcinoma, AC: adenocarcinoma.

Comparison based on log-rank test, SCC nCRT plus surgery *vs.* surgery alone, χ^2 7.4, df 1, p=0.006; AC nCRT plus surgery *vs.* surgery alone, χ^2 6.7, df 1, p=0.010.
Seven patients were excluded from this analysis because histological tumour type could not be determined.

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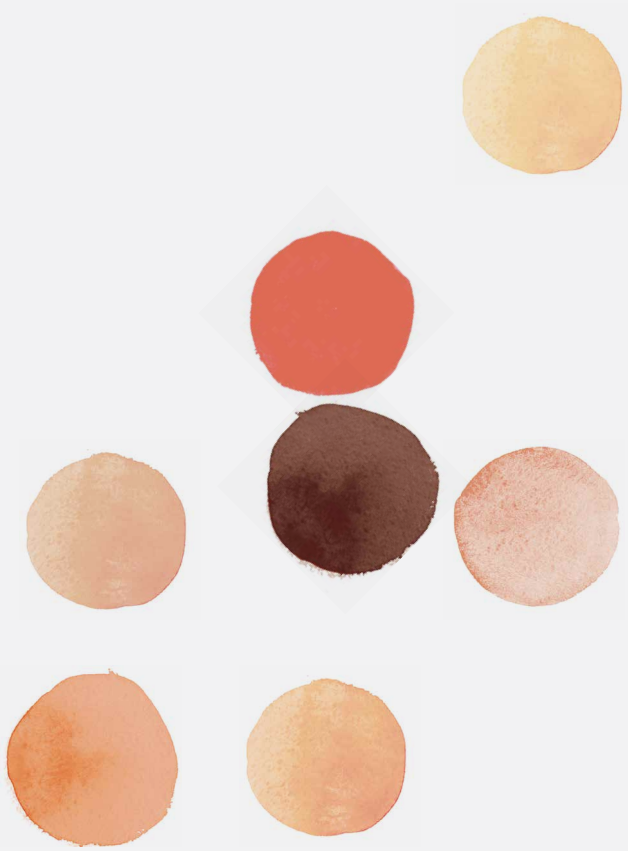
Chapter 7



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Residual esophageal cancer after neoadjuvant chemoradiotherapy frequently involves the mucosa and submucosa

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Abstract

Objective
To gain insight into the exact location of residual esophageal cancer in the esophageal wall and regional lymph nodes after neoadjuvant chemoradiotherapy (nCRT) and to determine the pattern of regression.

Background data
Data from the recently published CROSS trial showed that 49% of squamous cell carcinomas and 23% of adenocarcinomas had a pathologically complete response (pCR) in the resection specimen after nCRT. These results impose the ethical imperative to reconsider the necessity of esophagectomy with its substantial morbidity and mortality in patients with pCR. However, it remains challenging to accurately identify these patients before resection.

Methods
Between January 2003 and July 2011, all patients with esophageal cancer in a tertiary referral center, who underwent nCRT (5 weekly courses of carboplatin and paclitaxel plus 41.4 Gy concurrent radiotherapy) and surgical resection, were analyzed. The resection specimens were carefully re-evaluated by an experienced gastrointestinal pathologist. Tumor regression grade (TRG) was meticulously scored for each specific layer of the esophageal wall and for all removed lymph nodes.

Results
One-hundred and two consecutive patients were included. Seventy-one (70%) of 102 patients were non-complete responders (≥TRG2) and in 63 of these patients (89%) residual tumor cells were seen in the mucosa and/or submucosa. Five of eight patients without involvement of the mucosa and submucosa had isolated remnants in the muscle layer (5/102=5%); the other three patients had tumor cells only in a single lymph node (3/102=3%). The surrounding stroma showed the highest percentage of TRG1 (=pCR: 47%). In patients with pretreatment lymph node positivity, the percentage of TRG1 in all lymph nodes was also favorable (52%). Overall regression showed a non-random mixed pattern of both concentric regression and regression towards the lumen.

Conclusions
After neoadjuvant chemoradiotherapy for esophageal cancer both the mucosa and submucosa show frequent residual malignant involvement. The surrounding stroma and the regional lymph nodes show the highest percentage of pathologically complete response and the overall regression pattern is most frequently a mixed pattern of both concentric regression and regression towards the lumen. This overall regression pattern lends support to careful testing of a *wait-and-see* approach in a subgroup of esophageal cancer patients after nCRT.

Introduction

Over the last decades, the value of preoperative chemo- and/or radiotherapy for esophageal cancer has been studied. Neoadjuvant therapy has the potential for downstaging the tumor, which in turn increases the radical resectability rate, reduces the locoregional recurrence rate and possibly improves long-term survival. The recently published multicenter Dutch CROSS trial investigated the value of neoadjuvant chemoradiotherapy (nCRT) combining carboplatin and paclitaxel with 41.4 Gy concurrent radiotherapy[1]. Results showed a significant 13% increase in long-term survival for nCRT plus surgery as compared to surgery alone. Further analysis showed that 49% of the squamous cell carcinomas and 23% of the adenocarcinomas had a pathologically complete response (pCR) in the resection specimen (*i.e.* no viable tumor cells found, neither at the site of the primary tumor, nor in the resected regional lymph nodes, as determined by conventional histological examination).

Although surgical resection of the esophagus is still considered the cornerstone of intentionally curative treatment for esophageal cancer, it is associated with severe postoperative morbidity and a substantial impact on the quality of life[2-6]. Patients with pCR after nCRT plus surgery, achieve a favorable disease-specific 5-year survival rate of 68%[7]. However, it is questionable, whether patients with pCR after nCRT do have sufficient additional benefit to justify subsequent esophagectomy. Therefore, these high complete response rates impose a strong ethical imperative to clinically identify patients with pCR after nCRT. Under analogous conditions, a non-operative management in rectal cancer patients with a clinically complete response after nCRT has been shown feasible and safe, leading to organ-sparing treatment with low morbidity- and mortality rates and favorable long-term survival in a subset of these rectal cancer patients[8-10].

Several published studies have tried to identify esophageal cancer patients with pCR after nCRT using conventional endoscopy with histological biopsies, endoscopic ultrasonography (EUS), computed tomography (CT) and positron emission tomography (PET)[11-17]. So far, results from these studies have been mostly disappointing. It has been proven difficult to locate and to take targeted biopsies of limited amounts of

residual tumor in the esophagus, when restaging is performed only at an early single time point after completion of nCRT. Before a *wait-and-see* policy can be safely considered in a subgroup of patients with esophageal cancer after nCRT, a better insight into the exact location of residual tumor in the esophageal wall and regional lymph nodes is needed.

Therefore, the aim of the present study was (I) to describe the exact location of residual tumor in the esophageal wall and resected lymph nodes after nCRT, and (II) to describe the tumor regression pattern of esophageal cancer as induced by nCRT. In this way it will be possible to identify which layer(s) would yield the highest chance of detecting residual disease.

Patients and methods

Patients

All newly diagnosed patients with esophageal cancer at the Erasmus MC – University Medical Center, a tertiary referral hospital in Rotterdam, The Netherlands, who underwent nCRT according to the CROSS regimen followed by esophagectomy between January 2003 and July 2011[18, 19], were included (T2-3, N0-1, M0, max. tumor length 8 cm). This inclusion period contained three consecutive patient cohorts. The first cohort consisted of a consecutive subset of patients from a phase-II feasibility trial (February 2001 through January 2004)[18], the second cohort took part in the multimodality arm of a phase-III randomized controlled trial (March 2004 through December 2008)[1] and the third cohort received the CROSS regimen as standard treatment for resectable esophageal cancer in the period after completion of the two trials (January 2009 through July 2011). Patients with irresectable tumors (T4b) and/or disseminated disease (M1) were excluded as well as patients with tumors clinically limited to the mucosa or submucosa and without signs of positive lymph nodes during pretreatment workup (cT1N0). Pretreatment staging procedures included endoscopy with histological biopsy, EUS (with fine needle aspiration (FNA) of suspected lymph nodes, when indicated), CT scan of the neck, chest and abdomen and external ultrasonography of the neck (with FNA when indicated) in all patients. PET(-CT) scans were not routinely performed during this study period.

Neoadjuvant chemoradiotherapy and surgery

Patients received neoadjuvant chemoradiotherapy according to the CROSS regimen: carboplatin (area-under-curve=2) and paclitaxel (50 mg/m^2) administered by intravenous infusion on days 1, 8, 15, 22, and 29 plus concurrent external beam radiation with a total dose of 41.4 Gy, given in 23 fractions of 1.8 Gy, 5 fractions a week. Esophagectomy

was planned 4 to 6 weeks after completion of neoadjuvant chemoradiotherapy. For carcinomas proximal to or at the level of the carina a transthoracic esophageal resection with a two field lymph node dissection was performed. For carcinomas located well below the level of the carina, either a transthoracic approach with an extended lymph node dissection or a transhiatal approach was performed. For carcinomas involving the gastro-esophageal junction, a transhiatal esophageal resection was the preferred technique. In both approaches, an upper abdominal lymphadenectomy, including nodes along the hepatic artery, splenic artery and left gastric artery, was performed.

Histomorphological analysis

All resection specimens were initially processed using a standardized protocol[20]. During the original pathological examination, information was recorded concerning the macroscopic appearance and location and the proximal-, distal- and lateral extension of the tumor. If macroscopically no tumor could be identified, subtle lesions such as an ulcer or an irregular area covered by mucosa were embedded *in toto* together with surrounding areas in order to be adequately evaluated for the presence of residual tumor and/or secondary therapy effects.

For the present study, all 102 patients were systematically re-evaluated histomorphologically by an experienced gastrointestinal pathologist (FJWtK), irrespective whether a macroscopic lesion was identifiable. This re-evaluation also included re-assessment of all resected lymph nodes. Presence of vital tumor cells near the proximal- , distal- and circumferential resection margins, tumor type, tumor differentiation grade, depth of tumor invasion into the esophageal wall and the number of involved lymph nodes were rescored by the pathologist. Tumor cells were considered vital if the cytomorphological integrity of the tumor cells was intact. A radical resection (R_0) was defined as a minimal distance of 1 mm between vital tumor and the resection margins. The 7th edition of the *Union Internationale Contre le Cancer* (UICC) TNM-classification manual was used for tumor staging[21].

The original tumor area – before nCRT – was estimated based both on the extent of regressional changes (*e.g.* fibrosis, mucous lakes, keratin pearls and/or foreign body giant cell reactions) and on the presence of residual tumor cells in the resection specimen (figure 1), as has been described previously in rectal cancer patients[22-25] . These measurements were expressed as *preypT* and *preypN*, reflecting the assumed original depth of the primary tumor and the assumed number of originally involved lymph nodes, respectively. Some of the fibrotic changes seen in the post-nCRT resection specimen are caused by a preexistent desmoplastic reaction. This reaction is caused by the interaction between tumor cells and the surrounding stroma and cannot be easily

distinguished from therapy induced fibrosis. In order to determine the presence of regressional changes (*e.g.* mucous lakes, keratin pearls and/or foreign body giant cell reactions) as well as the amount of the preexisting desmoplastic reaction in the absence of nCRT, we investigated a set of 20 consecutive patients from the surgery-only arm of the CROSS trial. Tumors with invasion into the pleura and/or the diaphragm were not considered irresectable; therefore (pre)ypT4a tumors were categorized as (pre)ypT3.

The overall tumor regression grade (TRG) was evaluated using the modified Mandard scoring system as reported by Chirieac *et al.*[26]. The extent of tumor regression was divided into one of four categories, TRG1: no residual tumor cells found (pathologically complete response=pCR); TRG2: between 1-10% residual tumor cells; TRG3: between 11-50% residual tumor cells; TRG4: more than 50% residual tumor cells.

The TRG was also scored per individual esophageal wall layer and for all resected lymph nodes. For this purpose the esophageal wall was subdivided into four layers from superficial to deep: mucosa, submucosa, proper muscle layer and surrounding stroma (figure 2). The scoring was performed by first estimating the area with regressional changes for each individual layer and for all lymph nodes (using a 20x-40x magnification). In slides containing residual tumor cells, the area containing residual tumor cells was estimated relative to the area showing regressional changes. This was done for each layer and lymph node individually for all slides of the resection specimen. An average TRG was calculated for each individual layer of the esophageal wall and for all resected lymph nodes combined by averaging the TRG score of each individual layer in all slides.

To address the directionality in the pattern of regression within the esophageal wall, different potential patterns of regression after nCRT were investigated. This evaluation was performed by comparing the average TRG in two esophageal wall layers with the average TRG in the other two wall layers. A lower average TRG indicated a better response (*i.e.* more regression). The tested directions of regression were categorized as regression towards the lumen (*i.e.* more regression in the proper muscle layer and surrounding stroma as compared to the mucosa and submucosa); regression towards the invasive front (*i.e.* more regression in the mucosa and submucosa as compared to the proper muscle layer and surrounding stroma); concentric regression (*i.e.* more regression in the mucosa and surrounding stroma as compared to the submucosa and proper muscle layer) and random regression (*i.e.* comparable extent of regression in all layers) (figure 2).

The estimated distribution of residual tumor cells within the esophageal wall and lymph nodes was assessed by the relative distribution of regression between layers within each individual patient. TRG2 was set to 0.05 (=average 0-10% residual tumor cells), TRG3 was set to 0.30 (=average 10-50% residual tumor cells) and TRG4 was set to 0.75 (=average 50-100% residual tumor cells). Subsequently, the relative contribution of each layer was determined by dividing the score in that layer by the total score for all layers in that individual patient. For example, in a patient with TRG2, TRG3, TRG3, TRG1 and TRG1 in the mucosa, submucosa, proper muscle layer, surrounding stroma and lymph nodes, respectively, the relative percentage of residual tumor cells in the mucosa was determined to be 0.05/(0.05+0.30+0.30+0+0)=7.7%.

To estimate the potential clinical detectability of residual cancer cells after nCRT, patients with an overall TRG2 response were classified as major responders (*i.e.* less than 10% of original tumor cells remaining), while patients with an overall TRG3 or TRG4 response were classified as minor responders (*i.e.* more than 10% of original tumor cells remaining). It was assumed that major responders can probably not be detected reliably during clinical restaging, using either (cyto)histological sampling or PET-CT, due to the (very) low amount of residual tumor in the esophageal wall.

This study was performed on microscopy slides as used during regular patient diagnostics. None of the material used is traceable to individual patient data. The Ethics Council of the Erasmus MC – University Medical Center approves research conducted on diagnostic tissues, without special permission. Therefore, no additional ethical approval was sought for.

Statistical analysis

Grouped data were compared using the non-parametric Kruskall-Wallis *H* test or one-way ANOVA F test when appropriate with Bonferroni correction. Distributions of tumor regression grades per esophageal wall layer for all patients were compared using the non-parametric Friedman's test for related ordinal grades among all layers, with post-hoc testing using the one-on-one Wilcoxon's signed rank test with Bonferroni correction. For comparing distributions of pathologically complete response percentages, the non-parametric Cochran's Q test for related binary results was used, with post-hoc testing using the one-on-one McNemar's test with Bonferroni correction. For determining correlations between two ordinal variables Kendall's tau-b coefficient was used. The level of significance was set to $p<0.05$. All statistical tests were performed using IBM SPSS Statistics for Windows, Version 20.0 (IBM; Armonk, NY, USA).

Results

One-hundred and two consecutive patients who underwent neoadjuvant chemoradiotherapy (nCRT) followed by esophagectomy between January 2003 and July 2011 were included in this study. Their clinicopathological characteristics are summarized in Table 1. Median age was 62 years (range: 18 – 79 years), the majority of patients were male (72%) and had an adenocarcinoma (73%); clinical staging most frequently was cT3 (73%) and cN1 (62%). The tumor was located at the esophagogastric junction in 26/102 (25%) patients. The median delay between the end of nCRT and subsequent surgical resection was 49 days (range: 20 – 82 days). A microscopically radical (R_0) resection – with no tumor within 1 mm of the resection margins – was achieved in 95/102 (93%) patients. The median number of resected and identified lymph nodes was 19 (range: 2 – 58). The overall distribution of regression for all esophageal wall layers and lymph nodes combined according to Chirieac *et al.*[26] was TRG1=31 (30%), TRG2=35 (34%), TRG3=16 (16%) and TRG4=20 (20%). The pathologically complete response rate (pCR=TRG1) was 46% for squamous cell carcinomas and 24% for adenocarcinomas (Table 2a). Comparisons of clinicopathological characteristics between the three included time-based cohorts are also depicted in Table 1. Results show a significantly longer delay between nCRT and surgery in the post CROSS cohort (median 44, 44 and 55 days, respectively, p=0.007) and a significantly higher median number of lymph nodes identified in the resection specimen (median 12, 19 and 23 nodes, respectively, p<0.001).

Location of residual tumor in the esophageal wall and resected lymph nodes after nCRT

Thirty-one (30%) of the 102 included patients showed a pathologically complete response (=pCR) in the resection specimen, while 71 (70%) patients had residual tumor cells remaining after nCRT (figure 3a). The 31 patients with a pCR were 'excluded' from further analysis because they had no viable residual tumor cells left in the resection specimen and could therefore not contribute in localizing residual disease or determining direction of regression.

In the remaining 71 patients, the exact location of residual tumor was identified: the mucosa, submucosa, proper muscle layer, surrounding stroma and lymph nodes contained residual disease in 51 (72%), 53 (75%), 46 (65%), 30 (42%) and 26 (37%) patients, respectively (figure 3b). These results indicate that the mucosa, submucosa and proper muscle layer all contained residual tumor cells significantly more frequently than the surrounding stroma and regional lymph nodes (p≤0.002, figure 3b). Further analysis of histological subtypes in these 71 patients showed that the pathologically complete response rate per individual wall layer did not differ significantly between squamous cell- and adenocarcinomas (Table 2b).

The relative distribution of residual tumor within the esophageal wall and lymph nodes in individual patients is summarized for all 71 non-complete responders in figure 3c. These data show that 63 (89%) of 71 patients had residual tumor present in the mucosa, the submucosa or both. Eight (11%) patients had no residual tumor present in either mucosa or submucosa; five (5%) of whom had residual tumor involving the proper muscle layer and surrounding stroma and / or lymph nodes selectively and three (3%) patients had residual tumor only in a single lymph node. None of the analyzed patients had residual tumor in the surrounding stroma only or in multiple lymph nodes only.

However, it should be noted that these results do not distinguish between the absence of tumor due to actual tumor regression versus the absence of tumor because not all layers contained tumor initially (*i.e.* pretreatment). In order to analyze the biology of nCRT induced tumor regression (*i.e.* tumor regression pattern) one should focus only on the regression pattern in patients with pretreatment T3 tumors (preypT3): see below.

Tumor regression pattern within the esophageal wall and resected lymph nodes as induced by nCRT

To further characterize the tumor regression pattern, we specifically looked into those 57 patients from the 71 non-complete responders (figure 4), who showed regressional changes and/or residual tumor reaching into the surrounding stroma (preypT3), thereby selecting patients who had initial involvement of all layers of the esophageal wall and had residual tumor remaining after nCRT (figure 4a).

Results indicate that the surrounding stroma, as an individual layer, did show a significantly higher percentage of TRG1 (=pCR: 47%) in these patients as compared to the submucosa (21%; p=0.002) and proper muscle layer (26%; p=0.003) (figure 4b). The mucosa (TRG1=pCR=32%) did not differ significantly from the submucosa (p=0.180), proper muscle layer (p=0.664) or surrounding stroma (p=0.108). In these 57 patients there was no statistically significant difference in the pathologically complete response rate per individual wall layer between the two histological subtypes (Table 2c).

Subsequently, the overall regression pattern was analyzed as depicted in figure 2. Results show that regression towards the lumen was significantly more common as compared to regression towards the invasive front, 49% and 21%, respectively (p=0.012). However, only a non-significant trend was found in the percentage of patients with a TRG1 in

both deeper layers (*i.e.* proper muscle layer and surrounding stroma) as compared to both superficial layers (*i.e.* mucosa and submucosa), 25% and 14%, respectively (p=0.210). A concentric regression pattern was found in 46% of patients, while a random regression pattern (*i.e.* comparable regression in all layers) was much less common as compared to a non-random regression pattern (*i.e.* not all layers having a comparable TRG), 21% and 79%, respectively (p<0.001). These results indicate a mixed non-random pattern of both concentric regression and regression towards the lumen.

Fifty-four (53%) of the 102 included patients showed regressional changes and/or residual tumor in one- or more resected lymph nodes (Table 3), indicating pretreatment lymph node positivity. Of these 54 patients, 28 (52%) patients had a pathologically complete response (TRG1) in all resected lymph nodes, while 26 (48%) patients had residual tumor cells in one- or more of the resected lymph nodes, indicating the relatively high percentage of pathologically complete responders in regional lymph nodes as compared to the percentage of overall pathologically complete responders (30%). Thirteen of 54 patients had an overall pathologically complete response (including the lymph nodes), while positive nodes were found in 9/20 patients (45%) with an overall TRG2, 5/7 patients (71%) with an overall TRG3 and 12/14 patients (86%) with an overall TRG4. The correlation between the overall regression of the tumor and the percentage of patients with nodes that remain positive after nCRT was significant (τ_b=0.406, p=0.009).

In order to determine the presence of regressional changes (*e.g.* mucous lakes, keratin pearls and/or foreign body giant cell reactions) as well as the amount of the preexisting desmoplastic reaction in the absence of nCRT, we investigated a set of 20 consecutive patients from the surgery-only arm of the CROSS trial. Results show that none of the specific regressional changes were observed in these patients and that a desmoplastic reaction was seen to varying degrees, more pronounced in squamous cell carcinomas than in adenocarcinomas. The extent of the desmoplastic reaction was equally distributed across all layers of the esophageal wall and closely followed the contours of the tumor area, with little extension beyond the invasive front. The total area of the desmoplastic reaction in these patients was clearly smaller than the areas of fibrosis typically seen after nCRT.

Potential improvements for the identification of patients with a minor response after nCRT

We subsequently aimed to explain why previous studies had shown a relatively low success-rate for discrimination between complete versus non-complete responders after nCRT and to define potential ways for diagnostic improvements. Of the 102 included patients, 16 (16%) patients had an overall TRG3 response and 20 (20%) patients had an overall TRG4 response, respectively. Combined, these 36 patients were defined as minor responders. Of these 36 overall minor responders, 30 (83%), 30 (83%), 26 (72%), 16 (44%) and 10 (28%) patients also showed a minor response in the mucosa, submucosa, proper muscle layer, surrounding stroma and lymph nodes, respectively. This indicates that in patients with an overall minor response, the mucosa and submucosa most frequently showed a minor response as well, thus offering the highest chance of detecting residual tumor cells in these two specific layers. However, these percentages only apply to the 36 overall minor responders. The remaining 35 of the 71 non-complete responders had an overall major response (*i.e.* TRG2). Of these 35 overall major responders, 5(14%), 4 (11%), 2 (6%), 0 (0%) and 1 (1%) patients had a TRG3 or TRG4 (*i.e.* substantial residual disease) in the mucosa, submucosa, proper muscle layer, surrounding stroma and lymph nodes, respectively. Taken together, these results indicate that 30/36 overall minor responders plus 5/35 overall major responders =35/71 (49%) had substantial residual disease (more than 10% of residual tumor cells) in the mucosa which might potentially be detected by targeted mucosal biopsies alone.

During restaging, however, submucosal sampling, either by histological bite-on-bite biopsies or by cytological fine-needle aspirations, could be added to future restaging protocols. This would lead to a potential additional yield of 8 patients (=23% increase), who have a (probably undetectable) major response in the mucosa, but a (potentially detectable) minor response in the submucosa.

Discussion

Results show that after nCRT, 70% of patients had residual tumor cells in their resection specimen. In 89% of these patients with a non-complete response, the mucosa and/or submucosa contained residual tumor cells, while 5 patients had residual tumor cells limited to the proper muscle layer plus surrounding stroma / lymph nodes and 3 patients had residual tumor cells in a single lymph node only. Of all initially involved layers of the esophageal wall, the surrounding stroma showed the highest percentage of TRG1 (=pCR: 47%). In patients with pretreatment lymph node positivity, the percentage of TRG1 in all lymph nodes was also favorable (52%).

The overall regression pattern within the esophageal wall showed a non-random distribution between different layers. Overall regression showed a mixed pattern of both concentric regression and regression towards the lumen, indicating the preferential persistence of malignant cells in the (sub)mucosa and underlining the relatively high responsiveness of the surrounding stroma. This difference in response to neoadjuvant chemoradiotherapy between the different layers of the esophageal wall could possibly be explained by both cancer cell specific (intrinsic) factors and location specific (extrinsic) factors. An important intrinsic factor that could possibly explain the difference in responsiveness is selective advantage due to intratumoral genetic heterogeneity, as was recently shown for renal-cell carcinoma as a proof of principle[27]. This heterogeneity could be caused by microsatellite instability[28], loss of heterozygosity[29, 30] and copy number variations[31, 32]. Extrinsic factors include differences in oxygenation levels and cancer cell-stroma interactions. It has long been recognized that low tissue oxygenation levels are negatively correlated with cancer cell sensitivity to radiotherapy[33, 34] and chemotherapy[35]. The relative blood flow through the intestinal wall has been found lowest in the submucosa[36], offering further support to a hypoxia-related relative resistance of tumor cells in the submucosa. Another location specific mechanism by which differences in regression might arise is cancer cell-stroma interaction. The tumor microenvironment has been found an important player in determining cancer cell sensitivity to radiotherapy[37, 38] and chemotherapy[39]. Although the mechanisms of heterogeneous regression between the different layers of the esophageal wall are not exactly known, several of these intrinsic- and extrinsic factors might jointly contribute to differences in tumor cell sensitivity to nCRT between the different layers of the esophageal wall, as described here.

It might seem logical and self-evident that in the mucosa (and submucosa), being the presumed point of origin of both squamous cell- and adenocarcinomas of the esophagus, the largest amount of residual tumor was found. Interestingly however, a recent study which looked at the distribution of residual rectal cancer cells within the bowel wall after neoadjuvant chemoradiotherapy[40] found a pattern which was the exact opposite from ours. They describe the preferential clearing of residual cancer cells from both the mucosa and submucosa and report the highest percentage of residual cancer cells near the invasive front. We currently have no explanation for the apparent discrepancy between the two studies.

Our main motivation to undertake this study was the (surprisingly) but consistently high pathologically complete response (pCR) rate found in the CROSS trial[1]. In this trial it was found that 49% of squamous cell carcinomas and 23% of adenocarcinomas had a pCR in the resection

specimen. If one is willing to consider a *wait-and-see* approach in these patients it is of importance to know where residual tumor cells are most likely located. This might help to increase the chance of detecting residual and recurrent disease during initial clinical restaging after nCRT and subsequent surveillance by focusing tissue sampling not only at the mucosa but also at the submucosa. Also, these results in combination with the findings that the surrounding stroma and lymph nodes most frequently show a pCR suggest that a local recurrence might become clinically detectable before it becomes locoregionally irresectable.

However, it is unlikely that early after nCRT, pathologically complete responders (TRG1) could ever become clinically distinguishable from pathologically near-complete responders (TRG2), because differences between these two categories frequently constitute of no more than one- or two percent of residual tumor cells. Moreover, even a pathologically complete response does not guarantee long-term survival without locoregional recurrence. We showed earlier, that even after extended surgical resection pathologically complete responders carry a 13% risk for developing a locoregional recurrence[41]. Therefore, the main goal in the early period immediately following nCRT should perhaps rather be to identify minor responders (TRG3 and TRG4) first. If minor responders can readily be detected early after completion of nCRT, delay towards surgery would be minimal for these patients and their course of treatment would be unaltered. Over time, some patients with a near-complete response will recur locally or expose their (already) disseminated disease, while truly complete responders may only recur at distant sites. Therefore, time will be the discriminator between those who would benefit from delayed surgical resection and those who would not.

Several earlier studies have already investigated the sensitivity of discriminating between complete- and non-complete responders after nCRT[11,12,15,16], but only few focused on (cyto)histological confirmation. The first prospective study to evaluate the role of endoscopy with mucosal biopsies in detecting residual disease was performed by Bates *et al.*[42]. In this study 35 patients were included. Most were male (69%) and had a squamous cell carcinoma (80%). Neoadjuvant chemoradiotherapy consisted of cisplatin and 5-FU with 45 Gy concurrent radiotherapy. The pathologically complete response rate was 51%. Twenty-two patients underwent restaging by endoscopy with brushings and biopsies after nCRT. In these 22 patients, 17 (77%) patients had negative (cyto) histology. In these 17 negative patients, 7 (32%) patients had residual tumor cells in their resection specimen, while 10 (45%) patients had a pathologically complete response. These results indicate that the sensitivity of restaging with endoscopy and brushings and/or biopsies was

42% (5/12) and the accuracy of detection was 68%. The percentage of detected patients in this study (42%) approaches our own percentage of patients (49%) with a minor response (TRG3 or TRG4) in the mucosa, whom we might assume to have potentially detectable amounts of residual disease in the mucosa.

A second prospective study by Schneider *et al*.[14], investigated the accuracy of endoscopy, mucosal biopsies and EUS during restaging after nCRT. This study included 80 patients. Most patients were men (81%) and had locally advanced disease (uT3; 74%) of the squamous cell carcinoma subtype (61%). Neoadjuvant chemoradiotherapy consisted of cisplatin and 5-FU with 36 Gy concurrent radiotherapy. Of the 80 patients, 12 (15%), 21 (26%), 17 (21%) and 30 (38%) had an overall TRG1, TRG2, TRG3 and TRG4, respectively. The relatively low number of TRG1 responses and relatively high number of TRG4 responses in these patients (of whom a majority had squamous cell carcinoma) is not in line with more recent literature. However, this could possibly be related to the relatively low dose of radiation delivered in these patients. The overall sensitivity for histological identification of non-complete responders was 36% (20/55) which is lower than expected. It remains speculation what factors might have contributed to this low yield. The relatively short interval (2-3 weeks) between completion of nCRT and restaging might have increased the difficulty in finding positive histology within this study. In the period immediately following nCRT, the esophageal epithelium is frequently swollen and exhibits signs of acute inflammation, which might lead to more false-negative biopsy results.

Together, these two earlier studies show a relatively low sensitivity for detecting residual tumor cells by mucosal biopsies after nCRT, albeit higher in patients with a minor response. How can we potentially improve these unfavorable results before carefully attempting a *wait-and-see* approach after nCRT?

In several aspects the present data support the relative safety of delaying surgery temporarily. First, additional (cyto)histological sampling of the submucosa could increase the yield of detecting residual disease. The mucosa and submucosa together show residual tumor cell involvement in 89% of non-complete responders. In the present study 11% of non-complete responders have TRG1 or TRG2 in the mucosa while TRG3 or TRG4 is present in the submucosa. These patients are easily missed with regular endoscopic biopsies, but might be detected with bite-on-bite biopsies or fine-needle aspirations of the submucosa. Second, of all esophageal wall layers, the surrounding stroma has the highest percentage of TRG1, reflecting effective tumor downstaging with increased distance between the residual tumor and the circumferential resection margin and possibly allowing for a lag period before

the radicality of the circumferential resection margin is surgically threatened. Third, only three (3%) of 102 included patients in this study had isolated residual lymphatic disease and this was always limited to a single lymph node. No patient had isolated residual disease in multiple lymph nodes, indicating that the risk of missing isolated regional lymph node metastases is likely to be small.

Finally, a recent study by Furlong *et al*.[43] reported on their favorable results with a *wait-and-see* approach for high-risk esophageal cancer patients with a complete clinical response after neoadjuvant chemoradiotherapy. Taken together, these results strengthen our willingness to formally test a *wait-and-see* approach in esophageal cancer patients otherwise fit to undergo resection.

Limitations of the study

The first limitation of this study is that the reproducibility of the presented data was not tested. Having the slides reviewed by a single pathologist does limit the conclusions that can be drawn from this study. However, the measurements performed in this study are essentially the same as a standard pathological response evaluation (*e.g.* Mandard score[44]), which includes tumor regression grading (TRG) for the entire resection specimen. These measurements are currently being performed routinely in day-to-day clinical practice for many tumor types after neoadjuvant therapy. Our report of the TRG per individual wall layer thus uses this same scoring technique within different defined areas of the esophageal wall.

The second limitation is the assumption that the area of regressional changes plus residual disease truly represents the original (pretreatment) tumor location. Regressional changes such as mucous lakes and foreign body giant cell reactions were not observed in primarily resected patients and therefore seem to be exclusively correlated with neoadjuvant therapy. There might be a systematic overestimation of the original tumor area due to the presence of a desmoplastic reaction. However, this overestimation is probably small and most likely will not cause any skewing of the TRG data between different layers of the esophageal wall within the same patient. Also, the alternative gold-standard would be clinical staging based on EUS, which, although accepted as the most accurate clinical T-staging modality, is still considered relatively inaccurate, especially for less advanced tumors[45]. Therefore, we consider the staging method based on preypT (because of its high resolution) preferable for this study as opposed to clinical staging by EUS.

The third limitation is that all residual disease was scored based on the ratio between residual tumor area and residual fibrotic area. This ratio does not include information on the absolute size of either areas, because of technical limitations this was not feasible. A final limitation is that it is unknown what the described regression patterns would look like in patients treated with other neoadjuvant therapies, such as chemotherapy alone or cisplatin and 5-FU based chemoradiotherapy.

Conclusions

After neoadjuvant chemoradiotherapy for esophageal cancer both the mucosa and submucosa show relatively frequent residual malignant involvement, offering the opportunity for successful targeted sampling of both layers. Chemoradiotherapy induced regression of esophageal cancer within the esophageal wall and regional lymph nodes is heterogeneous. The surrounding stroma and the regional lymph nodes show the highest percentage of pathologically complete response and the overall regression pattern is most frequently a mixed pattern of both concentric regression and regression towards the lumen. This suggests that a local recurrence might become clinically detectable before it becomes locoregionally irresectable. Taken together these results point towards possible new improvements in identifying non-complete responders during clinical restaging and lend support to careful testing of a *wait-and-see* approach after nCRT in a subgroup of esophageal cancer patients, as has recently been proposed for rectal cancer patients.

Table 1 — Comparison of clinicopathological characteristics of 102 patients who underwent esophagectomy after neoadjuvant chemoradiotherapy according to CROSS[1] in the three time-based cohorts: CROSS I , CROSS II and post CROSS.

Parameter	Total	CROSS I	CROSS II	post	p
	n=102	n=19	n=31	n=52	
Median age (range), [years]	62 (18-79)	62 (41 - 74)	60 (44 - 74)	62 (18 – 79)	0.463
Gender					
Male	73 (72%)	17 (89%)	21 (68%)	35 (67%)	0.162
Female	29 (28%)	2 (11%)	10 (32%)	17 (33%)	
Histology					
Squamous cell carcinoma	28 (27%)	6 (32%)	13 (42%)	9 (17%)	0.047
Adenocarcinoma	74 (73%)	13 (68%)	18 (58%)	43 (83%)	
Preoperative T-stage*					
cT1	2 (2%)	-	1 (3%)	1 (2%)	0.067
cT2	26 (25%)	1 (5%)	9 (29%)	16 (31%)	
cT3	74 (73%)	18 (95%)	21 (68%)	35 (67%)	
Preoperative N-stage*					
cN0	32 (31%)	8 (42%)	9 (29%)	15 (29%)	0.216
cN1	63 (62%)	11 (52%)	22 (71%)	30 (58%)	
cN2	6 (6%)	-	-	6 (12%)	
cN3	1 (1%)	-	-	1 (1%)	
Median delay between nCRT and surgery (range), [days]	49 (20- 82)	44 (25 - 74)	44 (20 - 82)	56 (21 - 81)	**0.007**
Resection status∂					
R_0	95 (93%)	18 (95%)	28 (90%)	49 (94%)	0.819
R_1	6 (6%)	-	3 (10%)	3 (6%)	
R_2	1 (1%)	1 (5%)	-	-	
Median number of lymph nodes resected (range), [number]	19 (2-58)	12 (2 - 28)	19 (5 - 34)	23 (7 - 58)	**<0.001**
ypT°					
ypT0	34 (33%)	6 (32%)	11 (35%)	17 (33%)	0.969
ypT1	21 (21%)	4 (21%)	6 (19%)	11 (21%)	
ypT2	18 (18%)	3 (15%)	5 (17%)	10 (19%)	
ypT3	29 (28%)	6 (32%)	9 (29%)	14 (27%)	
ypN°					
ypN0	76 (73%)	15 (79%)	23 (74%)	38 (73%)	0.870
ypN1	17 (18%)	2 (11%)	5 (16%)	10 (19%)	
ypN2	6 (6%)	1 (5%)	3 (10%)	2 (4%)	
ypN3	3 (3%)	1 (5%)	-	2 (4%)	
TRG#					
TRG1	31 (30%)	6 (32%)	9 (29%)	16 (31%)	0.868
TRG2	35 (34%)	7 (37%)	13 (42%)	15 (29%)	
TRG3	16 (16%)	2 (10%)	5 (16%)	9 (17%)	
TRG4	20 (20%)	4 (21%)	4 (13%)	12 (23%)	

Legend table 1

* Preoperative T and N stages as determined by endoscopic ultrasonography, with or without cytohistological confirmation of suspected lymph nodes.

∂ Resection status: R_0 was defined as a minimal distance of 1 mm between vital tumor and the resection margins; R_1 was defined as vital tumor within 1 mm of the resection margins; R_2 was defined as a macroscopically irradical resection.

° ypT and ypN are the T- and N-stages as determined in the resection specimen after neoadjuvant chemoradiotherapy.

\# TRG: tumor regression grade as defined by Chirieac *et al.*[26]; TRG1: pathologically complete response, no viable tumor cells remaining; TRG2: ≤ 10% viable tumor cells remaining; TRG3: between 11 and 50% viable tumor cells remaining; TRG4: ≥ 50% viable tumor cells remaining.

Comparisons between groups were performed using the non-parametric Kruskall-Wallis *H* test or one-way ANOVA *F* test with Bonferroni correction. The significance level after correction was set at $p<0.017$ (=0.05/3). Significant P-values are highlighted in bold.

Table 2a — Overall distribution of tumor regression grade (TRG) per tumor type in 102 patients who underwent esophagectomy after neoadjuvant chemoradiotherapy according to CROSS[1].

Parameter	Total	Squamous cell carcinoma	Adenocarcinoma
		n=28	n=74
TRG			
TRG1	30% (31)	46% (13)	24% (18)
TRG2	34% (35)	29% (8)	36% (27)
TRG3	16% (16)	11% (3)	18% (13)
TRG4	20% (20)	14% (4)	22% (16)

Table 2b — Percentage of pathologically complete responders (TRG1) per individual wall layer for both tumor types in 71 patients with a pathologically non-complete response after neoadjuvant chemoradiotherapy according to CROSS[1].

	Squamous cell carcinoma	Adenocarcinoma	p
	n=15	n=56	
Mucosa	33% (5)	27% (15)	0.617
Submucosa	27% (4)	25% (14)	0.895
Proper muscle layer	33% (5)	36% (20)	0.864
Surrounding stroma	67% (10)	55% (31)	0.431

Table 2c — Percentage of pathologically complete responders per individual wall layer for both tumor types in 57 patients with an assumed pretreatment T3 tumor and a pathologically non-complete response after neoadjuvant chemoradiotherapy according to CROSS[1].

	Squamous cell carcinoma n=12	Adenocarcinoma n=45	p
Mucosa	33.3% (4)	31.1% (14)	0.883
Submucosa	33.3% (4)	17.8% (8)	0.240
Proper muscle layer	33.3% (4)	24.4% (11)	0.534
Surrounding stroma	58.3% (7)	44.4% (20)	0.394

Legend table 2a, 2b, 2c

TRG: tumor regression grade, as defined by Chirieac *et al.*[26]

TRG1: pathologically complete response, no viable tumor cells remaining; TRG2: ≤10% viable tumor cells remaining; TRG3: between 11 and 50% viable tumor cells remaining;

TRG4: ≥ 50% viable tumor cells remaining.

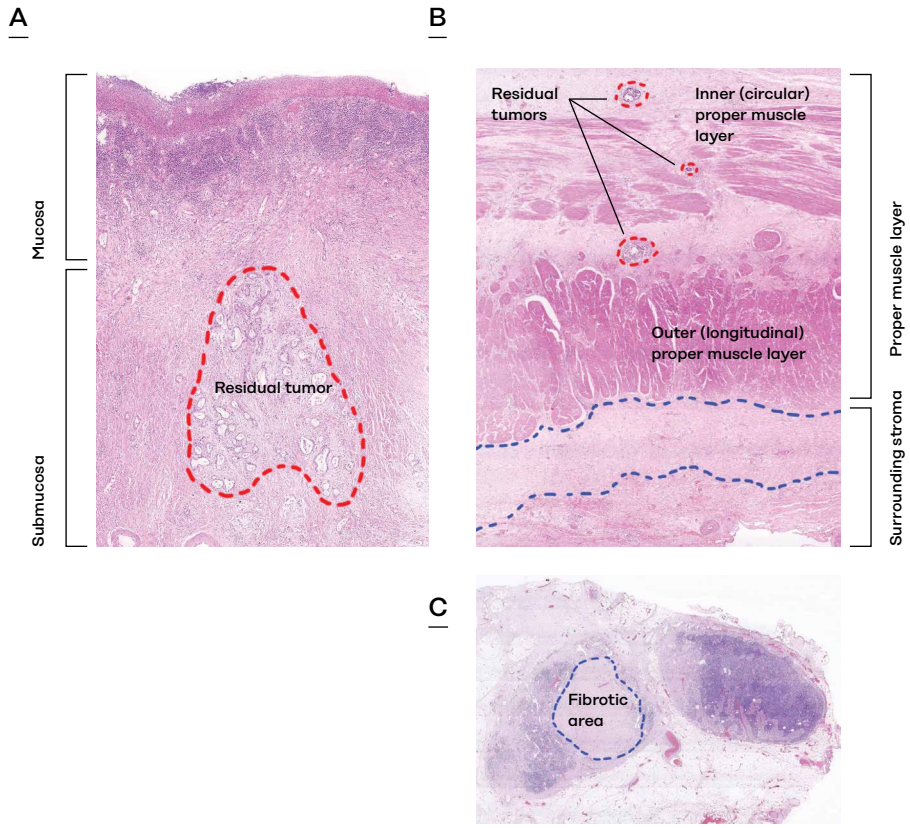
Table 3 — Estimate of pretreatment tumor extent in 102 patients who underwent esophagectomy after neoadjuvant chemoradiotherapy according to CROSS[1].

preypT		
preypT1	10	(10%)
preypT2	14	(14%)
preypT3	78	(76%)
preypN		
preypN0	48	(47%)
preypN1	39	(38%)
preypN2	11	(11%)
preypN3	4	(4%)

Legend table 3

The estimate of the extent of tumor before neoadjuvant chemoradiotherapy is based on the location of residual tumor and regressional changes in the esophageal wall and resected lymph nodes. These regressional changes include fibrosis, mucous lakes, keratin pearls and foreign body giant cell reactions. Both preypT and preypN categories were staged according to the pT- and pN-stages as defined by the 7th edition of the TNM staging manual[21].

Figure 1 — Histological examples of residual tumor and regressional changes in the esophageal wall and regional lymph nodes.



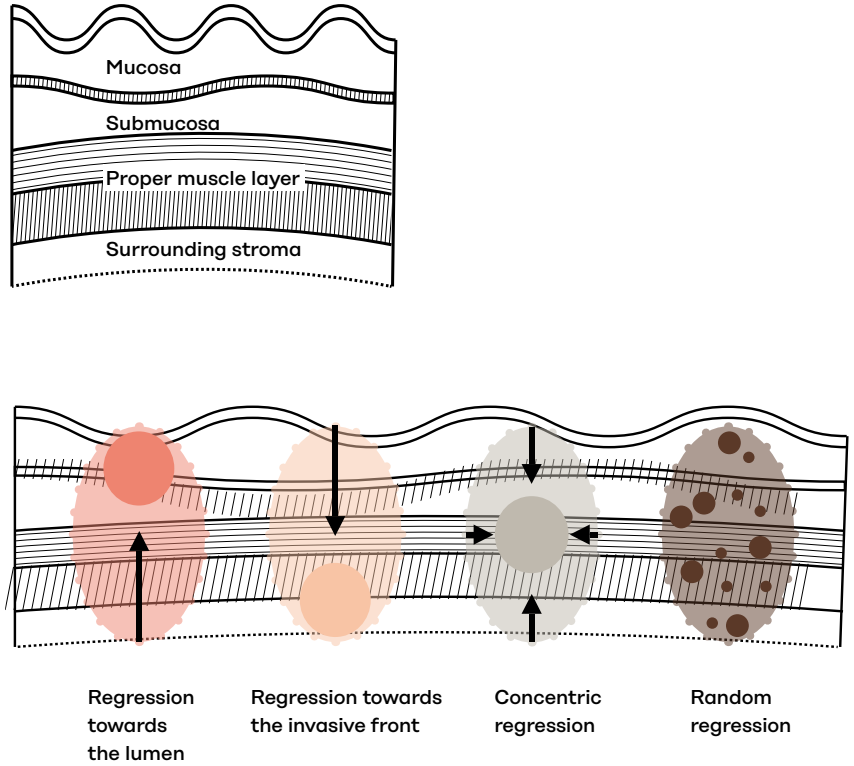
Legend figure 1

A. Example of substantial submucosal residual disease with a complete regression in the mucosa. The mucosa of this patient was scored TRG1 (=pCR) and the submucosa TRG3. The original mucosal surface was ulcerated, thus no epithelial lining was recognizable. The exact transition between mucosa and submucosa was undeterminable, due to extensive amounts of fibrosis; picture taken using 2.50x magnification. The red-dashed line indicates the edge of residual tumor.

B. Example of tumor downstaging. The surrounding stroma shows a major fibrotic area, indicating the initial involvement of this layer (preypT3) pretreatment. The deepest residual tumor cells reach well into the proper muscle layer, indicating a downstaging from preypT3 to ypT2. This patient was scored as an overall ypT2, TRG2; picture taken using 1.25x magnification. The red-dashed lines indicate the edge of residual tumor; the blue-dashed line indicates the edge of the major fibrotic area in the surrounding stroma; however, fibrotic areas are also seen in the proper muscle layer.

C. Example of two adjacent lymph nodes, with regressional changes in the left lymph node. Within the center of the left lymph node a large fibrotic area can be seen without any residual tumor cells. This node was assumed to have been tumor-positive pretreatment, but achieved TRG1 (=pCR) after nCRT. The right lymph node is an example of a normal, unaffected lymph node; picture taken using 1.25x magnification. The blue-dashed line indicates the edge of the major fibrotic area.

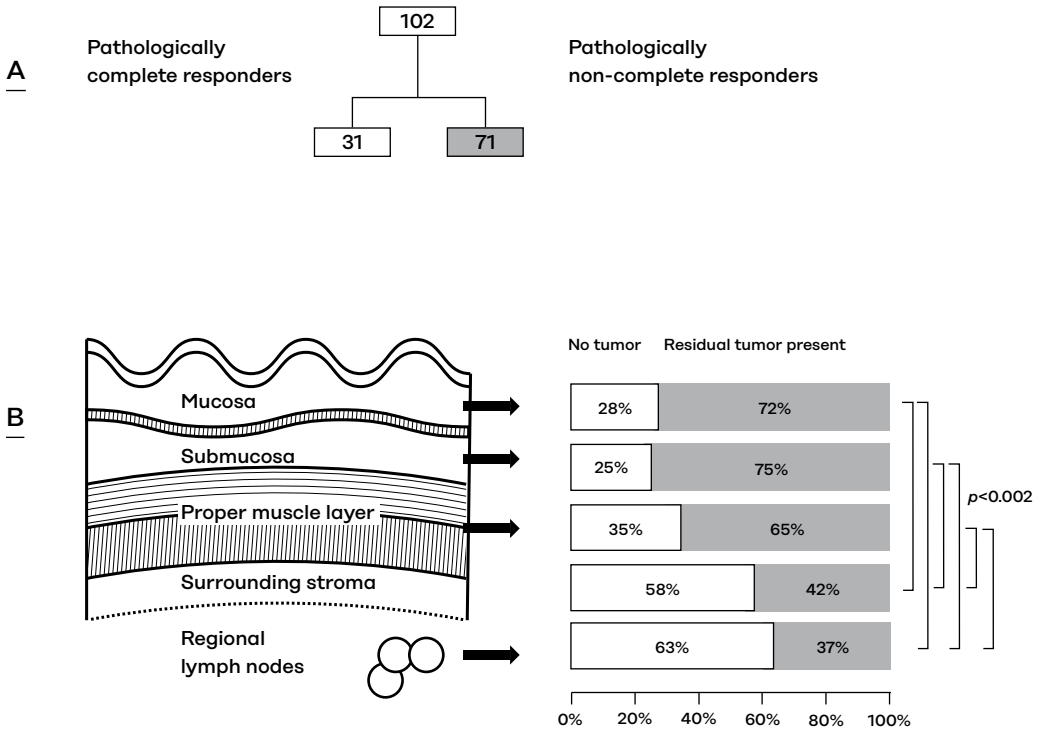
Figure 2 — Possible regression patterns within the esophageal wall after neoadjuvant chemoradiotherapy.



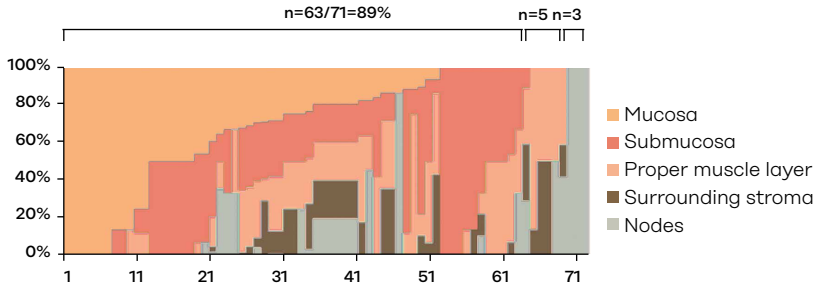
Legend figure 2

The esophageal wall consists of four layers. From the lumen: mucosa, submucosa, proper muscle layer and surrounding stroma. The transparently colored areas represent the areas of original (*i.e.* pretreatment) tumor extent. The darker colored areas represent the areas with residual tumor cells which are present after nCRT. Regression towards the lumen is defined as more regression in the proper muscle layer and surrounding stroma as compared to the mucosa and submucosa; residual tumor cells will be located mostly in mucosa and submucosa. Regression towards the invasive front is defined as more regression in the mucosa and submucosa, as compared to the proper muscle layer and surrounding stroma; residual tumor cells will be located mostly in the proper muscle layer and surrounding stroma. Concentric regression is defined as more regression in the mucosa and surrounding stroma, as compared to the submucosa and proper muscle layer; residual tumor cells will be located mostly in the submucosa and proper muscle layer. Random regression is defined as comparable extent of regression in all layers; residual tumor cells will be present to a comparable extent in all originally involved layers.

Figure 3 — Residual tumor location in 71 patients with a pathologically non-complete response in the resection specimen after neoadjuvant chemoradiotherapy according to CROSS[1].



C



Legend figure 3

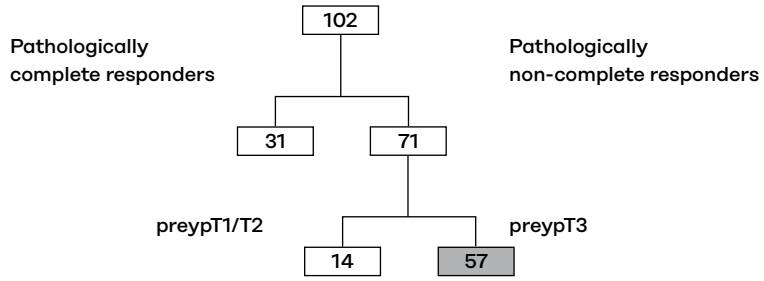
A. In 102 included patients, 31 patients had a complete response and 71 patients had a non-complete response.

B. In these 71 non-complete responders, residual tumor was scored for each individual layer of the esophageal wall and for all resected and identified lymph nodes. The mucosa, submucosa and proper muscle layer contained tumor significantly less frequently as compared to the surrounding stroma and lymph nodes. Bars indicate a significant difference between individual layers, all p-values ≤0.002.

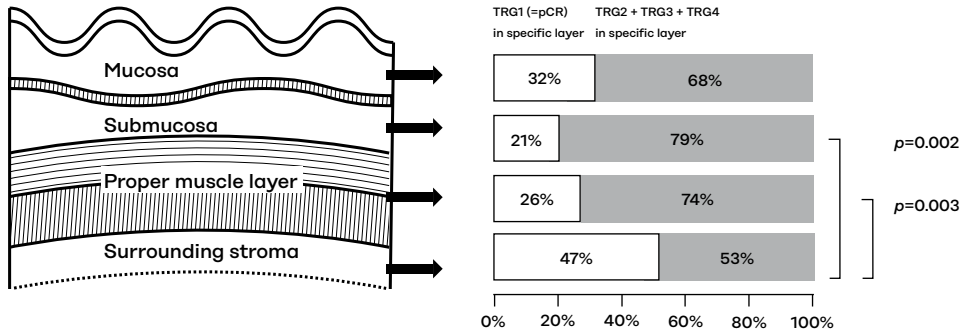
C. The estimated distribution of residual tumor cells within the esophageal wall and lymph nodes in all 71 non-complete responders; distribution of residual tumor cells per individual layer was assessed based on the relative distribution of regression between layers within each individual patient (see Methods for more details on the calculation method used); 63/71 (89%) patients had residual tumor cells in the mucosa, the submucosa or both; 8 patients had no residual tumor cells in the mucosa or submucosa, of whom 5/8 had residual tumor cells in deeper layers and 3/8 had residual disease in a single lymph node only.

Figure 4 – Residual tumor location in 57 patients with a pretreatment T3 tumor and a pathologically non-complete response in the resection specimen after neoadjuvant chemoradiotherapy according to CROSS[1].

A



B



Legend figure 4

A. Of 102 included patients, 31 patients had a complete response and 71 patients had a non-complete response. Of these 71 non-complete responders, 57 patients had initial involvement of all four esophageal wall layers (preypT3).

B. In these 57 patients, the tumor regression grade was scored for each individual layer of the esophageal wall. The surrounding stroma significantly more frequently showed a TRG1 as compared to the submucosa and proper muscle layer, as indicated by the bars, p=0.002 and p=0.003, respectively.

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Extra discussions

Discussants 1

Arnulf Hölscher (Cologne, Germany):
The article by Shapiro and colleagues represents a very detailed histopathology workup of resection specimens after neoadjuvant chemoradiotherapy (nCRT) for esophageal cancer. However, questions remain concerning the material and the suggested clinical implications. Most of the patients had transhiatal esophagectomy with lymphadenectomy only of the lower mediastinum. The first cohort had a median of just 12 resected lymph nodes for abdomen and chest. The nodal regression grading based on such low numbers is not representative. The analysis of regression in the 4 layers should focus on the 57 T3 carcinomas because only in these patients were all layers initially involved. In this group, no significant difference in pCR was found between mucosa and surrounding stroma. Furthermore, no significant difference in pCR was found comparing deeper and superficial layers in all patients. The authors suggest a '*wait-and-see*' approach in responders with negative endoscopic biopsies because they argue that the mucosa is very representative for residual cancer. However, results of histopathology workup of an esophagectomy specimen are not comparable with histology of endoscopic biopsies. Clinical studies report a sensitivity of 50% to 60% for endoscopy plus biopsy for detection of residual oesophageal cancer after nCRT.

If no cancer is found in endoscopic biopsies, the implications are either complete response with or without lymph node metastasis or regression grades 2 to 4 but without the presence of residual tumor in the mucosa or submucosa. In patientswith amajor response (<10% viable tumor), residual cancer is difficult to detect.However, it is these patients who benefit most from esophagectomy with a chance of cure that is similar to those with pCR. The risk of waiting for further development of cancer in these patients seems to be higher than the postoperative mortality of 3% in high-volume centers. Therefore, a '*wait-and-see*' approach based on the presented data is doubtful.

Response From Joël Shapiro (The Netherlands):
Thank you, Dr Hölscher, for your comments. We were faced with a significant number of patients in our cohortwho had no residual tumor in the resection specimen and we feel that it is our ethical imperative to try to find a solution and perhaps not operate on all of these patients. To try to detect minimal residual disease with a single clinical restaging shortly after completion of neoadjuvant chemotherapy is probably not the way to go as one will never be able to distinguish between a truly complete responder and those with minimal residual disease. We do not even know the clinical or biological significance of very small residual tumor deposits that are encased in fibrosis. Second, in those patients who have minimal residual disease, we think that it may be possible to perform serial clinical restaging and that the location of residual disease combined with the regression pattern would detect residual disease before it becomes clinically apparent or unresectable.

Discussants 2

Ronan O'connell (Dublin, Ireland):
Thank you for a very nice presentation andwhole mount preparation of the histology.Howmany pathologistswere involved and how reproducible were their assessments?

Response From Joël Shapiro (The Netherlands):
Thank you for your question. All these analyses have been performed by a single pathologist. We readily acknowledge that that is a limitation of this study; however, the method used was the same that is used all over the world to determine the tumor regression. We simply divided this tumor regression rate into individual layers of the oesophageal wall, so we believe that although this is a novel way of approaching the resection specimen, the technique itself has been used for many years.

Discussants 3

Carlos Pellegrini (Seattle, WA):
I have 2 brief questions. Did you get any idea of the volume of residual disease in these patients? There are some studies that have clearly shown that the amount of residual disease, 0% to 15%, 15% to 20%, or more than 50%, has a lot to do with the prognosis. Second, it seems to me, as pointed out by Dr Hölscher, that there is an unintended consequence in these findings. If you just did biopsies and adopted a wait and see approach, you would be excluding the patients who could be helped the most. So, with that in mind, is there anything other than a biopsy of the mucosa of the esophagus that you would recommend to determine whether or not residual disease is present? Have you used modern techniques or molecular imaging or any kind of radiological imaging, for example, PET scanning?

Response From Joël Shapiro (The Netherlands):
Thank you for your question. To answer your second question first, we believe that a full set of diagnostic modalities should be employed if one is willing to try and wait. That includes not only biopsies of the mucosa and fine-needle aspiration of the submucosa. The combination of PET-CT and biopsy seems to add information as opposed to a PET alone. So, yes, we think as much as possible should be done to not lose any patients with residual disease in the deeper layers, who as you pointed out, would benefit most from immediate resection. To assess the amount of tumor that is found in the resection specimen is technically quite difficult because there are many sections and then you have to determine the surface area. We determined the tumor regression rate on the basis of the modified tumor regression grade scoring system. TRG1 means zero present, then from 1% to 10%, from 10% to 50%, or more than 50% of residual tumor remaining. So every layer was scored according to that scoring system.

Discussants 4

Giovanni Zaninotto (Venezia, Italy):
I have 2 brief questions. When the operation was performed, after completion of the chemotherapy? We know from rectal cancer surgery the longer you wait, the better the response. The second question is whether you had any false positives in your group?

Response From J. Shapiro (The Netherlands):
The median time to operation was 49 days. There was some variation, of course, mostly for logistic reasons and indeed we did see a trend that the longer we waited the greater the percentage of pathological complete responders. To answer the second question, did you mean clinical restaging or did you mean false positives as pathologically staged? We based our results on the presence of viable tumor after resection. In that case, we did not have false positives because the criterion was viable tumor cells identified by histology. However, we have seen local and regional recurrences in some of the patients with a pathological complete response in whom we did not find a single tumor cell in the resection specimen.

Letter to the editor

Residual Esophageal Cancer After Neoadjuvant Chemoradiotherapy Frequently Involves the Mucosa and Submucosa.

To the Editor:
With great interest, we read the article by Shapiro et al[1] published in the November 2013 edition of *Annals of Surgery*. The authors meticulously reevaluated the resection specimens of 71 esophageal cancer patients showing pathological noncomplete response to neoadjuvant chemoradiotherapy (nCRT). For each layer of the esophageal wall and for all available lymph nodes, a tumor regression grade was appointed by a pathologist. The conclusion was drawn that after nCRT both the mucosa and the submucosa frequently show residual malignant involvement as opposed to the surrounding stroma. However, the data analysis and interpretation of results raise some questions. In particular, multiple concerns are raised regarding the conclusion of the authors that their findings support a *wait-and-see* approach in the subgroup of clinical complete responders.

First, in the overall population with clinical T3 esophageal tumors, residual disease may involve the surrounding stroma and the mucosa equally frequently. The authors found that in the subgroup of 57 patients with clinical T3 tumors (tumors where the initial cancer also involved the mucosa and surrounding stroma) the presence of residual tumor in the mucosa and surrounding stroma was not significantly different. Therefore, the conclusion that residual cancer is more often present in the mucosa than in the surrounding stroma should be interpreted with caution. The residual tumors in the surrounding stroma will not be detected by endoscopy.

Second, even superficial residues tend to be easily missed by endoscopic biopsies and endoscopic ultrasound. In studies investigating the value of endoscopic biopsies in identifying residual cancer after nCRT, consistently poor sensitivities of 31%,[2] 36%,[3] 42%,[4] and 59%[5] were reported. In this study by Shapiro et al,1 the authors suggest that post-nCRT bite-on-bite biopsies or fineneedle aspirations would improve the sensitivity of identifying noncomplete responders. Unfortunately, however, in patients with residual tumor in the submucosa but not in the mucosa, the normal appearing lumen may likely mask the underlying submucosal residual disease, resulting in a significant sampling error.

Endoscopic ultrasound has shown to be of limited additional value in the evaluation of treatment response,mainly because of the difficulty of differentiating fibrosis and inflammation from residual tumor, and is therefore not likely to overcome the sampling error problem. Therefore, a true sensitivity improvement by deeper biopsies is doubtful and warrants investigation before drawing any conclusions on postponement of surgical treatment. Notably, safety issues should be addressed first, as a high risk of esophageal perforations with accompanying morbidity and mortality is feared when applying deeper biopsies in a vulnerable irradiated esophagus.

Third, before considering a wait-andsee approach, the outcomes after salvage esophagectomy (in case of residual or recurrent disease) should be taken into account.

A recent meta-analysis by Markar et al[6] reported that salvage esophagectomy after definitive chemoradiotherapy is associated with a significantly increased incidence of postoperative mortality (10% vs 4%; p<0.001), anastomotic leakage (24% vs 14%; p=0.005), and pulmonary complications (30% vs 17%; p<0.001). Also, although studies from neoadjuvantly treated rectal cancer patients showed that a longer surgical delay (>8 weeks) was associated with a higher rate of pathological complete response (pCR), 2 studies in esophageal cancer showed that longer surgical delay (>8 weeks) did not result in a favorable pCR rate.[7,8] On the contrary, longer delay was associated with an increased amount of residual cancer (eg, higher tumor regression grade scores; p=0.024), and an inferior 5-year overall survival in a subgroup of clinical complete responders (35% vs 50%; p=0.038).[8]

Finally, because all current modalities yield unsatisfactory results in the assessment of response to nCRT so far, and salvage esophagectomy is associated with poorer outcome, one should first aim to assess the value of *improved* response evaluation techniques or even new modalities before testing a *wait-and-see* approach. More specifically, to justify a *wait-and-see* approach in *individual* patients, a test should first have a proven 'high enough' negative predictive value for detecting residual cancer, rather than a high sensitivity. In our opinion, it is doubtful that submucosal biopsies will improve the value of endoscopy, because of sampling error issues and the potential increased risk of perforations. New fields of improvement include 18F-fluorodeoxyglucose positron emission tomography studies combining spatial and metabolic information with computer-based comprehensive spatial-temporal features to evaluate tumor response to treatment. Also, functional magnetic resonance imaging may be a promising new tool for response evaluation as suggested by rectal cancer studies[9] and preliminary experience in esophageal cancer.[10]

In conclusion, we think that with current response evaluation tools initiation of a *wait-and-see* approach would imply deteriorated outcomes for patients that are erroneously portrayed as complete responders. In our opinion, these outcomes outweigh the benefits of an organ preservation regimen for the successfully identified group.

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Reply:

We thank van Rossum et al for their critical comments. Currently, it is unclear which patients benefit from surgical resection after neoadjuvant chemoradiotherapy (nCRT). One can reasonably assume that in a subset of patients esophagectomy is performed after neoadjuvant therapy without benefit, either because of unrecognized (but already present) distant dissemination, or because of complete absence of disease.

How then to preoperatively identify these patients in whom surgical resection would not be beneficial? The conceptual approach we suggest to be explored would be to postpone surgical resection until locoregional recurrence is detected during close surveillance, in the absence of disseminated disease. In such a *surgery-as-needed* approach, patients with a truly complete response and patients with disease manifestation at distant sites (irrespective of synchronous locoregional residual or recurrent disease)would not be exposed to an unbeneficial and potentially harmful surgical resection.

For such a surveillance strategy to be promising, the location and distribution of residual disease at the primary tumor site should be favorable. Presence of residual disease in the mucosa is helpful for early detection of (endoluminal) recurrence. On the contrary, presence of residual disease in the surrounding stroma would potentially be more harmful because of endangerment of the circumferential resection margin, if left undetected for extended periods of time.

In the 57 patients, as described in our article,[1] with initial pretreatment involvement of all layers of the esophageal wall (= preypT3) and an overall noncomplete response after

nCRT, the mucosa contained residual disease in 39 (68%) patients, whereas the surrounding stroma contained residual disease in 30 (53%) patients. This difference did not reach statistical significance. However, when the amount of residual disease is divided between major response (≤10% residual tumor cells; TRG1 + TRG2), and minor response (>10% residual tumor cells; TRG3 + TRG4), the incidence of minor response in the mucosa was significantly more frequent, as compared with the surrounding stroma: 26 (46%) versus 16 (28%) (p=0.021; χ2). Furthermore, of the 16 patientswith aminor response in the surrounding stroma, only 3 patients had a major response in the mucosa, whereas the remaining 13 patients had a concurrent minor response in the mucosa (see also Table 1). These results indicate that after nCRT only 5% (3/57) of patients with an initial pretreatment T3 tumor had more residual disease in the surrounding stroma than in the mucosa. Therefore, assuming equal chances of residual tumor outgrowth between all layers, only these 5% of patients are exposed to a clear and early endangerment of the circumferential resection margin, without the presence of substantial tumor in the mucosa.

Table 1

	Surrounding Stroma	
Mucosa	**Major Response**	**Minor Response**
Major response	28	3
Minor response	13	13

Major response: ≤10% residual tumor cells; (TRG1 + TRG2); minor response: >10% residual tumor cells; (TRG3 + TRG4).

Studies investigating the accuracy of endoscopic detection of residual disease after nCRT employed a single restaging strategy, relatively early after completion of nCRT. Results showed quite low concordance with the presence of residual disease in the resection specimen. However, these results are expected to improvewith repeated measurements at extended time points after completion of nCRT. Recent data from the CROSS study group show that a prolonged time to surgery after completion of nCRT up to at least 12 weeks was associated with an increased pathologically complete response (pCR) rate, without a significant rise in postoperative complications (Shapiro et al, data submitted for publication). Moreover, it has been shown in patients after definitive chemoradiotherapy (dCRT) that during intensive endoscopic surveillance protocols a primary site recurrence frequently presents as a newly developing erosion or submucosal tumor mass.[2]

Conceptually, *surgery-as-needed* after nCRT differs from salvage surgery after dCRT in the intent (and intensity) of chemoradiotherapy. The goal of nCRT is to downstage the tumor locoregionally to facilitate a radical resection, whereas the goal of dCRT is to maximize its impact and possibly to achieve cure. The practical difference is that with dCRT higher doses of radiation (50–65 Gy) are applied, with questionable improvements in pCR rates, as compared with nCRT regimens (36–45 Gy). Importantly, the higher doses of radiation generally show a negative impact on surgical outcome, although a recent publication reports on a favorable outcome after salvage surgery.[3] We disagree with van Rossum et al that data from salvage surgery after dCRT can be extrapolated to patients treated with nCRT.

We agree that improved restaging strategies are needed, but current restaging techniques have not yet been adequately tested. To this day, no prospective trial evaluated the predictive value of a full combination of currently available restaging techniques at an interval of at least 12 weeks from the end of nCRT to minimize false-positive signals due to radiation-induced inflammation. Such a prospective feasibility trial is currently being coordinated by our center in 5 Dutch high-volume centers (including the group of van Hillegersberg and colleagues from the UMC Utrecht), aimed at including 120 patients with both adenocarcinoma and squamous cell carcinoma of the esophagus or esophagogastric junction. All included patients will undergo 2 rounds of restaging, consisting of endoscopy with biopsies, endoscopic ultrasonography with tumor thickness measurements,[4] and fine-needle aspiration of suspected deeper lesions, guided by positron emission tomography-computed tomography. Results from this trial will demonstrate the predictive potential of a set of diagnostic modalities at 2 time points after completion of nCRT and will hopefully allow for the construction of a strong predictive model for pathologically (near-) complete response.

In conclusion, nearly 30% of patients in the multimodality arm of the CROSS trial5 had a pCR and another 32% of patients had a near-complete response (1%-10% residual tumor cells = TRG2) in the resection specimen. And yet, all these patients underwent a standard surgical resection, with its known morbidity and mortality. We feel an ethical imperative to try and identify those patients with a (near-)complete response because a substantial number of these patients probably do not have any benefit from standard surgical resection after nCRT. If results from our prospective feasibility trial will be promising (ie, sensitivity and specificity for predicting a pathologically (near-) complete response), we will compare a *surgery-as-needed* approach with standard surgical resection after nCRT in a subsequent, preferably randomized controlled trial.

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Chapter 8

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Prolonged time to surgery after neoadjuvant chemoradiotherapy increases histopathological response without affecting survival in patients with esophageal or junctional cancer

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Abstract

Objective
To determine the relation between time to surgery (TTS) after neoadjuvant chemoradiotherapy (nCRT) and pathologically complete response (pCR), surgical outcome and survival in patients with esophageal cancer.

Background data
Standard treatment for potentially curable esophageal cancer is nCRT plus surgery after 4-6 weeks. In rectal cancer patients evidence suggests that prolonged TTS is associated with a higher pCR rate and possibly with better survival.

Methods
We identified patients treated with nCRT plus surgery for esophageal cancer between 2001-2011. TTS (last day of radiotherapy to day of surgery) varied mainly for logistical reasons. Minimal follow-up was 24 months. The effect of TTS on pCR rate, postoperative complications and survival was determined with (ordinal) logistic, linear and Cox regression, respectively.

Results
In total, 325 patients were included. Median TTS was 48 days (p25-p75=40-60). After 45 days, TTS was associated with an increased probability of pCR (odds ratio, OR=1.35 per additional week of TSS, p=0.0004) and a small increased risk of postoperative complications (OR=1.20, p<0.001). Prolonged TTS had no effect on disease-free and overall survival (HR=1.00 and HR=1.06 per additional week of TSS, p=0.976 and p=0.139, respectively).

Conclusions
Prolonged TTS after nCRT increases the probability of pCR and is associated with a slightly increased probability of postoperative complications, without affecting disease-free and overall survival. We conclude that time to surgery can be safely prolonged from the usual 4-6 weeks up to at least 12 weeks, which facilitates a more conservative *wait-and-see* strategy after neoadjuvant chemoradiotherapy to be tested.

Introduction

Recent studies show that, in patients with potentially curable esophageal or esophagogastric junction cancer, the addition of neoadjuvant chemoradiotherapy (nCRT) to surgery improves locoregional control and long-term survival[1,2]. Therefore, nCRT plus surgery has become the standard of care for patients with potentially curable esophageal or junctional cancer in many countries.

Reasons for prolonged time to surgery (TTS) after nCRT include patients' malnutrition or poor physical status, comorbidities such as infections and cardiopulmonary problems, and logistical problems, such as hospital bed and operating room availability.

Tumor response to neoadjuvant therapy is classified in several tumor regression grading (TRG) systems[3-6]. A pathologically complete response (pCR; *i.e.* no viable tumor cells found in the resection specimen) has been recognized as a valuable prognostic factor for long-term survival[3,7-10]. It remains unclear, however, whether prolonged TTS has an impact on pCR rate, on short-term surgical outcome and on long-term survival.

Theoretically, prolonged TTS might increase pCR rate and possibly improve disease-free survival because of a prolonged effect of nCRT. Conversely, prolonged TTS might lead to residual tumor outgrowth, increased difficulty of surgical resection with a higher postoperative complication rate and possibly a worse overall survival.

In rectal cancer patients, several studies have shown that prolonged TTS probably increases the percentage of pathologically complete responders[11-18]. Perioperative morbidity and -mortality, disease-free survival and overall survival, seem less clearly correlated with TTS[12,13,15-22]. Comparable studies in esophageal cancer patients have shown no effect of TTS on pCR rate and no (or little) effect on disease-free survival.[23,24]

We aimed to investigate the impact of TTS after nCRT on pCR rate, short-term surgical outcome and disease-free and overall survival in a cohort of patients with potentially curable esophageal or junctional cancer, who underwent neoadjuvant chemoradiotherapy according to CROSS[2] followed by surgical resection.

Patients and Methods

Patients

We included patients treated with nCRT according to CROSS[2] followed by surgical resection within a previously published single-center phase-II trial (February 2001 – January 2004)[25], a multi-center randomized controlled phase-III trial (March 2004 – November 2008)[2] and as standard therapy in the period after completion of the two trials (March 2009 - December 2011) at the Erasmus MC – University Medical Center Rotterdam (a tertiary referral hospital in Rotterdam, The Netherlands). Patients who did not receive at least 80% of planned nCRT or who received a different nCRT regimen were excluded. Also, patients with irresectable tumors (cT4b) and/or disseminated disease (M1) were excluded.

Treatment and pathological assessment

The neoadjuvant treatment regimen has been described before in detail[2]. Esophagectomy was planned 4-6 weeks after completion of nCRT. The resection specimens (primary tumor and all resected lymph nodes) were processed according to a standardized protocol[26]. The tumor regression grade (TRG) was scored with the modified Mandard scoring system as reported by Chirieac *et al.*[3,4]. A microscopically radical resection (R_0) was defined as a tumor-free resection margin ≥ 1 mm.

Follow-up and data collection

Clinical, surgical and histopathological characteristics were retrieved from prospectively maintained institutional databases. TTS was determined as the interval between the last day of radiotherapy and the day of surgery. After surgery, patients were routinely followed; every three months during the first postoperative year, every six months during the second postoperative year, and yearly until the fifth postoperative year. During follow-up, diagnostic investigations were performed only when recurrence was suspected clinically. Recurrences were scored at the time of first failure. Survival was determined using hospital records and municipal registers.

Surgical outcome

Surgical outcome was described by duration of operation in minutes, intraoperative blood loss in milliliters and length of hospital stay in days. Postoperative complications were categorized according to the Clavien-Dindo classification[27,28], a postoperative complication ranking system: grade-0: no complications; grade-I: complications requiring no or minimal (non-pharmacological) treatment; grade-II: complications requiring pharmacological treatment; grade-III: complications requiring surgical, endoscopic or radiological intervention; grade-IV: life-threatening complications requiring ICU-level care; grade-V: postoperative death.

Potential delay-related confounders

To correct for the possibility that variations in TTS were not only caused by random logistical difficulties, but also by patient-related characteristics (*i.e.* intentionally longer TTS in more vulnerable patients), effects of TTS on pCR and on surgical outcome were adjusted for three potential delay-related confounders. First, this was the Charlson comorbidity index[29] at diagnosis, an indicator of comorbidity. For this index, each condition present at diagnosis is assigned a standard score of 1, 2, 3 or 6 (determined by the estimated risk of dying from this condition). The total sum per patient is predictive for mortality. Second, the Karnofsky performance status[30,31] at the end of nCRT, an indicator of general well-being. The scale ranges from 100 (normal health) to 0 (death). A score of 90 indicates minor signs and/or symptoms of disease, 80 indicates mild signs and/or symptoms of disease, etc. Third, weight loss during nCRT, defined as the difference in body weight (in kilograms) between the start of the first cycle of chemotherapy and the start of the fifth cycle of chemotherapy. Weight gains were set to zero loss.

Statistical analysis

Baseline data were described as medians with the interquartile range in case of continuous variables and as frequencies with percentages in case of categorical variables. Missing baseline data were imputed with a single imputation technique based on correlations with relevant baseline variables and outcome. TTS was truncated at 5 and 95% to reduce leverage effects of outliers. The effect of TTS on pCR and on surgical outcome was determined using univariable and multivariable logistic regression. Non-linearity of the effect of TTS was assessed with restricted cubic splines (three knots)[32]. To correct for the possibility that TTS was intentionally longer in more vulnerable patients, the effects of TTS on surgical outcome was adjusted in multivariable regression analysis for the three potential delay-related confounders together with surgical approach (=adjusted odds-ratio, aOR). Subsequently, the effects of pCR and TTS on disease-free survival and overall survival were assessed using Cox regression with adjustment for the three potential delay-related confounders alone and in combination with postoperative complications (=adjusted hazard ratio, aHR).

Results

Patient and treatment related characteristics

In total, 325 patients with potentially curable esophageal or junctional cancer were included who underwent nCRT according to CROSS[2] followed by resection between February 2001 and December 2011. Fifty-one included patients participated in the phase-II trial[25], 157 patients participated in the multimodality arm of the randomized phase-III trial[2] and 117 patients were treated with nCRT followed by resection as standard therapy in the period following completion of the phase-III trial. In total, 344 patients completed nCRT within the three cohorts. Of these, four patients did not undergo surgery (primarily due to patient preferences) and fifteen additional patients did not undergo resection (due to disease progression).

Clinical, surgical and histopathological characteristics of included patients are summarized in Table 1. Median age at diagnosis was 60 years. The majority of patients were male (78%), had an adenocarcinoma (76%), and were clinically staged as cT3 (77%), cN1 (63%). Before truncation, TTS ranged from 18 to 291 days, with a median of 48 days (interquartile range 40-60 days), as also shown in figure 1. Median TTS in irresectable patients was 46 days (interquartile range 40-61 days), which was similar to TTS in resected patients (p=0.99). Surgical resection was performed regularly via both transhiatal approach (48%) and transthoracic approach (50%). Median duration of operation was 368 minutes, median volume of intraoperative blood loss was 900 ml and median length of hospital stay was 14 days. Postoperative complications requiring pharmacological, interventional or surgical treatment (≥ grade-II) were reported in 55% of patients. Postoperative in-hospital mortality was 4%. Histopathological staging showed frequent ypT0 and ypT3 stages (34% and 32%, respectively), while ypN0 was the most frequent nodal stage (68%). A pCR (=TRG1) was found in 28% of patients. The microscopically radical resection rate (R_0) was 93%.

The potential delay-related confounders are summarized in Table 2. The majority of patients had a pretreatment Charlson comorbidity index of 0 (75%) and a Karnofsky performance score at the end of nCRT of 90 (64%). Median weight loss during nCRT was one kilogram, (p25-p75: 0 – 3).

Effect of TTS on pCR

Median TTS was 48 days (p25-p75: 40-60 days, p5-p95: 18-83 days). The association of TTS with pCR was non-linear (p=0.025). After approximately 45 days (Supplementary figure 1), additional TTS was associated with an increased probability of pCR (odds ratio, OR, 1.35 per additional week after 45 days, p=0.0004).

In itself a pathologically complete response was associated with a significantly better disease-free survival and overall survival (hazard ratio, HR, 0.29, p<0.0001 and 0.44, p<0.0001, respectively). The effect of pCR on overall survival was sustained after correction for the three potential delay-related confounders (adjusted HR, aHR, 0.44, p<0.0001).

Effect of TTS on short-term surgical outcome

The effect of TTS on short-term surgical outcome was reasonably linear (non-linearity, p=0.920). Increased TTS was associated with prolonged duration of operation (12 minutes per additional week of TTS, p<0.001), prolonged length of hospital stay (1.55 day per additional week of TTS, p=0.006) and more severe postoperative complications (OR 1.20 per additional week of TTS, p<0.001). No association was found with intraoperative blood loss (-3 ml per additional week of TTS, p=0.898). However, these associations were partly explained by the three potential delay-related confounders together with surgical approach. After adjustment, the effects were reduced for duration of operation (6 minutes per additional week of TTS, p=0.031), length of hospital stay (1.03 days per additional week of TTS, p=0.071) and for postoperative complications (aOR 1.10 per additional week of TTS, p=0.132, Table 3).

Effect of TTS on survival

The effect of TTS on disease-free survival and overall survival was reasonably linear (non-linearity, p=0.566). Increased TTS was not associated with disease-free survival, (HR 1.00, p=0.976; aHR 0.98, p=0.620), nor with overall survival (HR 1.06, p=0.139; aHR 1.03, p=0.465, table 4).

Discussion

Results show that prolonged TTS, beyond 45 days after completion of nCRT, significantly increased the probability of pCR. Longer TTS was also associated with a small increased risk of postoperative complications. Furthermore, pCR in the resection specimen was associated with significantly improved disease-free survival. However, prolonged TTS (with increased probability of pCR) was not associated with improved disease-free survival.

Although prolonged TTS was associated with an increased pCR rate, disease-free survival was not accordingly improved. The comparable disease-free survival between patients with shorter and longer TTS implies that their disease is biologically similar, despite differences in pCR rate. The increase in pCR after prolonged TTS is attributed to patients

who presumably would have been non-complete responders at shorter TTS. Interestingly, these patients, who become pathologically complete responders relatively late after completion of nCRT, do not contribute to an improved disease-free survival after prolonged TTS. This can be explained if 'late complete responders' would already behave as biologically complete responders at shorter TTS, despite a pathologically non-complete response in their resection specimens. Therefore, the (minimal) residual disease found at histological examination after shorter TTS in these late complete responders is presumed to be biologically non-relevant. We hypothesize that prolonged TTS does not truly improve the biological pCR rate, but that prolonged TTS simply allows for a more precise histopathological distinction between viable residual tumor cells and biologically non-relevant tumor cell debris by the pathologist.

In this cohort, prolonged TTS was significantly associated with a prolonged duration of operation, a prolonged length of hospital stay and increased postoperative complications. However, these associations were partly explained by the three potential delay-related confounders, *i.e.* Charlson comorbidity index[29] at diagnosis, Karnofsky performance status[30,31] at the end of nCRT and weight loss during nCRT (besides surgical approach; Table 3). These potential confounding factors were chosen to reflect the overall condition of patients at the end of nCRT, which might have influenced the surgeons' decision to delay surgical resection after nCRT, independent of logistical planning. Therefore, these results indicate that the association of prolonged TTS with negative short-term surgical outcome and increased postoperative complications is partly the result of an intentionally prolonged TTS in patients with a worse overall condition at the end of nCRT and that prolonged TTS has at most a small association with short-term surgical outcome and postoperative complications.

Two previous studies investigated the association between TTS and surgical and oncological outcome in patients with esophageal cancer. Both studies did not find a correlation with pCR rate[23,24]. This discrepancy with the present study might be related to the heterogeneity of nCRT protocols used in both previous studies. In our cohort, all patients completed the same nCRT protocol, and in all patients there was a similar initial intention to undergo surgery after 4-6 weeks, possibly allowing for a clearer delineation of the effect of TTS on pCR rate. Also, the present study investigated the association between TTS and pCR in all patients using logistic regression, resulting in more power to detect subtle effects as compared to subgroup analyses. Last, the present study focused on the association between TTS and disease-free survival, which was lacking in the study by Kim and colleagues[24].

Currently, controversy exists concerning the management of cancer patients with a clinically complete response after neoadjuvant therapy. For rectal cancer patients, more conservative strategies have already been proposed and tested in clinically complete responders[33-36]. The goal of these *wait-and-see* approaches is organ preservation and overall reduction in treatment-related morbidity and mortality. However, it has remained difficult to clinically distinguish truly complete responders from nearly-complete responders. Patients belonging to the latter group might be incorrectly identified as clinically complete responders and could thus be exposed to potentially harmful disease progression during the *wait-and-see* period. For esophageal cancer, the challenge to distinguish complete from nearly complete responders seems even more difficult[37-40]. However, if surgery can be safely postponed beyond the usual 4-6 weeks, without negatively affecting oncological outcome as suggested in the present study, more time is allowed to identify non-complete responders. Such a strategy would not focus on distinguishing complete from non-complete responders at a single instance, but would rather use intense surveillance, with targeting of the mucosa and submucosa[41] during repeated instances, for the detection of residual or recurrent disease. Thereby, possibly allowing more time to differentiate between truly complete responders and non-complete responders.

Limitations of the study

The first limitation is that the present study was a retrospective analysis. However, all parameters in this study were collected prospectively. Furthermore, all three cohorts used the same neoadjuvant CROSS regimen and all three cohorts had a similar intention to perform surgery at 4-6 weeks after completion of nCRT. The second limitation of this study is that the number of patients with a TTS beyond 10 weeks was relatively small. Therefore, a subtle effect of TTS on disease-free and overall survival cannot be excluded in patients with a TTS beyond 10 weeks.

Conclusions

In patients with potentially curable esophageal or junctional cancer, prolonged time to surgery (beyond 45 days) after neoadjuvant chemoradiotherapy increases the probability of a pathologically complete response, without improving disease-free and overall survival. It might also slightly increase the probability of postoperative complications, which can be partly explained by intentional postponement of surgery in some high-risk patients with worse overall condition. We hypothesize that prolonged time to surgery allows for more accurate determination of histopathological response due to continued disintegration of nonviable cells in some patients, without affecting actual prognosis after neoadjuvant chemoradiotherapy. We conclude that time to surgery can be safely prolonged from the usual 4-6 weeks up to at least 12 weeks, which facilitates a more conservative *wait-and-see* strategy after neoadjuvant chemoradiotherapy to be tested.

Table 1 — Clinical, surgical and histopathological characteristics of 325 included patients with potentially curable esophageal or junctional cancer, treated with neoadjuvant chemoradiotherapy according to CROSS[2] followed by surgical resection.

Characteristic	n (total)	n		%*
Age [years]	325			
median (p25-p75)		60	(55 – 67)	
Gender	325			
Male		253		78%
Female		72		22%
Tumor type	325			
Squamous cell carcinoma		73		23%
Adenocarcinoma		247		76%
Other		5		2%
cT-stage§	323			
cT1		9		3%
cT2		60		19%
cT3		250		77%
cT4		4		1%
cN-stageǂ	324			
cN0		120		37%
cN1		204		63%
Time to surgery [days] ₪	325			
median (p25-p75)		48	(40 – 60)	
Surgical approach	325			
Transhiatal approach		156		48%
Transthoracic approach		164		50%
Thoraco-phreno-laparotomy		5		2%
Duration of operation [min]	308			
median (p25-p75)		368	(262 – 388)	
Intraoperative blood loss [ml]	281			
median (p25-p75)		900	(550 – 1350)	
Length of hospital stay [days]	319			
median (p25-p75)		14	(12 – 23)	
Clavien–Dindo classification [27, 28] ∧	324			
Grade-0		72		22%
Grade-I		73		23%
Grade-II		82		25%
Grade-III		43		13%
Grade-IV		40		12%
Grade-V		14		4%

Characteristic	n (total)	n	%*
ypT-stage•	324		
ypT0		110	34%
ypT1		49	15%
ypT2		59	18%
ypT3		103	32%
ypT4		3	1%
ypN-stage•	325		
ypN0		222	68%
ypN1		74	23%
ypN2		21	7%
ypN3		8	3%
Tumor regression grade	325		
(TRG) ¥		90	28%
1		102	31%
2		77	24%
3		56	17%
4			
Radicality◊	325		
R_0		303	93%
R_1		22	7%

Legend Table 1

* Percentages may not add up to 100 due to rounding

§ cT-stage: clinical T-stage as defined by endo-ultrasonography and/or CT-scanning according to the *Union Internationale Contre le Cancer* (UICC) TNM Cancer Staging, 7th edition.

ǂ cN-stage: clinical N-stage as defined by endo-ultrasonography and/or CT-scanning and/or FDG-PET-scanning according to UICC TNM Cancer Staging, 6th edition.

₪ Time to surgery was determined as the interval between the last day of radiotherapy and the day of surgery.

Δ Clavien-Dindo classification, a postoperative complication ranking system. Grade 0: no complications, grade I: complications requiring none or minimal (non-pharmacological) treatment, grade II: complications requiring pharmacological treatment, grade III: complications requiring surgical, endoscopic or radiological intervention, grade IV: life-threatening complications requiring ICU-level care, grade V: postoperative death.

• ypT and ypN-stage: pathological T-stage and pathological N-stage in the resection specimen following neoadjuvant chemoradiotherapy, according to UICC TNM Cancer Staging, 7th edition.

¥ Tumor regression grade was defined as: TRG1: no residual tumor cells found (pathologically complete response=pCR); TRG2: 1-10% residual tumor cells; TRG3: 11-50% residual tumor cells; TRG4: > 50% residual tumor cells.

◊ R_0 was defined as a tumor-free resection margin ≥ 1 mm. R_1 was defined as a macroscopically radical resection, with a microscopically tumor-free resection margin < 1 mm.

Table 2 — Three potential delay-related confounders in 325 included patients with potentially curable esophageal or junctional cancer, treated with neoadjuvant chemoradiotherapy according to CROSS[2] followed by surgical resection.

Characteristic	n (total)	n	%*
Charlson comorbidity index[29] § at initial diagnosis	324		
0		243	75%
1		63	19%
2		15	5%
3		2	1%
4		1	0%
Karnofsky performance status[30,31] ǂ at the end of nCRT	269		
100		80	30%
90		171	64%
≤80		18	7%
Weight loss [kg] • during nCRT	297		
median (p25-p75)		1 (0 – 3)	

Legend Table 2

* Percentages may not add up to 100 due to rounding

§ Charlson comorbidity index, an indicator of comorbidity. Each condition present at diagnosis is assigned a standard score of 1, 2, 3 or 6 (determined by the estimated risk of dying from this condition). The total sum per patient is predictive for mortality.

ǂ Karnofsky performance status, an indicator of general well-being. The scale ranges from 100 (normal health) to 0 (death). A score of 90 indicates minor signs and/or symptoms of disease, 80 indicates mild signs and/or symptoms of disease, etc.

• Weight loss during nCRT was defined as the difference in body weight (in kilograms) between the start of the first cycle of chemotherapy and the start of the fifth cycle of chemotherapy. Weight gains were set to zero loss.

Table 3 — Effect of time to surgery on short-term surgical outcome and postoperative complications in 325 included patients treated with CROSS[2] followed by surgical resection, adjusted for the three potential delay-related confounders together with surgical approach.

Outcome	beta	p	adjusted beta	p
	(per additional week)		(per additional week)	
Duration of operation (minutes)	12	<0.001	6	0.031
Intraoperative blood loss (ml)	-3	0.898	-3	0.898
Length of hospital stay (days)	1.55	0.006	1.03	0.071
Postoperative complications (according to the Clavien-Dindo classification)	1.20*	<0.001	1.10*	0.132

Legend Table 3

*Odds ratio.

Adjustment for the three delay-related confounders (Charlson comorbidity index[29] at diagnosis, Karnofsky performance status[30,31] during the last week of nCRT and weight loss during nCRT) together with surgical approach.

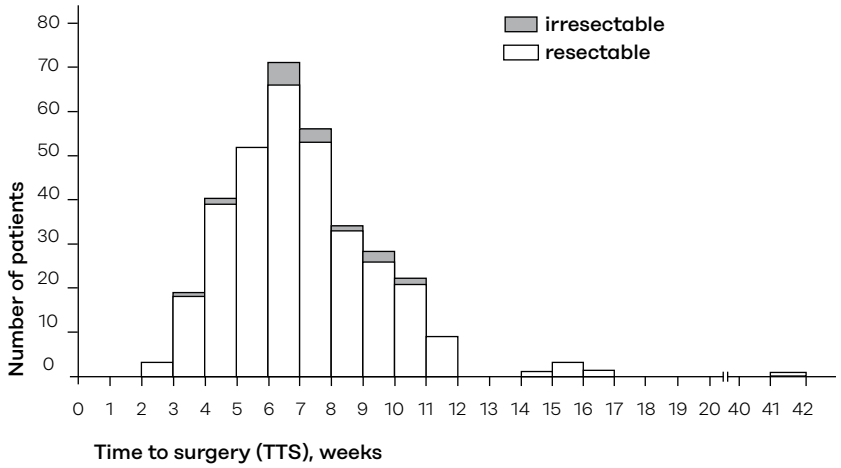
Table 4 — Effect of time to surgery on overall survival in 325 included patients with potentially curable esophageal or junctional cancer, treated with neoadjuvant chemoradiotherapy according to CROSS[2] followed by surgical resection.

Time to surgery	Hazard ratio for OS	p
	(per additional week)	
Univariate	1.06	0.139
Adjusted for three potential delay-related confounders	1.04	0.387
Adjusted for three potential delay-related confounders + postoperative complications (shows effect of TTS on overall survival via increased pCR)	1.03	0.465

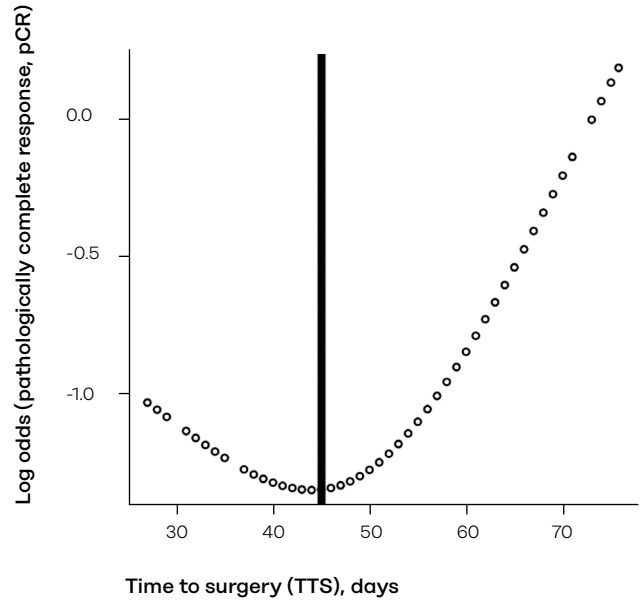
Legend Table 4

OS: overall survival; TTS: time to surgery; pCR: pathologically complete response
Delay-related confounders: Charlson comorbidity index[29] at diagnosis, Karnofsky performance status[30,31] during the last week of nCRT and weight loss during nCRT.

Figure 1 — Distribution of time to surgery (before truncation) in 340 patients who completed neoadjuvant chemoradiotherapy according to CROSS[2] and underwent surgery for potentially curable esophageal or junctional cancer, 15 of these patients were irresectable due to disease progression, while 325 patients underwent resection.



Supplementary figure 1 — Effect of time to surgery (after truncation) on pCR using a restricted cubic splines model (3 knots) for all 325 included patients with potentially curable esophageal or junctional cancer, treated with neoadjuvant chemoradiotherapy (nCRT) according to the CROSS regimen[2] followed by surgical resection.



Legend supplementary figure 1

Time to surgery is expressed in days. The y-axis represents the relative probability (log odds) of reaching pCR. A higher log odds indicates a higher probability of reaching pCR. After approximately 45 days the probability of reaching pCR increases.

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Extra discussions

Discussants 1

T. Sano (Tokyo, Japan):
This is an interesting study examining the relationship between the time to surgery after neoadjuvant CRT and the rate of pathological complete response in resectable esophageal carcinoma. The strong point is the large number of patients treated with a fixed regimen of CRT. The weak point is that TTS was not prospectively determined or randomized for analyses, showing a wide variation with diverse background differences. I agreewith the authors' conclusion in the abstract book that prolonged TTS allows for a more accurate determination of histopathological response without affecting actual prognosis but am not convinced by their conclusions in the manuscript that TTS can be safely prolonged for a more conservative wait-and-see strategy. What is the core clinical message from this study? After safely completed nCRT, should we wait longer? Or, should we operate on the patient with an appropriate TTS like 6 weeks?

Some questions:

1. The definition of DFS or OS is unclear. Is it the duration from the start (or end) of nCRT or from the date of surgery? In the former case, the term 'disease-free' is inappropriate because the disease does exist before surgery. 'Progression-free' would be suitable. In the latter case, the DFS or OS is not appropriate for comparison due to lead time bias caused by different TTS.
2. In the CROSS study, the survival benefit of nCRT was definitely larger in squamous cell carcinoma than in adenocarcinoma. Patients with SCC are usually older and more comorbid than those with adenocarcinoma. In this study, how did the balance of histology in the long and short TTS groups? Doesn't this affect the pCR rate or survival analysis?
3. In the CROSS trial and this study, TTS was planned to be 4 to 6 weeks, but the actual median TTS was 7 weeks, that is, the surgery was delayed in the majority of cases. Was this due to inappropriate trial planning? Or due to lax quality control? In the phase II study preceding the CROSS trial, the median TTS was 42 days (6 weeks).What was the standard in the clinical practice after the CROSS trial?
4. The follow-up policy (no diagnostic investigation unless recurrence is suspected) may not be suitable in clinical studies having DFS as an endpoint.

Response From J. Shapiro, J. van Lanschot (Rotterdam, Netherlands):
Thank you for your comments. I will start with the first question, which was about how survival was calculated. In this study, survival was calculated from the start of neoadjuvant therapy. We fully agree that progression-free survival would have been a more appropriate term.

The second question was about the differences between squamous cell carcinomas and adenocarcinomas. We didn't perform this subanalysis, which I think would have been interesting to do. Although, I don't think that time to surgery would have differed based on the histology. We discussed this with the involved surgeons, and there is no policy to preferentially treat squamous cell carcinomas or adenocarcinomas with different delays. So, I think that in this way tumor subtype did not influence the time to surgery itself, although I do agree that patients with squamous cell carcinoma are usually more comorbid. However, this we hopefully corrected for with these factors that we now included.

Your third question was about how it's possible that we had longer time to surgery intervals than was scheduled in the protocol. This is I think largely due to logistical problems that were present in The Netherlands during the years of these trials.We did do a subgroup analysis for treatment center, which did not show an effect. I think that these logistical problems were really national.

Your fourth question was a comment about how we did our follow-up. I guess that this is a local mentality that recurrences are not actively looked for. I think the good thing is that in The Netherlands, we have very low loss to follow-up. So, if a patient does develop a recurrence, we do find out. Although it's true that we might have slightly longer disease-free survival intervals than would be strictly or formally correct.

Discussants 2

R. van Hillegersberg (Utrecht, Netherlands):
Thank you for this very interesting study. We know from daily practice that if you operate on patients who have had a longer interval between chemoradiotherapy and surgery, the surgical dissection may be more difficult. Indeed, you showed that the incidence of complications is also higher in those patients. At the same time, you didn't show any survival benefits or disease-free survival benefits in the delayed group. So, what are your arguments for waiting longer after chemoradiotherapy? Shouldn't we go directly to surgery after 6 weeks?

Response From J. Shapiro, J. van Lanschot (Rotterdam, Netherlands):
Thank you for your question. I don't necessarily propose to wait longer, although I think you would obtain a clearer image in the resected specimen. I think that this study adds to the evidence that if you have a reason towait, then you can do this safely for up to 12weeks after completion of neoadjuvant therapy. It is difficult to test howgood our correction of the effect of time to surgery was using the patients' condition at the end of neoadjuvant therapy, although the patients' condition is considered a valid confounder since it confounded both time to surgery and our outcome - in this case, surgical outcome. We tested for the impact of the 3 potential delay-related factors. I have to say that weight loss during neoadjuvant therapy was borderline significant. The other 2 were significant in their impact on both the time to surgery and on postoperative outcomes. However, it remains a valid point that surgery might become more difficult, if you wait longer.

Chapter 9

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Accuracy of detecting residual disease after CROSS neoadjuvant chemoradiotherapy for esophageal cancer (preSANO trial): rationale and protocol

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Abstract

Background
Results from the recent CROSS trial showed that neoadjuvant chemoradiotherapy (nCRT) significantly increased survival as compared to surgery alone in patients with potentially curable esophageal cancer. Furthermore, in the nCRT arm 49% of patients with a squamous cell carcinoma and 23% of patients with an adenocarcinoma had a pathologically complete response in the resection specimen. These results provide a rationale to reconsider and study the timing and necessity of esophagectomy in (all) patients after application of the CROSS-regimen. We propose a *surgery as needed* approach after completion of nCRT. In this approach, patients will undergo active surveillance after completion of nCRT. Surgical resection would be offered only to those patients in whom residual disease or a locoregional recurrence is highly suspected or proven. However, before a *Surgery As Needed approach in Oesophageal cancer patients* (SANO) can be tested in a randomized controlled trial, we aim to determine the accuracy of detecting the presence or absence of residual disease after nCRT (preSANO trial).

Methods/Design
This study is set up as a prospective, single arm, multicenter, diagnostic trial. Operable patients with potentially curable squamous cell- or adenocarcinoma of the esophagus or esophago-gastric junction will be included. Approximately 4-6 weeks after completion of nCRT all included patients will undergo a first clinical response evaluation (CRE-I) including endoscopy with (random) conventional mucosal biopsies of the primary tumor site and of any other suspected lesions in the esophagus and radial endo-ultrasonography (EUS) for measurement of tumor thickness and -area. Patients in whom no locoregional or disseminated disease can be proven by (cyto)histology will be offered a postponed surgical resection, 6-8 weeks after CRE-I (*i.e.* approximately 12-14 weeks after completion of nCRT). In the week preceding the postponed surgical resection a second clinical response evaluation (CRE-II) will be planned, which will include a whole body PET-CT, followed again by endoscopy with (random) conventional mucosal biopsies of the primary tumor site and any other suspected lesions in the esophagus, radial EUS for measurement of tumor thickness and —area and linear EUS plus fine needle aspiration of PET-positive lesions and/ or suspected lymph nodes. The main study parameter is the correlation between the clinical response assessment during CRE-I and CRE-II and the final pathological response in the resection specimen.

Results
Enrolment began July 23, 2013, results expected January 2016.

Discussion
If this preSANO trial shows that the presence or absence of residual tumor can be predicted reliably 6 or 12 weeks after completion of nCRT, a randomized trial comparing nCRT plus standard surgery versus chemoradiotherapy plus *surgery as needed* will be conducted (SANO trial).

Registration
Netherlands Trial Register (NTR4834)

Introduction

Cancer of the esophagus remains a highly lethal malignancy, as reflected by an average overall five-year survival of 17%[1]. In the Netherlands, the incidence of esophageal cancer resembles the growing trend in Western countries, with an estimated incidence of 15/100,000 for men and 6/100,000 for women[2], and more than 2,500 new cases diagnosed nationally each year.

At present, surgical resection is still considered the cornerstone of curative treatment for patients eligible with stage cT1b-4aN0-3M0 disease. The reported five-year survival rate for patients who undergo an esophagectomy ranges from 20 to 50%, but rarely exceeds 35%[3-7]. Esophagectomy is associated with postoperative mortality rates of 1-5% in high-volume centers, severe postoperative morbidity and a substantial impact on the quality of life[8-13]. In order to improve the radicality of surgical resection and the long term survival after surgical resection many trials have been performed to study the effect of (neo-) adjuvant chemo- and/or radiation therapy[14-17]. One of the largest trials is the recently published CROSS trial. This randomized trial compared neoadjuvant chemoradiotherapy (nCRT) plus surgery to surgery alone[18].

During a five-year period 366 patients from 5 academic and 2 non-academic high-volume teaching hospitals in the Netherlands were included. This study showed that the addition of nCRT (Carboplatin AUC2, Paclitaxel 50 mg/m^2 and 41.4 Gy of concurrent radiotherapy) to surgery significantly increases long term survival as compared to surgery alone. Median overall survival of patients who received nCRT plus surgery was 49 months, compared to 24 months for those who received surgery alone and the 3-year overall survival was superior in the nCRT arm (hazard ratio (HR)=0.66; 95% confidence interval (CI) 0.50-0.87; p=0.003). Therefore, nCRT plus surgery is now considered the therapy of choice

in the Netherlands and several other countries for potentially curable esophageal cancer (cT2-3N0-3M0 and cT1N1-3M0, according to the UICC TNM classification, 7th ed.). In subsequent analyses of secondary endpoints of the CROSS trial an interesting observation was made. In the nCRT arm, 49% of patients with a squamous cell carcinoma (SCC) and 23% of patients with an adenocarcinoma (AC) had a pathologically complete response (pCR) in the resection specimen (*i.e.* no viable tumor cells were found, neither at the site of the primary tumor nor in the resected regional lymph nodes, as determined by conventional histological examination)[18]. Therefore, these results provide a rationale to reconsider and study the timing and necessity of standard esophagectomy in (all) patients after application of the CROSS regimen.

We propose a *surgery as needed* approach after completion of nCRT for carcinoma of the esophagus. In this *surgery as needed* approach, patients will undergo active surveillance after completion of nCRT. Surgical resection would be offered only to those patients in whom a locoregional recurrence is highly suspected or proven, in the absence of any signs of distant dissemination. Such an organ-preserving strategy would clearly have great advantages. Postoperative mortality and severe morbidity (grade ≥3 according to the Clavien-Dindo classification[19]) after esophagectomy in the Netherlands is 5% and 60%, respectively. Thus, a non-surgical treatment strategy in patients with a clinically complete response after nCRT, theoretically saves 5% mortality and 60% severe morbidity in this patient group. Moreover, this approach might improve quality of life and might lead to a reduction in health care costs. However, this *surgery as needed* approach is only favorable if long term survival would be comparable to that of the trimodality approach comprising nCRT followed by standard surgery. Before a *surgery as needed* approach can be tested in a randomized trial, we aim to determine the feasibility of accurate detection of residual disease after chemoradiotherapy.

Objective

The aim of this present prospective, multicenter, diagnostic study (preSANO study) is to determine the accuracy by which we can detect the presence or absence of residual disease after nCRT. The results of this trial will inform us about the percentage of patients with a clinically complete response after nCRT, and will help to estimate the number of patients needed for a subsequent randomized controlled trial. This future so called SANO-trial (*Surgery As Needed in Oesophageal cancer patients*) will randomize patients to nCRT plus surgery versus nCRT followed by an active surveillance strategy.

Methods/Design

Study design

The preSANO trial is a prospective, multicenter, diagnostic trial including 120 patients, using a single arm. Five high-volume centers in the Netherlands are currently participating in this study (Erasmus Medical Center, Rotterdam; Academic Medical Center, Amsterdam; University Medical Center, Utrecht; Catharina Cancer Center, Eindhoven; Atrium Medical Center, Heerlen). The study has been approved by the medical ethics committee of the Erasmus Medical Center (MEC2013-211) and has been registered in the Netherlands Trial Register (NTR4834).

Study population

We plan to include individuals from a population of operable patients with potentially curable SCC or AC of the esophagus or esophago-gastric junction. All patients who are planned to undergo nCRT according to the CROSS regimen[18], followed by surgical resection are eligible to participate. Patients with dementia or altered mental status prohibiting the understanding and giving of informed consent will be excluded from participation in this study. Patients will undergo conventional pre-treatment selection (including at least a 'partial body' F18-FDG PET-CT to assess the avidity of the primary tumor process; figure 1 and Table 1).

Study algorithm

All included patients will receive nCRT according to the CROSS protocol (Carboplatin, Paclitaxel and 41.4 Gy of concurrent radiotherapy)[18]. Patients will be re-evaluated either once or twice before undergoing surgical resection during clinical response evaluations (CRE). The aim of these CREs will be to identify those patients in whom residual and/or disseminated disease is present.

CRE-I

The first CRE (CRE-I) will be performed 4-6 weeks after completion of chemoradiotherapy (figure 1). During CRE-I, all patients will undergo esophagogastroduodenoscopy (EGD) with registration of endoscopic images for future reference and biopsies of any suspected lesions, including mucosal biopsies at the site of the primary tumor (one regular biopsy per centimeter in each of the 4 quadrants), radial endoscopic ultrasonography (EUS) for measurement of maximal tumor thickness and area and linear EUS. Patients with (cyto)histological evidence of locoregional residual disease, but without evidence of disseminated disease, will be offered immediate surgical resection. These patients have no clear benefit from postponement of surgical resection and should therefore have no delay according to current recommendations. Patients without (cyto)histological evidence of locoregional residual

disease and without disseminated disease will be considered to be *clinically complete responders* and will be offered a postponed surgical resection. In these patients a surgical resection will be postponed for an additional 6-8 weeks, allowing patients more time to reach a better condition for surgery.

CRE-II

In the week preceding the planned postponed surgical resection a second clinical response evaluation (CRE-II) will be scheduled. CRE-II will be performed only in patients who were considered to be clinically complete responders (*i.e.* no viable tumor found) at CRE-I. CRE-II will consist of a PET-CT (standard for all patients at CRE-II and only for tumor positive patients at CRE-I), an EGD with registration of endoscopic images for future reference and biopsies of any suspected lesions, including (random) mucosal biopsies at the site of the primary tumor, radial endoscopic ultrasonography (EUS) for measurement of maximal tumor thickness and -area and linear EUS plus fine needle aspiration (FNA) of PET-positive lesions and/ or suspected lymph nodes.

An important difference between CRE-I and CRE-II will be that during CRE-I clinically complete responders will be offered a postponed surgical resection, whereas after CRE-II both locoregionally complete- and non-complete responders will be advised to undergo a surgical resection (figure 1). In other words, all patients who are considered clinically complete responders at CRE-I and are therefore allowed to postpone their surgery by an additional 6-8 weeks, will undergo CRE-II followed by the postponed surgical resection, irrespective of the locoregional findings during CRE-II. The diagnostic results from CRE-II will later be compared with results from both CRE-I and the final pathological analysis of the resection specimen. However, patients with (cyto)histological evidence of disseminated disease during CRE-I or CRE-II will be excluded from further curative therapy and will be referred for palliative care.

If after CRE-II the planned operation is postponed for more than 4 weeks (*e.g.* because the patient has not yet sufficiently recovered from the nCRT), a CRE-III (comparable to CRE-II) will be performed one week before the (further) postponed operation.

Surgery

Surgical resection will be attempted immediately after CRE-I only in those patients who present at CRE-I with (cyto)histologically proven residual disease after completion of nCRT, without any signs of disseminated disease. All other patients will undergo surgical resection after CRE-II in the absence of distant metastases.

A transthoracic esophageal resection or a transhiatal approach can be performed, depending on both patient characteristics and local expertise and preference. Both open and minimally invasive techniques are allowed.

A wide local excision including the regional lymph nodes is carried out in both techniques including a standard dissection of the lymph nodes around the coeliac axis. The continuity of the digestive tract will preferably be restored by a gastric tube reconstruction or if required by a colonic interposition.

At least 15, but preferably 23 or more lymph nodes should be aimed to be removed in every patient. First, because it has been shown that long-term survival is maximized with the removal of at least 23 nodes[20]. Moreover, the risk of understaging the tumor in these patients should be minimized. If an insufficient number of nodes is removed, the patient might be erroneously staged as ypN0, while in fact ypNpos nodes have been left *in-situ* (stage migration).

Pathology

All resection specimens will be revised centrally by two independent expert pathologists, using a standard protocol. In case of a discordant outcome, the specimens will be reviewed by a third independent expert pathologist. A final diagnosis will be made only if at least two pathologists agree. Also, all the CRE-II biopsies of patients who were considered negative at CRE-II, but who had >10% residual tumor in their resection specimen will be revised centrally following the same strategy. In these specimens special attention will be given to the effects of the preoperative chemoradiation, *i.e.* tumor reduction and therapy effects. The lymph node dissection should contain at least 15, but preferably 23 or more nodes derived from both mediastinum and upper abdomen which are essential for correct ypTNM staging. The resection margins, especially the circumferential margin, will be evaluated with a 1mm cut-off point for vital tumor. This implies that the tumor-free margin should be >1mm in order to be classified as R_0. If vital tumor is present at ≤1mm from the surgical resection margin it is considered microscopically positive (R_1).

Interim analysis

An interim analysis will be performed by an independent safety committee after a total inclusion of 60 patients in order to carefully monitor serious complications during CRE-I and CRE-II and to assess the achieved radicality of the performed operations.

Main study parameter/endpoint
The main study parameter in this study is the correlation between the clinical response assessment during CRE-I and CRE-II and the final pathological response in the resection specimen, as measured by the modified tumor regression grading (TRG) system of Chirieac; no residual carcinoma (TRG1), 1-10% residual carcinoma (TRG2), 11-50% residual carcinoma (TRG3), 51-100% residual carcinoma (TRG4) [21].
We propose that in this study TRG2 residual tumors may be missed as long as we expect them to be detectable reliably as soon as they have outgrown from TRG2 to TRG3-4 during follow up. The risk that TRG2 residual tumors will lead to irresectability in the short-term is likely to be small/negligible. However, we do propose that TRG3 and TRG4 residual tumors should be detected without further delay in order to prevent short-term loss of resectability and to minimize the risk of long-term distant disease dissemination. The validity of these assumptions can only be determined in a future SANO trial, in which an active surveillance strategy will be compared with standard surgery in all patients after nCRT.

Statistical analysis

Sample size calculation
As was seen in the previous CROSS trial approximately 40% of the included patients will have TRG3 or TRG4 residual tumor in the resection specimen[18]. With a total inclusion of 120 patients, approximately 45 patients will have TRG3 or TRG4 residual tumor. We consider 45 patients a sufficiently large sample for determining the accuracy of individual and/or combined diagnostic tests. In order to estimate the distribution of 120 patients planned to be included, data were used from the CROSS trial as indicated in figure 2. Furthermore, several assumptions were made:

- We assume that during the first clinical response evaluation (CRE-I), clinically complete responders will comprise patients with TRG1 or TRG2 (as taken from the pathological response data of the CROSS trial), whereas clinically non-complete responders will be patients with TRG3 or TRG4.
- The percentage of patients with SCC and AC with TRG1 or TRG2 in the CROSS trial was 78% and 57%, respectively. This means that approximately 60% of included patients are expected to have negative (cyto)histology at CRE-I.
- In a trial by Blom *et al.*[22] approximately 10% of patients who were re-evaluated by PET-CT after completion of nCRT had newly discovered disseminated disease. We assume less newly found disseminated disease with positive (cyto)histology at CRE-II, because a number of these patients are expected to be discovered during CRE-I.
- We assume that approximately 25% of clinically complete responders will refuse to undergo the postponed resection and choose to undergo an active surveillance strategy if no alarming results are found during CRE-II.

These calculations indicate that approximately 60 patients will show a clinically complete response after combined diagnostic investigations, during CRE-I and CRE-II (including EUS-FNA with tumor thickness measurements and PET-CT). Of these, approximately 15 patients will refuse to undergo surgery and will undergo active surveillance and approximately 30 patients will have a pathologically complete response (TRG1). The 15 remaining patients are expected to have residual disease, of whom approximately 12 patients will have TRG2 residual tumor and approximately 3 patients will have TRG3 or TRG4 residual tumor. As we proposed above, TRG2 residual tumors may be missed. Therefore, we expect that approximately 3 patients with clinically relevant residual disease (TRG3 or TRG4) will be missed.

In case of unexpected aberrant distribution of patients in the preSANO-trial that leads to decreased TRG3 and TRG4 rates, results of the first 120 patients will be analyzed following the present protocol. If these results are promising but do not reach statistical significance, possibly due to a lack of power, inclusion of extra patients will be considered. If inclusion of extra patients is desirable, the protocol will be amended and assessed by the medical ethics committee.

Data analysis
The clinical response evaluation will consist of different diagnostic modalities. Results of each diagnostic modality will be presented as categorical or continuous data, depending on the outcome measure of each diagnostic modality. These results will be correlated to the (categorical) tumor regression grading in the resection specimen, using a chi-square based test (categorical-categorical) or a one-way ANOVA test (continuous-categorical) with post-hoc testing.

Results

The first patient has been enrolled on July 23, 2013 and results are expected in January 2016.

Discussion

The uniqueness of this study lies in the prospective evaluation of a sufficiently large number of patients, using multiple diagnostic modalities on different time points. Although (cyto)histological assessment of biopsies and / or FNAs is the most objective parameter, several studies have shown that the response to nCRT is reflected by tumor size or volume as assessed by EUS[23-26]. The rationale to include a second clinical response evaluation before a planned surgical resection is to allow for a comparison between multiple measurements and to increase the chance of detecting residual- and/or disseminated disease. It is expected that during CRE-II (due to an extended time period from the end of nCRT) the F18-FDG PET-CT signal will have a more favorable signal-to-noise ratio than has been described previously[27-32], because after 12 weeks the artefacts due to radiation-induced inflammation are expected to have largely dissolved. This allows for identification of suspected lymph nodes to be targeted by FNA during CRE-II.

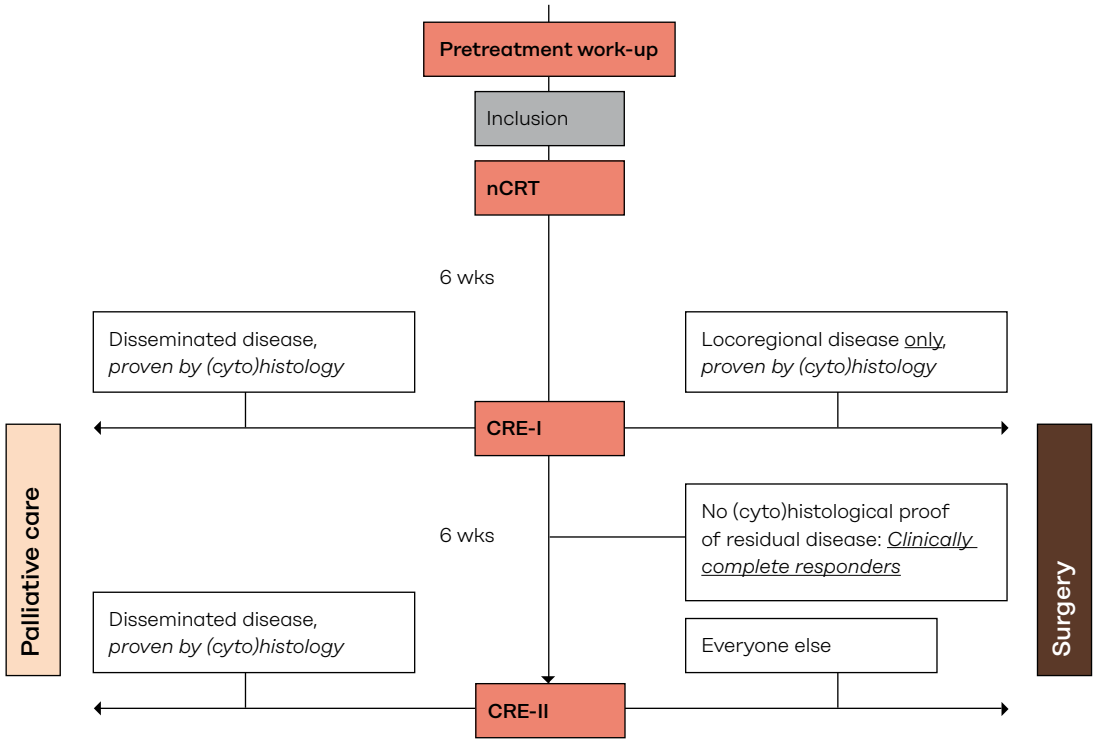
The reason to include patients with SCC as well as patients with AC in the preSANO trial, is that the CROSS regimen has been shown to be effective in both groups of patients. The pCR rate of 49% in patients with SCC and 23% in patients with AC in the CROSS trial, provide a rationale for a SANO approach in both histological subtypes . Furthermore, together with the low frequency of toxic effects of the CROSS regimen (91% received the full treatment regimen of nCRT), these high pCR rates advocate the use of the relatively low dose of 41.4 Gy radiotherapy[18].

Although we have not yet clearly shown that we are able to detect a clinically threatening residual cancer 4-6 weeks after nCRT, there are several arguments why it is not deemed necessary to do so before we can further delay the planned surgical resection with an additional 6-8 weeks. Recently, it was shown that prolonged time to surgery after nCRT up to at least 12 weeks had no effect on disease-free- and overall survival (HR=1.00 and HR=1.06 per additional week, p=0.976 and p=0.139, respectively). Moreover, prolonged time to surgery increased the probability of pCR in the resection specimen (odds ratio, OR=1.35 per additional week of time to surgery, p=0.0004)[33]. Comparable results have been published by other groups[34, 35].

Postoperative mortality and severe morbidity (grade ≥3 according to the Clavien-Dindo classification [19]) after esophagectomy in the Netherlands is 5% and 60%, respectively. Thus, a non-surgical treatment strategy in patients with a clinically complete response after nCRT, theoretically saves up to 5% mortality and 60% severe morbidity in this patient group. Moreover, this approach might improve quality of life and might lead to a reduction in health care costs. Therefore, we will consider this study as successful when the results of the combined diagnostic modalities lead to a maximum percentage of clinically false-negative TRG3 and TRG4 tumors of twice the postoperative mortality, *i.e.* 10%. If more than 10% of TRG3 or TRG4 tumors will be missed, the SANO trial will be reconsidered.

If the preSANO trial shows that TRG3 and TRG4 residual tumor can be predicted reliably, a randomized trial comparing nCRT plus standard surgery versus chemoradiotherapy plus *Surgery As Needed in Oesophageal cancer patients* (the SANO trial) will be conducted. Hopefully, this SANO trial will result in an organ-preserving treatment strategy for a selected group of patients and therefore reduce treatment related morbidity and mortality, improve quality of life and lead to a reduction in health care costs.

Figure 1 — Study algorithm



Legend figure 1

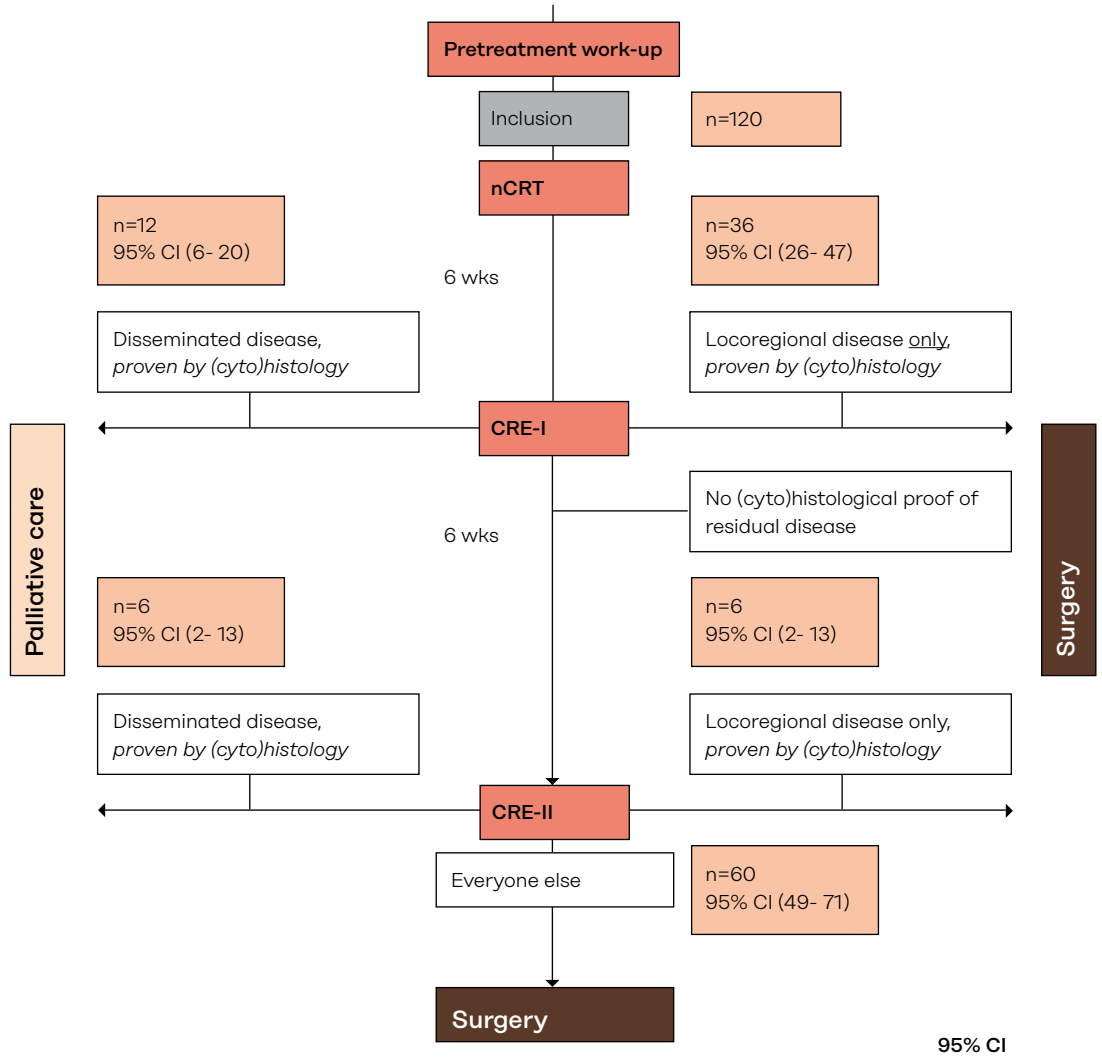
Pretreatment work-up and clinical response evaluations include:
- '*partial body*' or '*whole-body*' F18-FDG PET-CT[1]
- OGD (with biopsies)
- EUS (with FNA)[2]
- Dedicated CT of neck, thorax, abdomen and pelvis (in pretreatment work-up and on indication)
- External US of the neck (in pretreatment work-up and on indication).

[1] During the pretreatment work-up, it suffices when a '*partial body*' F18-FDG PET-CT of the esophagus will be performed (to test for avidity of the primary lesion); if it is preferred to make a *'whole-body*' PET-CT not only after, but also before the neoadjuvant chemoradiotherapy in order to detect distant metastases at an earlier stage, the indication for performing an external US with FNA of the neck can be limited to those patients who have a suspected lymph node on the PET-CT [22]. In the period after neoadjuvant therapy one *whole-body* F18-FDG PET-CT will be performed either at CRE-I (for the clinically non-complete responders) or at CRE-II (for the clinically complete responders at CRE-I).

[2] EUS with FNA of suspected lymph nodes only during CRE-II, not during CRE-I.

CRE: clinical response evaluation; CT: computed tomography; EUS: endoscopic ultrasonography; FNA: fine-needle aspiration; nCRT: neoadjuvant chemoradiotherapy; EGD: esophagogastroduodenoscopy; PET: positron-emission tomography; US: ultrasonography.

Figure 2 — Expected distribution of patients (based partly on CROSS-trial data).



		95% CI
TRG 1	30	(22- 38)
TRG 2	12-15	(6- 23)
≥ TRG3	0-3	(0- 8)
Refusal	15	(9- 23)

Legend figure 2

All numbers are based on an inclusion of 120 patients. CI: confidence interval; CRE: clinical response evaluation; nCRT: neoadjuvant chemoradiotherapy; N: number of patients; TRG: tumor regression grade, as measured by the modified TRG system of Chirieac[21]. Of the 45 patients who will undergo a postponed resection following CRE-II, 15 patients are expected to have a pathologically incomplete response (at least TRG2).

2b

Inclusion		CRE-I		CRE-II		Histology	
Positive	120	Positive	36	Positive	6	Positive	15
Negative	-	Negative	72	Negative	60	Negative	30
Disseminated	-	Disseminated	12	Disseminated	6	Disseminated	-
						Refuses surgery	15

Table 1 — Study algorithm

Parameter	Pretreatment	First clinical response evaluation (CRE-I)	Second clinical response evaluation (CRE-II)
History, Physical Examination	X	X	X
Performance status	X	X	X
Haematology[1]	X		
eGFR	X		
Biochemistry[2]	X		
Endoscopy + (random) biopsies	X	X	X
Radial EUS[3]	X	X	X
Linear EUS (+FNA)[4]	X		X
CT of neck, thorax, abdomen and pelvis	X		
PET-CT	X 'partial body'	X[7] 'whole body'	X[8] 'whole body'
Pulmonary function tests	X		
Bronchoscopy[5]	X		
ECG	X		
Toxicity[6]	Baseline		

Legend table 1

[1] Hematology: CBC, differential
[2] Biochemistry: serum protein, albumin, magnesium, electrolytes, serum creatinin, bilirubin, alkaline phosphatase, AST, and pregnancy test if indicated at baseline only
[3] Radial EUS: with measurement of maximum tumor thickness and –area
[4] Linear EUS: with fine-needle aspiration (FNA) of any suspected lymph nodes
[5] Bronchoscopy: when tumor is located above the carina and when there is suspicion for invasion of the tracheo-bronchial tree
[6] Toxicity: to be evaluated after each cycle (incidence and grade according to CTC toxicity scale)
[7] PET-CT: during CRE-I, after EGD and EUS, only for clinically non-complete responders, to exclude disseminated disease
[8] PET-CT: during CRE-II, prior to EGD and EUS, for all patients (all were clinically complete responders during CRE-I) to guide EGD and EUS in targeting suspected locoregional lesions and to exclude disseminated disease.

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Chapter 10

Future perspectives

Survival of patients undergoing primary surgical resection for esophageal cancer has steadily increased over the last decades, from a five-year overall survival rate of approximately 12% in the 1960s and 1970s[1] and approximately 20% in the 1980s and 1990s[2,3] to approximately 35% in the last two decades.[4,5] Survival improved during this period mainly due to improved preoperative diagnostic modalities, more restrictive patient selection, intensified perioperative patient management and improved surgical technique, thus lowering peri-operative mortality and increasing locoregional control. New multimodality treatment regimens, such as the Dutch CROSS neoadjuvant chemoradiotherapy (nCRT) regimen, have further increased the five-year overall survival rate of patients undergoing esophagectomy for esophageal or junctional cancer to approximately 47%.[5,6] However, when considering the entire group of esophageal cancer patients (including patients with disseminated disease at presentation) the five-year overall survival rate still remains sobering at approximately 10%.[7]

Therefore, the current challenge in esophageal cancer treatment is to further improve survival (at an acceptable loss in quality of life) for those selected patients who have locally advanced esophageal cancer and are planned to undergo nCRT plus surgery with curative intent.

Should we extend the surgical resection?

The main goal of surgical therapy with curative intent for oncological disease is maximization of locoregional control. However, with many tumor types and especially with esophageal cancer, achieving maximum locoregional control with surgery alone has proven difficult. Many factors add to this, such as the frequent deep invasion of the primary tumor at presentation, the early regional (lymphatic) dissemination and the surrounding critical structures which do not allow for wide excision. Therefore, current neoadjuvant chemo(radio)therapy strategies have focused on achieving tumor downstaging, thus allowing for a more radical surgical resection. This was clearly shown in the CROSS trial, where radical resection percentages increased from 69% in the surgery alone group to 92% in the nCRT plus surgery group.[5] These percentages have increased even further in recent years due to increasing experience with post chemoradiotherapy resections. Therefore, in the current era of multimodality treatment, radicality of resection has reached acceptable percentages and is thereby no longer a major determinant of survival.

Should we perform an extended lymphadenectomy?
Talsma *et al*.[8] have shown that in the surgery alone group of the CROSS trial the number of resected nodes was significantly associated with survival, while in the nCRT plus surgery group that association was no longer present. Also, the positive association between the number of nodes resected and the number of positive nodes identified, which was significant in the surgery alone group was absent in the nCRT plus surgery group. These results indicate that in patients treated with nCRT plus surgery, there is no clear survival benefit of maximizing the number of lymph nodes resected. However, to adequately quantify the potential therapeutic impact of extended lymphadenectomy for patients treated with nCRT plus surgery, a new randomized controlled trial is needed in which patients are randomized after nCRT between a limited (transhiatal) lymphadenectomy and a more extended (transthoracic) lymphadenectomy.

Should we intensify the neoadjuvant chemoradiotherapy regimen?
Oppedijk *et al*.[9] have shown that after nCRT (according to CROSS) plus surgery only 5% of patients develop infield recurrences, while only 1% of patients develop infield recurrences without synchronous distant failure. Some 2% of patients develop borderline recurrences and 6% develop regional outfield recurrences. These results indicate that intensifying the nCRT regimen might improve survival in approximately 1% of patients and extending the radiation field might improve survival in approximately 2% of patients. However, the marginal survival benefit that could possibly be achieved by intensification of chemotherapy and radiotherapy or by the extension of the radiation field would be offset by a significant increase in nCRT related morbidity. Therefore, it does not seem beneficial to routinely increase the nCRT dose or to routinely extend the radiation field. However, it should be of interest for future research to try and identify those patients, pretreatment, who stand to benefit from intensification of chemotherapy and/or radiotherapy or from the extension of the radiation field.

Can we minimize iatrogenic morbidity and mortality?
Results from the initial phase II feasibility trial[10] and the subsequent randomized phase III CROSS trial5 have shown that the nCRT treatment regimen according to CROSS is generally well tolerated, with 100% and 94% of patients, respectively, completing neoadjuvant therapy without delay or dose-reduction. Furthermore, the nCRT treatment regimen is associated with a relatively low frequency of high-grade toxic effects. These results suggest that morbidity and mortality from nCRT is already low and might therefore not be reduced much further.

Standard esophagectomy, which is associated with severe postoperative morbidity and a substantial impact on the quality of life[11-14], might be reconsidered as a necessity for all patients following nCRT. In the nCRT group of the CROSS trial5 49% of patients with a squamous cell carcinoma and 23% of patients with an adenocarcinoma had a pathologically complete response in the resection specimen (*i.e.* no viable tumor cells were found at the site of the primary tumor or in the resected regional lymph nodes, as determined by conventional histological examination). Therefore, these results provide a rationale to reconsider and study the timing and necessity of esophagectomy in (all) patients after application of the CROSS regimen. Under analogous conditions, a non-operative management in rectal cancer patients with a clinically complete response after nCRT has been shown feasible and safe, leading to organ-sparing treatment with low morbidity and mortality rates and favorable long-term survival in a subset of these rectal cancer patients.[15-17] Currently a single arm diagnostic feasibility trial (preSANO, Dutch Trial Register NTR4834)[18] is underway in several Dutch high volume centers, which aims to determine the accuracy of clinically detecting or predicting the presence of residual disease after nCRT. If indeed results from the preSANO trial will be encouraging, a new randomized trial comparing nCRT plus standard surgery versus chemoradiotherapy plus surgery as needed in esophageal cancer patients (the SANO trial) will be conducted. Results from the future SANO trial will show whether an organ-preserving treatment strategy (for a selected group of patients) is safe and whether such a treatment strategy also reduces treatment related morbidity and improves quality of life.

Can we improve distant disease control?
Most patients with locally advanced esophageal cancer, who undergo nCRT plus surgery with curative intent, will die due to distant disease recurrence. In the nCRT plus surgery group of the CROSS trial, 49% (87/178) of patients showed disease recurrence, of whom 22% (39/178) showed locoregional disease recurrence and 39% (70/178) showed distant disease recurrence (22 patients showed both locoregional and distant disease recurrence).[6] These results emphasize the importance of distant disease recurrence as one of the major determining factors in overall survival.

Perhaps adding adjuvant treatment, either as chemotherapy or as targeted therapy[19] (or a combination of both), in a selected (high-risk) group of patients might reduce the number of distant disease recurrences, thereby increasing overall survival in these patients. However, the addition of adjuvant treatment should always be balanced with its cost in quality-of-life. It is to be expected that the addition of adjuvant

treatment, after neoadjuvant treatment and surgical resection, will carry a heavy burden for patients, especially for those patients with significant comorbidities.

In conclusion, five-year overall survival for patients with locally advanced esophageal cancer, who undergo nCRT plus surgery with curative intent has improved in recent decades to approximately 47%. The neoadjuvant CROSS regimen has proven to be effective for tumor downstaging yet well tolerable for patients. In-field and borderline recurrences are rare and therefore do not justify intensifying the nCRT regimen. Perhaps, in the near future, an organ-preserving treatment strategy (for a selected group of patients) will prove to be safe, with hopefully an associated reduction in treatment-related morbidity and improvement in quality-of-life. However, what continues to endanger our patients is distant disease recurrence, as approximately 40% of patients with locally advanced esophageal cancer, treated with nCRT plus surgery will develop distant disease recurrence during follow-up. Therefore, we should focus our research efforts on effective yet mild chemotherapy and/or targeted therapy treatment regimens, specifically geared towards increasing our distant disease control rate. Finally, although not the topic of this dissertation, patients with disseminated esophageal cancer at presentation, who do not get the benefit of potentially curative treatment should also be the benefactors of continued research in order to maximize their survival and minimize their suffering.

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Chapter 11
Summary

The research described in this thesis addresses prediction of survival and new treatment strategies for patients with esophageal or junctional cancer. Results will be discussed in two parts, **Part I** focusses on the prediction of survival using conventional and more novel prognostic factors in patients with esophageal or junctional cancer. While **Part II** focusses on the long-term survival benefit of neoadjuvant (*i.e.*preoperative) chemoradiotherapy plus surgery, as compared to surgery alone and on the feasibility of a new treatment strategy for a subset of patients with potentially curable esophageal or junctional cancer.

Part I

Prognostication for esophageal and junctional cancer

In patients with cancer of the esophagus or esophagogastric junction, several prognostic factors for survival have been identified and prediction models have been developed to predict survival in individual patients, based on these prognostic factors.[1,2] However, most of these prognostic factors have been identified and validated in the era of primary surgical resection. In **chapter 1** we show that most of these conventional prognostic factors lose their prognostic importance in patients treated with neoadjuvant chemoradiotherapy (nCRT) plus surgery. Only the pretreatment number of suspected tumor positive lymph nodes and the posttreatment number of resected tumor positive lymph nodes remain as independent prognostic factors. Interestingly, the pretreatment number of suspected tumor positive lymph nodes was an independent prognostic factor, despite its relative inaccuracy.[3] Therefore, to further study the possible relevance of pretreatment tumor extent we describe and validate a novel method of determining pretreatment tumor extent in **chapter 2**. In this chapter we show that the pretreatment tumor extent could be reproducibly determined based on the location of tumor scarring (*i.e.* fibrosis) and on the location of residual tumor cells in the resection specimen after surgery. Especially the number of pretreatment tumor positive lymph nodes carries much prognostic information. For example, patients who had pretreatment nodal involvement and became node-negative due to nCRT had a worse survival compared to patients without any pretreatment nodal involvement. Resecting more lymph nodes was previously shown to improve prediction of survival[4,5] and even survival itself[6,7] in patients with esophageal cancer. However, it is unclear whether after nCRT resecting more lymph nodes is still indicated. Therefore, we compared the prognostic impact of total number of resected lymph nodes and the number of resected tumor positive lymph nodes between patients who underwent nCRT plus surgery and surgery alone in **chapter 3**. Results

show that tumor involvement of lymph nodes, especially if persistent after nCRT, is a strong negative predictor of survival, confirming our results from chapters 1 and 2. Also, the number of resected nodes (irrespective of tumor positivity) was shown to be an independent prognostic factor for survival in patients treated with surgery alone, but not in patients treated with nCRT plus surgery. Therefore, these data question the benefit of maximization of lymph node removal after nCRT. In order to better predict survival for patients after primary surgery, we studied activation of certain genes in patients' tumor tissue as biomarkers for survival. Activation of these genes was demonstrated using a labelling technique (immunohistochemistry) which can be visualized using a microscope, as described in **chapter 4**. In this chapter we confirm that a simple panel of three biomarkers can predict survival in patients with esophageal adenocarcinoma, independently from other patient and tumor related variables and that this panel of three biomarkers also predicted survival in patients from other continents.

Part II

New treatment strategies for esophageal and junctional cancer

The CROSS trial compared nCRT plus surgery to surgery alone in patients with esophageal or junctional cancer. Initial results showed that 13% more patients were alive after five years in the nCRT plus surgery group as compared to the surgery alone group. These results made nCRT plus surgery the new treatment of choice for esophageal and junctional cancer in many countries, including The Netherlands. In **chapter 5** we confirm the initial results of the CROSS trial using longer patient follow-up and we also show that patients in the nCRT plus surgery group develop less disease recurrence at the site of the original tumor and also less recurrence in other organs (*i.e.* metastases). Still today surgical removal of the esophagus carries a risk of death between 2% and 5%. Even at highly specialized centers this type of surgery is associated with severe and frequent complications, with a substantial impact on the quality of life. Interestingly, after nCRT approximately 30% of patients have no residual tumor in their esophagus (*i.e.* complete response). Therefore, it is questionable whether patients with a complete response in the resection specimen after nCRT have sufficient additional benefit to justify subsequent standard surgical removal of the esophagus. Several studies have tried to identify patients with a complete response after nCRT using different diagnostic modalities, such as endoscopy.[8-14] However, results from these studies have been mostly disappointing. Before we can consider a watchful waiting policy (instead of a standard surgical removal of the esophagus) in a subgroup of patients with esophageal cancer, a better insight into the location of residual tumor is needed. Therefore, in **chapter 6** we describe the exact location of residual tumor in the esophageal wall and resected lymph nodes after nCRT and we describe the tumor regression pattern of esophageal cancer as induced by nCRT. A possible determinant of the complete response rate might be the waiting time between the end of nCRT and the day of surgery. Theoretically, a prolonged waiting time might increase the complete response rate and possibly improve survival because of a prolonged effect of nCRT. Conversely, a prolonged waiting time might lead to residual tumor outgrowth, increased difficulty of surgical resection due to inceased tissue scarring with a higher postoperative complication rate and possibly a worse overall survival. Therefore, we investigate the impact of waiting time on the complete response rate, short-term surgical outcome and survival in **chapter 7**. In this chapter we show that a prolonged waiting time (beyond 45 days) increases the probability of a complete response, slightly increases the probability of postoperative complications and does not impact survival. Finally, in **chapter 8**, we describe the study protocol of a single arm diagnostic feasibility trial (preSANO, Dutch Trial Register NTR4834)[15] which is currently running in several Dutch high volume centers and aims to determine the accuracy of clinically detecting or predicting the presence of residual disease after nCRT.

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Chapter 11
Samenvatting

Het onderzoek dat in dit proefschrift beschreven is, behandelt de voorspelling van overleving en nieuwe behandelstrategieën voor patiënten met een slokdarm- of junctiecarcinoom. De resultaten worden besproken in twee delen. **Deel I** richt zich op de voorspelling van overleving, waarbij gebruik wordt gemaakt van conventionele en nieuwe prognostische factoren bij patiënten met een slokdarm- of junctiecarcinoom. **Deel II** richt zich op het lange-termijn overlevingsvoordeel van neoadjuvante (*i.e.* preoperatieve) chemoradiotherapie gevolgd door een chirurgische resectie, in vergelijking met *al*leen een chirurgische resectie. Tevens richt dit deel zich op de haalbaarheid van een nieuwe behandelstrategie voor (een deel van de) patiënten met een slokdarm- of junctiecarcinoom.

Deel I

Voorspelling van overleving bij patiënten met een slokdarm- of junctiecarcinoom

Bij patiënten met kanker van de slokdarm en slokdarm-maag overgang zijn verschillende prognostische factoren geïdentificeerd om overleving te voorspellen voor individuele patiënten na voltooiing van behandeling en zijn modellen ontwikkeld op basis van deze prognostische factoren.[1,2] Echter, het merendeel van deze conventionele prognostische factoren werden geïdentificeerd in een tijdperk van primaire chirurgische resectie (dus zonder additionele chemo- of radiotherapie). In **hoofdstuk 1** tonen wij dat het merendeel van de conventionele prognostische factoren hun voorspellende waarde verliezen bij patiënten die behandeld worden met neoadjuvante chemoradiotherapie (nCRT) gevolgd door een chirurgische resectie. Alleen het aantal verdachte tumor positieve lymfeklieren bij start van de behandeling en het aantal tumor positieve lymfeklieren in het resectiepreparaat bij voltooiing van behandeling blijven waardevolle prognostische factoren in het tijdperk van nCRT gevolgd door resectie. Opvallend is dat het aantal verdachte tumor positieve lymfeklieren bij start van de behandeling een waardevolle prognostische factor bleek te zijn, ondanks de relatieve onnauwkeurigheid waarmee dit kan worden bepaald.[3] Om de potentiële waarde te bestuderen van de initiële uitgebreidheid van de tumor, lokaal en in naburige lymfeklieren, beschrijven wij in **hoofdstuk 2** een nieuwe methode om de initiële uitgebreidheid van de tumor nauwkeurig te bepalen. In dit hoofdstuk tonen wij dat de initiële uitgebreidheid van de tumor nauwkeurig bepaald kan worden in het resectiepreparaat op basis van tumorrestanten en op basis van verlittekening van de tumor (*i.e.* fibose). Vooral het aantal verdachte tumor positieve lymfeklieren bij start van de behandeling bevat

veel prognostische informatie. Bijvoorbeeld, patiënten die bij start van de behandeling tumor positieve lymfeklieren hebben, maar na afloop van de behandeling géén tumor positieve lymfeklieren meer hebben, houden toch een slechtere overleving dan patiënten die nooit tumor positieve lymfeklieren hebben gehad. Eerder onderzoek toonde dat het verwijderen van meer lymfeklieren het voorspellen van overleving verbetert4,5 en zelfs de overleving zelf verbetert[6,7] bij patiënten met slokdarmkanker. Echter, het is onduidelijk wat het nut is van lymfeklier verwijdering na nCRT. Daarom vergeleken wij in **hoofdstuk 3** de prognostische waarde van het aantal verwijderde lymfeklieren en het aantal verwijderde tumor positieve lymfeklieren tussen patiënten die behandeld werden met nCRT gevolgd door chirurgische resectie en patiënten die behandeld werden m*et a*lleen een chirurgische resectie. Resultaten tonen dat tumor positiviteit in lymfeklieren, met name aanhoudende positiviteit na nCRT, sterke negatieve voorspellers zijn van overleving. Dit bevestigde onder andere de resultaten uit hoofdstukken 1 en 2. Het aantal verwijderde lymfeklieren (onafhankelijk van het aantal tumor positieve lymfeklieren) was een waardevolle voorspeller van overleving bij patiënten die behandeld werden m*et a*lleen een chirurgische resectie, maar niet bij patiënten die behandeld werden met nCRT gevolgd door chirurgische resectie. Derhalve dient men zich op basis van deze resultaten af te vragen wat de waarde is van uitgebreide lymfeklierverwijdering na nCRT. Om beter de overleving van patiënten een met slokdarm- of junctiecarcinoom te kunnen voorspellen na een chirurgische resectie, bestudeerden wij de activatie van bepaalde genen in het tumorweefsel van patiënten als biomarkers voor overleving. Activatie van deze genen werd bepaald middels een labeltechniek (immunohistochemie), waarmee de activatie zichtbaar wordt onder een microscoop, zoals beschreven in **hoofdstuk 4**. In dit hoofdstuk bevestigen wij dat een eenvoudig panel van drie biomarkers de overleving van patiënten met een slokdarm- of junctie adenocarcinoom kan voorspellen, onafhankelijk van andere patiënt- en tumor gerelateerde factoren.

Deel II

Nieuwe behandelstrategieën voor patiënten met een slokdarm- of junctiecarcinoom

De CROSS trial vergeleek nCRT gevolgd door chirurgische resectie m*et a*lleen een chirurgische resectie bij patiënten met een slokdarm- of junctiecarcinoom. De initiële resultaten toonden dat bij patiënten die behandeld werden met nCRT gevolgd door chirurgische resectie, na vijf jaar 13% meer patiënten in leven waren. Door deze resultaten werd nCRT gevolgd door chirurgische resectie in Nederland en veel andere landen de standaardbehandeling voor patiënten met een slokdarm- of junctiecarcinoom. In **hoofdstuk 5** bevestigen wij deze initiële resultaten van de CROSS trial bij een langere follow-up. Ook laten wij zien dat er bij patiënten die behandeld worden met nCRT gevolgd door chirurgische resectie minder recidieven optreden in het gebied van de originele tumor en ook minder afstandsmetastasen ontstaan. Vandaag de dag is het risico op overlijden bij een chirurgische resectie van de slokdarm nog steeds twee tot vijf procent. Zelfs in hoog gespecialiseerde centra zijn dit soort ingrepen geassocieerd met ernstige en frequente complicaties met een significante impact op de kwaliteit van leven. Opvallend is dat bij ongeveer 30% van de patiënten die een slokdarmresectie ondergaan na nCRT geen tumorresidu meer gevonden wordt in de verwijderde slokdarm (*i.e.* complete respons). Het is daarom de vraag of patiënten met dergelijke complete respons nog wel genoeg voordeel hebben van een slokdarmresectie, om deze ingreep standaard bij alle patiënten uit te voeren. Verscheidene onderzoeken hebben geprobeerd om deze patiënten met een complete respons te identificeren na voltooiing van de nCRT, voorafgaand aan een resectie.[8-14] Resultaten van deze onderzoeken waren voornamelijk teleurstellend. Voordat men een meer selectieve strategie kan overwegen (in plaats van een slokdarmresectie bij iedere patiënt) is een beter inzicht nodig in de locatie van tumorresidu na nCRT. In **hoofdstuk 6** beschrijven wij de exacte locatie van tumorresidu in de slokdarmwand en in de omliggende lymfeklieren. Tevens beschrijven wij in dit hoofdstuk het tumorregressiepatroon na blootstelling aan nCRT. Een mogelijke factor die van invloed is op het complete respons percentage in het resectiepreparaat is de wachttijd tussen voltooiing van de nCRT en de chirurgische resectie. Theoretisch zou een langere wachttijd door een langer effect van de nCRT het complete respons percentage en mogelijk zelfs de overleving positief kunnen beïnvloeden. Daarentegen, zou een langere wachttijd ook kunnen leiden tot hernieuwde tumor uitgroei, een bemoeilijkte chirurgische resectie (door toegenomen tumorverlittekening) en mogelijk zelfs een verminderde overleving. Daarom onderzoeken wij in **hoofdstuk 7** de invloed van de wachttijd op het complete respons percentage, op de korte-termijn chirurgische uitkomsten en op de overleving. In dit hoofdstuk tonen wij dat een wachttijd langer dan 45 dagen de kans op een complete respons verhoogt, de kans op postoperatieve complicaties minimaal verhoogt, maar er geen effect is op de overleving. Tot slot, beschrijven wij in **hoofdstuk 8** een nieuw onderzoek (preSANO, Dutch Trial Register NTR4834)[15] dat op dit moment in enkele Nederlandse hoog volume centra wordt uitgevoerd, waarbij wordt bepaald wat de nauwkeurigheid is waarmee met behulp van diagnostiek kan worden voorspeld of er bij een patiënt na voltooiing van de nCRT nog tumorresidu aanwezig is in de slokdarm.

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PhD portfolio

Name PhD student:	J. Shapiro
Erasmus MC department:	Surgery
PhD period:	October 2010 - September 2014
Title thesis:	Prognostication and new treatment strategies for esophageal and junctional cancer
Promotors:	Prof.dr. J.J.B. van Lanschot
Date defense thesis:	June 1, 2016

PhD Training

Oral presentations (0.5 points each)	Year	ECTS
Wnt signaling and cancer stemness in esophageal adenocarcinoma: friends or foes? United European Gastroenterology Week, Vienna	2014	0.5
Impact of neoadjuvant chemoradiotherapy on conventional prognostic factors and on individual prediction of survival in patients with esophageal or junctional cancer. International Society for Disease of the Esophagus, Vancouver	2014	0.5
Prolonged time to surgery after neoadjuvant chemoradiotherapy in patients with esophageal or junctional cancer. International Society for Disease of the Esophagus, Vancouver	2014	0.5
Lymph node retrieval during esophagectomy with and without neoadjuvant chemoradiotherapy; prognostic and therapeutic impact on survival. International Society for Disease of the Esophagus, Vancouver	2014	0.5
Prolonged time to surgery after neoadjuvant chemoradiotherapy in patients with esophageal or junctional cancer. NVvH Chirurgendagen, Veldhoven	2014	0.5
Prolonged time to surgery after neoadjuvant chemoradiotherapy in patients with esophageal or junctional cancer. European Surgical Association, Athens	2014	0.5
Impact of neoadjuvant chemoradiotherapy on prognostic factors for survival in patients with esophageal or junctional cancer. Voorjaarsvergadering NVGE, Veldhoven	2014	0.5
'*Surgery as needed*' after neoadjuvant chemoradiotherapy for esophageal or junctional cancer. Stafdag Heelkunde Erasmus MC, Rotterdam	2013	0.5
Accuracy of detecting residual disease after neoadjuvant chemoradiotherapy for oesophageal cancer (preSANO). Dutch Oesophagogastric Cancer Group Meeting, Utrecht	2013	0.5

PhD Training

Oral presentations (0.5 points each)	Year	ECTS
Residual esophageal cancer after neoadjuvant chemoradiotherapy mainly involves the (sub)mucosa. European Surgical Association, Beaune	2013	0.5
Timing of surgery and location of residual tumor after neoadjuvant chemoradiotherapy (nCRT) for esophageal cancer. Voorjaarsvergadering NVGE, Veldhoven	2013	0.5
Neoadjuvant chemoradiation plus '*surgery as needed*' for squamous cell carcinoma of the oesophagus. Dutch Oesophagogastric Cancer Group Meeting, Rotterdam	2012	0.5
Search for cancer stem cells in ESO26, an esophageal adenocarcinoma cell line. Stafdag Heelkunde Erasmus MC, Rotterdam	2011	0.5

Poster presentations (0.5 points each)		
Residual esophageal cancer after neoadjuvant chemoradiotherapy frequently involves the (sub)mucosa. European Society for Disease of the Esophagus, Rotterdam	2013	0.5
More pathologically complete responders after a longer interval between chemoradiotherapy and surgery for esophageal cancer. European Cancer Congress, Amsterdam	2013	0.5
Search for cancer stem cells in ESO26, an oesophageal adenocarcinoma cell line. European Society of Esophagology, Newcastle upon Tyne	2011	0.5

In-depth courses (0.3 points/day)		
Basiscursus regelgeving en organisatie klinisch onderzoekers (BROK), Erasmus MC, Rotterdam	2014	1.0
Integriteit in wetenschappelijk onderzoek, Erasmus MC, Rotterdam	2013	0.6
Systematisch literatuuronderzoek, Erasmus MC, Rotterdam	2013	0.6
Epigenetics in health and disease, LUMC, Leiden	2012	1.0
Methodologie van patiëntgebonden onderzoek en voorbereiding van subsidieaanvragen, Erasmus MC, Rotterdam	2012	0.3

Teaching	Year	ECTS
Supervising MSc students at the Erasmus MC	2011-2014	3.0

International conferences (0.3 points/day)		
- United European Gastroenterology Week, Vienna	2014	1.5
- International Society for Disease of the Esophagus, Vancouver	2014	1.0
- European Surgical Association, Athens	2014	1.0
- European Cancer Congress, Amsterdam	2013	1.0
- European Society for Disease of the Esophagus, Rotterdam	2013	1.0
- European Surgical Association, Beaune	2013	1.0
- International Society for Disease of the Esophagus, Venice	2012	1.0
- European Society for Disease of the Esophagus, Newcastle upon Tyne	2011	1.0

Scientific meetings (0.3 points/meeting)		
- Dutch Oesophagogastric Cancer Group Meeting	2012-2014	1.0
- NVvH Chirurgendagen	2010-2014	1.0
- Voorjaarsvergadering NVGE	2011-2014	1.0

List of publications

1. Bosman LW, Koekkoek SKE, Shapiro J, Rijken BF, Zandstra F, van der Ende B, et al. Encoding of whisker input by cerebellar Purkinje cells. J Physiol. 2010; 588(19): 3757-83.

2. Jongen JL, Pederzani T, Koekkoek SKE, Shapiro J, van der Burg, J, De Zeeuw CI, et al. Autofluorescent flavoprotein imaging of spinal nociceptive activity. J Neurosci. 2010; 30(11): 4081-7.

3. Shapiro J, van Lanschot JJB. Definitive chemoradiation therapy. In 'Innovation in Esophageal Surgery', editor L. Bonavina; Springer International Publishing 2012: 85-94.

4. 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(12): 1576-82.

5. Shapiro J, ten Kate FJW, van Hagen P, Biermann K, Wijnhoven BPL, van Lanschot JJB. Residual esophageal cancer after neoadjuvant chemoradiotherapy frequently involves the mucosa and submucosa.
Ann Surg. 2013; 258(5): 678-88.

6. Shapiro J, van Hagen P, Lingsma HF, Wijnhoven BPL, Biermann K, ten Kate FJW, 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(5): 807-13.

7. Talsma AK, Shapiro J, Looman CW, van Hagen P, Steyerberg EW, van der Gaast A, et al. Lymph node retrieval during esophagectomy with and without neoadjuvant chemoradiotherapy: prognostic and therapeutic impact on survival.
Ann Surg. 2014; 260(5): 786-92.

8. Shapiro J, Wijnhoven BPL, van Lanschot JJB.
Reply to letter: "Residual esophageal cancer after neoadjuvant chemoradiotherapy frequently involves the mucosa and submucosa".
Ann Surg. 2015; 262(2): e84-e85.

9. 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(8): 1047-53.

10. 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 Research Protocols. 2015; 4(2): e79.

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(9):1090-8.

12. Shapiro J, van Lanschot JJB, Hulshof MCCM, van der Gaast A. Effectiveness of neoadjuvant chemoradiotherapy for early-stage esophageal cancer.
J Clin Oncol. 2015; 33(3): 288-9.

13. Talsma AK, Shapiro J, Wijnhoven BPL, van Lanschot JJB. Goals of surgical therapy for esophageal cancer.
In 'Minimally invasive foregut surgery for malignancy', editors SN Hochwald and M Kukar; Springer International Publishing 2015: 35-49.

14. Toxopeus EL, Nieboer D, Shapiro J, Biermann K, van der Gaast A, van Rij CM, et al.
Nomogram for predicting pathologically complete response after neoadjuvant chemoradiotherapy for oesophageal cancer.
Radiother Oncol. 2015; 115(3): 392-8.

15. Shapiro J, Biermann K, van Klaveren D, Meijer SL, Offerhaus GJA, ten Kate FJW, et al. Prognostic value of pretreatment tumor extent in patients treated with neoadjuvant chemoradiotherapy plus surgery for esophageal or junctional cancer.
Ann Surg. 2016; [epub ahead-of-print].

16. 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; [epub ahead-of-print].

17. Shapiro J, van Klaveren D, Lagarde SM, Toxopeus ELA, van der Gaast A, Wijnhoven BPL, et al. Prediction of survival in patients with esophageal or junctional cancer: impact of neoadjuvant chemoradiotherapy on conventional prognostic factors.
Accepted for publication.

About the author

Joël Shapiro was born on December 2, 1983 in Rotterdam, the Netherlands. After obtaining his high school degree at the Erasmiaans Gymnasium, Rotterdam in 2002, he worked and travelled for one year, spending a summer studying epidemiological methods at the University of California, Berkeley.

In 2003 he started Medical school at the Erasmus University Rotterdam, during which he successfully obtained his Master in Neuroscience degree (supervisors dr. S.K.E. Koekkoek and prof. dr. C.I. de Zeeuw). As part of his research he spent two months at the Department of Neuroscience, University of Minnesota, Minneapolis, studying cerebellar cortical activity using autofluorescent flavoprotein imaging (supervisor: prof. dr. T.J. Ebner).

He spent his final clinical rotation at the Department of Surgery of the IJsselland Hospital, Capelle aan den IJssel and two elective rotations in the USA, one at the Department of Surgery, Memorial-Sloan Kettering Cancer Center, New York and one at the Department of Surgery, Mayo Clinic, Rochester. He obtained his Medical degree in 2010 (cum laude). In that same year he started his PhD training with prof. dr. J.J.B. van Lanschot and prof. dr. R. Fodde, investigating the role of prognostication and exploring new treatment strategies in the era of neoadjuvant chemoradiotherapy, while experimentally studying the role of cancer stem cells in esophageal cancer. In 2014, after four years of full-time research, with his PhD work nearly complete, he returned to the IJsselland Hospital to work as a surgical resident 'not in training' (ANIOS). On July 1, 2015, Joël started his surgical training (AIOS) at the Erasmus MC (residency coordinator: dr. B.P.L. Wijnhoven) and from July 1, 2016 he will continue his surgical training at the IJsselland Hospital (residency coordinator: dr. I. Dawson).

He is happily married to Clara Shapiro-Koss, a psychiatrist-in-training, with whom he has two beautiful monsters, Adam (6) and Hannah (3).

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