PREDICTIVE FACTORS OF PATHOLOGICAL COMPLETE RESPONSE AFTER LONG-COURSE NEOADJUVANT CHEMORADIATION THERAPY FOR RECTAL CANCER

A THESIS
SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF THE UNIVERSITY OF MINNESOTA
BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
MASTER OF SCIENCE

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MAY 2012

Acknowledgements

I wish to express my sincere gratitude to all those who have supported me during the process of writing this thesis. I especially want to thank:

My mentors, Professors **Russell Luepker, David Rothenberger, Ann Lowry** and **Anders Mellgren** for excellent guidance, and generosity of their time and expertise;

Renee Kaufmann together with **Ann Lavallee** and **Janelle Willard** for coordinating and making the impossible possible;

Andrea Kish, who has helped me and so many in the Masters program in Clinical Research; and

Most importantly, **Jennifer**, my wife for her unconditional support and my children **Isabella**, **Sebastian** and **Lukas,** who are the best part of my life.

Abstract

Background: Preoperative chemoradiation therapy (CRT) in patients with rectal cancer results in pathologic complete response (pCR) in about 10-30% of patients. Predictive factors for obtaining pCR may influence the selection of patients for this therapy. The aim of this study was to evaluate the impact of tumor size, stage, location, circumferential extent, patient characteristics and pretreatment CEA levels on development of pCR after CRT.

Methods: 530 patients treated with preoperative CRT and radical surgery for rectal adenocarcinoma 1998-2011 were identified. A total of 469 patients remained after excluding patients with a history of pelvic radiation (n=2), previous transanal endoscopic microsurgery or polypectomy of the primary lesion (n=15), concurrent malignant tumor (n=14), and no information about pre- or post-treatment T stage in the chart (n=30). The clinical tumor stage and size were assessed by endorectal ultrasound (90%), MRI (10%), pelvic CT (5%), flexible sigmoidoscopy/colonoscopy (100%), chest X-ray (100%) and PET-CT (1%). Pathologic complete response was defined as absence of viable tumor cells in the rectal wall and in any of the resected lymph nodes. CRT consisted of a 5-fluorouracil-based regimen and external beam radiation with a mean radiation dose of 50 Gy given over a mean of 5.7 weeks. Mean time between completion of CRT and surgery was 8.7 weeks (SD, ±7,4).

Results: Ninety-six patients (20%) were found to have pCR in the operative specimen. Low pretreatment CEA (3.4 vs. 9.6 ng/ml; $p<0.008$) and smaller mean tumor size (4.2 vs.

4.7 cm; $p<0.02$) were significantly associated with pCR, but only low CEA level remained a significant predictor of pCR in the multivariate analysis. When stratifying for smoking status, low CEA was significantly associated with pCR only in the group of non-smokers ($p=0.02$).

Conclusions: The current study indicates that non-smoking rectal cancer patients with a low CEA level have an improved chance to obtain pCR after CRT. Further studies are necessary to determine whether a low CEA level can aid in identifying patients who are candidates for close follow-up rather than surgery after CRT.

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Introduction

Colorectal cancer (CRC) is the second most common cause of cancer related death and the third most common cancer in the world. Geographically there are some variations in the incidence of CRC with the highest occurrences in Western Europe and North America and the lowest in Asia, Africa and South America. However, these variations in incidence are more obvious in colon cancer than in rectal cancer.

The risk of developing CRC increases with age. Genetic and environmental factors are also important risk factors for developing CRC. The two most known colorectal cancer syndromes are familial adenomatous polyposis (FAP) and hereditary non-polyposis colorectal cancer (HNPCC). Only a minority, about 1-3 % of all incident cases of CRC, are caused by hereditary CRC syndromes. Apart from these genetic CRC syndromes, the risk of developing CRC is increased in the presence of a family history of CRC, especially if a close relative or multiple relatives were diagnosed with CRC before the age of 55 (Strate and Syngal, 2005).

Other risk factors for developing CRC include colorectal polyps, history of cancer, smoking, and inflammatory bowel disease. Several environmental factors have been associated with an increased risk of developing CRC however the evidence of acquired risk factors, particularly dietary factors, and their influence on the incidence of CRC is controversial. Dietary factors that potentially increase the risk of CRC include low vegetable, fruit, and fiber intake or high red meat or saturated fat consumption and excessive alcohol intake.

Histopathological characteristics of Colorectal Cancer

The most common histopathological type of CRC is adenocarcinoma. Other subtypes like mucinous adenocarcinoma, medullary carcinoma, signet ring cell, squamous cell carcinoma, and adenosqaumous carcinoma are less common. Tumor grade describes the level of cell differentiation and is usually divided into four groups; well-, moderately-, poorly- and undifferentiated. Of the different tumor grades the only demonstrated independent prognostic factor for CRC is poor differentiation (Compton et al., 2000), which is identified by decrease in tubular formation, severe cell atypia and numerous mitotic figures. Most of the colorectal adenocarcinomas are moderately differentiated. The CRC morphology is typically heterogeneous in that tumor grade tends to vary within the same tumor. There is also a considerable inter-observer variation in the interpretation of tumor grade between different pathologists. Consequently, classification of tumor grade is sometimes subjective and not always a reliable measure, which contributes to its limited clinical significance as a prognostic factor in CRC. The old Duke's classification of CRC has mainly been replaced by the TNM (Tumor, Node, and Metastasis) system initially recommended by Beart and Wood et al. (Beart et al., 1978; Wood et al., 1979). The American Joint Committee on Cancer latest edition (7^{th}) is described in detail in Table 1.

Treatment of CRC

The four main treatment modalities for CRC include surgery, radiation therapy, chemotherapy and other targeted therapies like monoclonal antibodies. The final choice

of treatment modality should always be individualized and based on the stage and location of the disease as well as the patient's age and general health condition. Most often, in the treatment of localized CRC, two or more types of treatments are used either concurrently or followed by each other.

To optimize the treatment for CRC, several disciplines should be involved. A multidisciplinary approach has been associated with decreased local recurrence rates in rectal cancer (Nicholls, 2008; Valentini et al., 2005; Wille-Jorgensen and Bulow, 2009) and in most countries it has become the gold standard for treatment. The aim of this multidisciplinary approach is to improve the preoperative staging and treatment selection by including oncologists, surgeons, radiologists and specialized nurses in the decision making progress.

Surgery

Surgery is the most important predictor that influences the patient's overall survival. The type of surgical procedure for CRC is usually tailored to the extent and location of the disease. In rectal cancer, the introduction of total mesorectal excision (TME) has significantly improved the outcome after rectal cancer surgery (Heald, 1988). Rectal cancer surgery using the TME technique, sometimes together with radiation or chemoradiation therapy (CRT), has become the recommended standard treatments for rectal cancer (1997; 2001; Group, 1997; Kapiteijn et al., 2001). The TME technique focuses on a precise and sharp dissection, under direct vision, in the avascular plane outside the mesorectum. This technique ensures the removal of an intact package of the

tumor, with its main lymphatic drainage, and central ligation of the main supplying vessels. This technique is recommended when performing surgery on tumors located in the middle or low rectum.

The three main surgical procedures performed for the treatment of rectal cancer are anterior resection with anastomosis, anterior resection without anastomosis (Hartman's procedure) and abdominoperineal resection. An anterior resection is usually recommended for tumors located in the upper rectum (11-15 cm), while tumors located in the mid or low rectum can be operated with a low anterior resection if a satisfactory tumor free distal margin can be achieved. Hartmann's procedure, a low anterior resection without an anastomosis, is usually considered in patients with a high surgical risk or in patients with poor anal sphincter function. Patients with low rectal tumors an abdominoperineal resection is usually recommended. This includes a synchronous resection of the rectum, anal canal, sphincter complex and the maturing of a permanent sigmoidostomy. In addition to these three types of surgery, there are other endoanal procedures usually only recommended for the treatment of early rectal cancer. Transanal Endoscopic Microsurgery (TEM) is one of the most commonly performed procedures for the treatment of localized rectal tumors with limited invasion into the rectal wall.

Radiotherapy

Radiotherapy can be given both before and after surgery for rectal cancer and it has in randomized clinical trials been demonstrated to prolong survival and reduce the risk of

local recurrence (2001; Glimelius et al., 2003). Furthermore, preoperative radiotherapy, as compared with postoperative, may be more effective and has been associated with fewer adverse effects (2001; Frykholm et al., 1993; Sauer et al., 2004).

One disadvantage of preoperative radiotherapy is that it relies on an accurate preoperative staging and may therefore over- or undertreat patients. Depending on the local extent of the tumor preoperative radiotherapy is usually given in two different ways: as a short or as a long course. The short course radiotherapy is given in 5 fractions of radiotherapy in one week (5 x 5 Gray) usually followed by surgery within the following 2-4 days. Long course radiotherapy is given over 5 weeks (25 x 1.8 Gray) and typically accompanied by low dose chemotherapy to sensitize the radiotherapy. Surgery after long course chemo-radiotherapy (CRT) is usually performed 6-8 weeks after the completion of CRT to allow tumor shrinkage and potential side effects to settle.

There is debate about the two different approaches to preoperative therapy, and the trend in Europe is that short course radiotherapy is more commonly used than in the US. Due to the higher likelihood of downsizing of the tumor after long course CRT, or after delayed surgery, some authors have suggested a stratified approach. After characterizing the clinical stage and the local extent of the disease rectal tumors can be divided into three main groups; the ‘good’, the ‘bad’ and the ‘ugly’ (Blomqvist and Glimelius, 2008). This group characterization will determine whether pre- or postoperative treatment is recommended (figure 1). Early stage or ‘good’ rectal cancer ($<T_{3a}N_0$), 30-40% of all rectal cancers, have a low risk of local recurrence and radiotherapy is not routinely recommended. The intermediate or ‘bad’ group, 40-60% of all rectal tumors, are $T_{3b\text{-}c}$

tumors with clear CRM and local lymph node metastasis (N_1N_2) and patients with these are offered preoperative radiotherapy since the local recurrence risk is high. Advanced or 'ugly' rectal cancers, 10-20% of all rectal cancers, are T4 tumors or T3 tumors with lymph node metastases and a CRM less than 1 mm. In these 'ugly' tumors there is a high risk of a non radical resection because the surgical planes have usually been invaded by the tumor. The recommended treatment for this group is long radio-chemotherapy (50.4 Gy in 1.8 Gy fractions combined with 5-Fluorouracil) or short radiotherapy (5x5 Gy) with delayed surgery in an effort to achieve a downstaging and ultimately a radical resection of the tumor.

Chemotherapy

The primary goal of chemotherapy is to eradicate tumor cells that already have metastasized, or, when given in addition to radiotherapy, to sensitize malignant cells and enhance the cytotoxic effect of the radiotherapy. Accordingly, in rectal cancer chemotherapy serves to achieve systemic control compared to radiotherapy that serves to obtain local control of malignant cells.

In patients curatively operated for colon cancer stage III the recurrence rate can exceed 50%. Adjuvant chemotherapy, 5-FU in combination with folinic acid (Leucovorin), has been demonstrated to reduce recurrence rates by 30% (Moertel et al., 1990) and is therefore routinely recommended. If 5-FU and Leucovorin are combined with an additional chemotherapeutic drug (capesitabin or oxaliplatin) the recurrence rate can be further reduced, but with the risk of increased side-effects (Andre et al., 2004; Twelves et

al., 2005). In patients without lymph node metastasis (stage I and II), the expected 5 year survival rates are 75% to 95% and adjuvant therapy is not recommended. However, in patients with high risk features in stage II disease (obstruction, perforation, inadequate lymph node sampling or T4 disease) adjuvant chemotherapy is usually recommended (Figueredo et al., 2008). There is a need to define the high risk features in stage II CRC patients that can be used to select patients for adjuvant therapy. Currently there is lot of research attempting to identify different molecular characteristics that can be used to stratify patients into low or high risk groups for recurrence in patients with stage II disease.

In patients with rectal cancer who have received preoperative CRT there is no direct evidence from randomized trials supporting any benefit from adjuvant chemotherapy (Bosset et al., 2006; Collette et al., 2007; Valentini et al., 2002), and therefore chemotherapy is not generally recommended as an adjuvant treatment after curative TME surgery in rectal cancer.

Pathologic Complete Response

About 15-25% of all patients receiving neoadjuvant therapy will have pathological complete response (pCR) without any detectable tumor cells in the resected specimen (Carlomagno et al., 2009; Rosenthal et al., 2008; Shivnani et al., 2007). Pathological complete response is associated with increased disease-free survival in some non-randomized studies (Maas et al., 2010; Rodel et al., 2005; Yeo et al., 2010; Zorcolo et al., 2011) and the role of adjuvant therapy in this group is still unclear.

Some authors have suggested that adjuvant chemotherapy could potentially be avoided in patients with pCR (Capirci et al., 2008). On the other hand, pCR in patients treated with neoadjuvant CRT could hypothetically be a predictor for good response to chemotherapy, indicating that patients with pCR might particularly benefit from adjuvant therapy (Collette et al., 2007). Nevertheless, when comparing the incidence rate of pCR between different neoadjuvant treatments confounding factors like volume of given radiotherapy/chemotherapy, imaging modalities used to define the preoperative stage, and the time interval between neoadjuvant therapy and surgery are always a concern and a source of bias. This is particularly true for retrospective studies.

The time interval between completion of radiation therapy and surgery has been proven to influence the degree of downstaging of the tumor in one randomized control trial (Francois et al., 1999). Another factor influencing the results is the quality of the pathology and how thoroughly i.e. how many sections of the specimen that is performed and examined by the pathologist. This will ultimately influence the likelihood of finding a specimen with pCR (Quirke et al., 2007).

Several methods of assessing tumor response to neoadjuvant CRT have been suggested. The most used method is tumor regression grade (TRG), which was described by Dworak and Mandard et al. (Dworak et al., 1997; Mandard et al., 1994). TRG estimates the local treatment efficacy of neoadjuvant CRT and has been demonstrated to be an independent prognostic factor for overall and disease free survival after preoperative CRT in patients with rectal cancer (Rodel et al., 2005; Suarez et al., 2008; Vecchio et al., 2005).

Today, the standard practice is to offer surgery after CRT regardless of how the patient responds to CRT. It has been demonstrated that downstaging of the tumor and pCR after preoperative CRT followed by definitive surgical resection for rectal cancer results in decreased recurrence and improved disease-free survival (Garcia-Aguilar et al., 2003; Valentini et al., 2002). The median follow up time in these studies averaged from 33 to 67 months. This suggests that patients with clinical complete response after neoadjuvant CRT not undergoing rectal surgery may also have a decreased recurrence rate.

The challenge is to identify which patients have complete pathological response, without removing the rectum. It is not clear whether a rectal resection will improve overall or disease free survival in patients with pCR, and there are some obvious ethical challenges in performing a randomized controlled clinical trial trying to answer this question.

A group in Brazil has been studying operative versus non-operative treatment for distal rectal cancer following CRT by using clinical complete response as a surrogate marker for pCR. Their results indicate, in stage 0 rectal cancers (clinical complete response), excellent long-term results irrespective of treatment strategy. With a median follow-up of 57.3 months the overall and disease-free survival was 100% and 92% in the observation group compared to 88% and 83% in the resection group respectively (Habr-Gama et al., 2004). The results were updated in 2005 and 2006 including three additional patients (five in total) that developed local recurrence (Habr-Gama, 2006; Habr-Gama et al., 2005).

Predictors of pCR

It is difficult to correctly identify patients with pCR after CRT, and currently we have no method to accurately exclude the presence of viable tumor cells at the primary tumor site and surrounding lymphnodes without performing surgery. Furthermore, to be able to individualize therapy it would be useful to be able to predict the response to CRT. This will potentially aid in identifying responders as well as non-responders, or even patients that progress in their disease during CRT. Consequently, identifying patients that will develop pathologic complete response after CRT could both influence the decision to undergo or avoid surgery, and possibly identify patients that will not respond to CRT. Clinically this is also important in that it might provide the clinician with information to dissuade patients from receiving CRT before surgery.

There are a few published studies evaluating clinical predictors for pathologic complete response in rectal cancer after CRT (Das et al., 2007; Kalady et al., 2009; Moreno Garcia et al., 2009; Park et al., 2009; Park et al., 2006b; Yoon et al., 2007). All these are of a retrospective nature and the largest study to date by *Das et al*., included a total of 562 patients (Das et al., 2007). In this study, the authors concluded the circumferential extent of the tumor (<60%) to be a significant predictor of pCR and the distance of the tumor from the anal verge (≤5cm) together with circumferential extent <60% were predictors of tumor downstaging. CEA level ≤2.5 ng/ml was only significantly associated with pCR in the univariate analysis.

Four other studies have confirmed the result of CEA as a predictive factor for response to CRT (Kalady et al., 2009; Moreno Garcia et al., 2009; Park et al., 2009; Park et al., 2006b; Yoon et al., 2007). In two of these studies (Kalady et al., 2009; Moreno Garcia et

al., 2009) , the same CEA cut-off level (≤2.5 ng/ml) was used as in the study by *Das et al*. In the other two studies (Park et al., 2009; Park et al., 2006b; Yoon et al., 2007), a CEA cut-off level of 5ng/ml or several cut-off levels (≤ 3 ng/ml, 3-6ng/ml, 6-9 ng/ml, > 9ng/ml) were used.

The above mentioned studies suggest that CEA can serve as a predictor of response to CRT. However, smoking is a known factor that may increase the CEA levels in serum (Fukuda et al., 1998) and a higher reference level of CEA is therefore used for smokers in clinical practice (2.5 ng/ml vs. 5 ng/ml). It has also been suggested that CEA levels increase with the number of cigarettes smoked per day (Sajid et al., 2007).

At the same time, smoking may not only elevate the CEA levels but also negatively influence the efficacy of radiochemotherapy by increasing the levels of carboxyhemoglobin resulting in tissue hypoxia and subsequent inhibition of apoptosis. This has been hypothesized as one explanation for the negative effect on outcome in patients that smoke while receiving radiochemotherapy for the treatment of anal cancer (Mai et al., 2007). Similar observations about the impact of smoking and CRT have been demonstrated in other cancer forms (Kanai et al., 2009; Videtic et al., 2003) suggesting that smokers or patients with a history of smoking might have a poor response to CRT compared to non-smokers. Subsequently, there is a concern that CEA might be a confounding factor that needs to be considered when analyzing the predictive value of CEA for response to CRT in rectal cancer.

Primary aim of the study

The primary aim of this study was to evaluate the impact of tumor size, stage, location, circumferential extent, patient characteristics and pretreatment CEA levels on development of pCR in patients treated with neoadjuvant therapy for rectal cancer.

Patients and Methods

We performed a retrospective review of medical charts and identified 530 patients with primary rectal adenocarcinoma operated with a total mesorectal excision (TME) resection without any evidence of distant metastasis and treated with preoperative CRT and radical surgery for rectal adenocarcinoma between 1998 and 2011. All the patients were operated by colorectal surgeons in the Division of Colon and Rectal Surgery, University of Minnesota, with operations performed at the University of Minnesota Medical Center, the Minneapolis Veterans Affairs Medical Center and affiliated private hospitals in Minneapolis and St. Paul, Minnesota. During the study period TME resection was the standard technique among the colorectal surgeons when performing a rectal resection. TME was defined as a sharp dissection under direct vision in the avascular embryological plane surrounding the mesorectum. Rectal cancer was defined as a histologically biopsy proven adenocarcinoma with a distal extent $\leq$ 15 cm proximal to the anal verge. A total of 469 patients remained for inclusion in the study after excluding patients with any of the following: history of pelvic radiation treatment (n=2), previous transanal endoscopic microsurgery or polypectomy of the primary lesion (n=15), concurrent malignant tumor

(n=14), and no information about pre- or post-treatment T-stage in the chart (n=30). Initial tumor stage was assessed prior to CRT by endorectal ultrasound (ERUS), MRI, CT, chest X-ray and PET-CT. All patients were evaluated with a physical exam, flexible sigmoidoscopy/colonoscopy, and proctoscopy. Tumor height and length were estimated by proctoscopy and the extent of circumferential tumor growth and tumor depth by direct measure with EAUS. If EAUS was not performed or missing measures of the tumor were obtained from MRI images or sigmoidoscopy/colonoscopy. CEA levels were determined before CRT. The study was approved by the Institutional Review Board.

Pathologic staging

Tumor grade was assessed from the initial tumor biopsy and grouped into one of three categories: well, moderate and poor differentiation. Histopathological features of signet ring cells, and mucinous component (>50%) were also documented. Perineural growth, vascular and lymphatic invasion as well as tumor-budding and infiltrating tumor border were recorded in the final pathology specimen. The maximum tumor size measured as the maximum size of viable residual tumor cells in millimeters was documented. In all patients with pCR the number of performed sections of the specimen by the pathologist was recorded. Patients with only a few clusters of viable malignant residual cells in the specimen (<1mm) were also recorded. The pathologic stage was defined according to the 7^{th} edition from the American Joint Committee on Cancer (Edge and Compton, 2010) (table 1).

Definition and rationale of endpoints

Pathologic complete response

Pathologic complete response (pCR) was defined as no presence of viable tumor cells in the rectal wall or in any of the resected lymphnodes. The presence of acellular mucin at the previous tumor site or in lymphnodes was considered as pCR. Pathologic complete response was used as the primary endpoint.

Tumor downstaging

Our secondary endpoint, tumor downstaging, was defined as decreased T-stage independent of N-downstaging. This endpoint was chosen because together with pCR it is the most commonly used endpoint when evaluating predictors for tumor response to CRT. However, T-downstaging is not always a reliable measure of tumor response to CRT since it only describes the change in the location of tumor cells within the rectal wall, which does not necessary equal a change in the actual tumor size (figure 2a and 2b).

Reduction in tumor size ≥ 75%

The reduction in tumor size ≥ 75% between the maximum pre-and post treatment diameter in the pathology specimen was used as an additional secondary endpoint. We chose 75% reduction in tumor size as a cut-off, since this is a clinically significant decrease in tumor size and this cut-off was stated *a priori*. The limitations of using this endpoint is that it only measures the tumor in one of three possible dimensions and it is dependent both on the pretreatment and pathological estimation of the tumor size.

Particularly, in a retrospective design the accuracy of the pre- and post CRT tumor measurement are limited increasing the risk of misclassification bias. However, the potential benefit of measuring tumor size reduction is – separate from tumor downstaging –that it gives an estimation of the response to CRT independent of the actual location of the residual tumor in the rectal wall.

Treatment

During the study period two different standardized protocols for the radiation treatment were implemented. For the patients receiving radiotherapy between 1998-2005 (57% of the patients), a four field technique (posterior-anterior and two lateral fields) was used to deliver 15 to 18 megavolt photons at 1.8 Gy/day for five days per week for 5-6 weeks followed by a perineal boost. Patients treated between 2006-2011 (43% of the patients) received intensity modulated radiation therapy utilizing 6 megavolt photons to limit the radiation to surrounding organs and tissues. The delivered target dose for both of these protocols was 45 Gy to the rectal tumor with a boost of 5.4 Gy limited to the mesorectum. A median radiation dose of 50.4 Gy (range, 18 to 59.4 Gy) over a mean duration of 5.6 weeks was given. Thirty nine patients (8%) also received an additional boost restricted to the tumor, receiving up to a total of 54 Gy.

Concurrent 5-fluorouracil (5-FU) based therapy was administered either as a continuous intravenous infusion during the initial five weeks of radiotherapy (72%) or as a bolus infusion (12%) or oral 5-FU (9%) throughout the radiotherapy. In 19 (4%) of the patients 5-FU was combined with oxaliplatin, irinotecan or mitomycin.

Statistical analyses

The association between categorical variables was evaluated for significance by chi-square test. Univariate and multivariate analyses were performed using student's t-test and logistic regression to identify continuous predictors for the endpoints of pCR, tumor downstaging and tumor size reduction.

A cut-off value of 5ng/mL and 8 weeks was used when evaluating the predictive value of CEA and the duration between completion of CRT and surgery respectively. The rationale for using these cut-off values were for CEA that this cut-off is used for a positive test result in smokers and has also been demonstrated in previous studies to be of prognostic value (Park et al., 2006a; Slentz et al., 1994). The duration of 8 weeks between completion of CRT and surgery has been associated with a higher rate of pCR (Kalady et al., 2009). For all analyses a p-value <0.05 was considered significant. Data were analyzed using STATISTICA software (version 7.1, StatSoft Inc, Tulsa, OK, USA).

Sample size calculation

A sample size calculation was performed for both the primary (pCR) and secondary endpoint (tumor downstaging) to detect a difference in CEA (≤ 5 or >5 ng/mL) with a power of 80% and a two sided α-level of 0.05 respectively. The estimated effect sizes were achieved for pathological complete response from a study by *Park et al* (Park et al.,

2006b) and for tumor downstaging from a study by Yoon et al (Yoon et al., 2007). The statistical software “R” was used for the computations.

Sample size calculation for pCR

No study using CEA cut-off 5ng/mL with the endpoint pCR was found. For that reason, the estimate of the incidence proportion of complete responders in patients with CEA levels ≤5ng/ml and > 5ng/ml were based on a previous report by *Park et al.* using the endpoints of “good responders” (complete or near complete response) and “poor responders” (partial or no response). The proportion of patients in each group was assumed to be equal.

The estimated total number of patients needed in the study to demonstrate a difference in pCR and tumor downstaging between patients with CEA levels ≤5ng/ml and > 5ng/ml were 266 and 78 patients respectively (table 2).

Results

A total of 309 (66%) men and 160 (34%) women were included in the study. The mean age was 59 years (SD, ±12 years). The most commonly performed pretreatment staging method was endorectal ultrasound (90%). The mean duration between completion of CRT and surgery was 8.7 weeks (SD, ± 7.4 weeks) and the mean radiation dose given was 50 Gray. CEA levels were only available for 267 patients (57%) of the patients included in the study (table 3).

Ninety-six patients (20%) had pCR, 84 patients (18%) had only microscopic residual disease in the pathology specimen and 265 patients (59%) and 181 patients (40%) had a lower T and N-stage after CRT respectively (table 4).

Predictors for pCR

Patients with and without pCR were similar in terms of age, gender, duration between CRT and surgery, circular extent of tumor growth, duration between completion of CRT and surgery, smoking status and pretreatment T-and N-stage. Small pretreatment tumor size of 4.2cm (95% C.I 3.9-4.5 cm) vs. 4.7 cm (95% C.I 4.5-4.9 cm) and a low CEA level 3.4 ng/ml (95% C.I 2.4-4.4 ng/mL) vs. 9.6 ng/mL (95% C.I 7.3-11.9 ng/ml) were significant predictors for pCR. There was a non-significant trend of more patients who interrupted or had a break in their chemotherapy in the group who did not develop pCR (p=0.05) (table 5).

When analyzing CEA as a categorical variable using the cut-off level 5 ng/ml the Odds Ratio (OR) for pCR was 0.86 (95% C.I 0.77-0.97) (table 6a), this was still significant in the multivariate analyses adjusting for break/interruption in CRT and tumor size OR 0.87 (95% C.I 0.76-0.98) (table 6b).

When stratifying for smoking status low pretreatment CEA levels were associated with pCR in non-smokers 2.9 ng/mL (95% C.I 2.0-3.8 ng/mL) vs. 8.3 ng/mL (95% C.I 5.8-10.4 ng/mL) (p=0.022) but not in smokers 7.0 ng/mL (95% C.I 1.9-12.0 ng/mL) vs. 14.8 ng/mL (95% C.I 8.9-20.6 ng/mL) (p=0.31) (Figure 3a and 3b).

Predictors for tumor downstaging and reduction in tumor size

The total number of patients with available data for the estimation of tumor downstaging and tumor size reduction ≥ 75% was 468 and 408 respectively. Pretreatment T-stage and tumor size was significantly associated with tumor downstaging ($p<0.001$ and $p=0.03$) whereas no predictors were demonstrated to be significantly associated with tumor size reduction ≥ 75% (table 7and 8).

Discussion

In our study, low levels of CEA demonstrated to be a predictor of pCR in patients diagnosed with rectal cancer receiving neoadjuvant CRT. This result is consistent with previous studies where CEA has been proven, independent of other clinicopathologic features, to be associated with pCR after CRT (Moreno Garcia et al., 2009; Park et al., 2009; Park et al., 2006b; Yoon et al., 2007). In a study by *Das et al*. including 562 patients where the total number of patients with available CEA values was not specified, pretreatment CEA was only significantly associated with pCR in the univariate analysis. Different from these studies, we also analyzed the influence of smoking status on the predictive value of CEA and confirmed, after stratifying for smoking, that CEA was significantly associated with pCR in patients that stated they did not smoke but not in patients who declared they were smokers. This result might reflect a true difference of the predictive value of CEA between smokers and non-smokers but could also be a consequence of a type II error because of the small number of smokers included in our analysis. Another study by Perez et al. (Perez et al., 2009) did account for smoking in their analyses of CEA as a predictor of response to CRT and demonstrated a correlation between post-chemotherapy CEA levels and complete response but not for pre-treatment CEA levels. This study included patients with pathological complete response as well as clinical complete response and given the relatively short follow up time (median, 38 months), late recurrences could have been missed leading to an overestimation of the rate of pathologic complete response in the group with clinical complete response.

In our study, a smaller pretreatment tumor size was a predictor for pCR in the univariate analysis, but when adjusting for pretreatment CEA this was no longer significant. Regardless, the clinical significance of this finding is questionable since the difference in mean tumor size between patients with and without complete response was only 0.5 centimeters, which is too small to be useful as a predictive marker in a clinical setting. When analyzing predictors for tumor downstaging only pretreatment tumor size and T-stage was significantly associated with tumor downstaging. Even though our study was adequately powered for detecting the predictive value of pretreatment CEA for tumor downstaging, this was not significant. These results are different from other studies that demonstrate a value of CEA as a predictor for tumor downstaging (Das et al., 2007; Yoon et al., 2007). Arguably, tumor downstaging is not an accurate method for measuring tumor shrinkage as response to CRT since it basically describes the change of the location of tumor cells within the rectal wall which is not necessarily equal to the change of the actual tumor size.

We did not find any predictors in our study for reduction in tumor size. There are no studies that specifically use this endpoint in the assessment of predictors for response to CRT in rectal cancer, and there are two obvious disadvantages to using this method: 1) it only measures the tumor in one of three possible dimensions; 2) it is dependent on the accuracy of the pretreatment imaging modality and the pathological assessment of the tumor size which might be difficult to assess after CRT. The reason we choose to include reduction in tumor size as a secondary endpoint was that in some respects it gives an estimate of the response to CRT independent on the actual location of the residual tumor in the rectal wall compared to using tumor downstaging.

One limitation in our study was that in the pathology report, tumor regression grade (TRG) was not assessed. TRG has been shown to be a predictor of disease-free survival after preoperative therapy in patients with rectal cancer (Vecchio et al., 2005). Since TRG is independent of the preoperative staging method, it hypothetically provides a more accurate measure of the tumor response to CRT. Other limitations in our study are missing CEA values in a substantial proportion of the patients and the possible miss-classification of smoking status. We were only able to obtain the CEA value from 267

(57%) patients of the included in the study thus potentially introducing a selection bias. However, we could not demonstrate (data not shown) any significant association between the endpoints (pCR, downsizing or downstaging) or any other of the important preoperative variables (T-stage, tumor size, circumferential growth) with whether CEA was available from the medical record charts or not. This does not exclude the possibility of selection bias yet supports the theory that patients with missing CEA values are not different from those with available CEA values in our study.

In conclusion we demonstrated an association between low pretreatment CEA levels and pCR in non-smoking patients treated with neoadjuvant CRT for locally advanced rectal cancer. The predictive value of CEA in smokers can be limited and further studies are needed to evaluate the impact of smoking on the predictive value of CEA for pCR in rectal cancer.

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Appendix

Stage	TNM Classification	Five-Year Survival
I	T1-2, N0,M0	>90%
IIA	T3, N0, M0	60-85%
IIB	T4, N0, M0	
IIIA	T1-2, N1, N0	15-65%
IIIB	T3-T4, N1, M0	
IIIC	T (any), N2, M0	
IV	T (any), N (any), M1	5-7%

Primary tumor (T)	
Tx	Primary tumor cannot be assessed
Tis	Carcinoma in situ
T1	Tumor invades submucosa
T2	Tumor invades muscularis propria
T3	Tumor penetrates muscularis propria and invades subserosa
T4	Tumor directly invades other organs or structures or perforates visceral peritoneum
T4a	Perforates visceral peritoneum
T4b	Directly invades other organ or structures

Nodal status (N)	
Nx	Regional lymph nodes cannot be assessed
N0	No metastases in regional lymph nodes
N1a	Metastases in one regional lymph nodes
N1b	Metastases in 2-3 regional lymph nodes
N1c	Satellites in subserosa, without regional lymph nodes
N2	Metastasis in 4 or more regional lymph nodes
N2a	4-6 lymph nodes
N2b	7 or more lymph nodes

Distant metastases(M)	
Mx	Presence or absence of distant metastases cannot be determined
M0	No distant metastases
M1a	Distant metastases in one organ
	Distant metastases in more than one organ or peritoneum

Table 1.TNM staging American Committee on Cancer 7th edition

Sample size calculation for pCR	**Sample size calculation for tumor downstaging**
R-code: >power.prop.test(power=.80,p1=.24,p2=.11) Two-sample comparison of proportions power calculation n = 132.9193 p1 = 0.24 p2=0.11 sig.level = 0.05 power = 0.8 alternative = two.sided	R-code: >power.prop.test(power=.80,p1=.58,p2=.27) Two-sample comparison of proportions power calculation n = 38.71794 p1 = 0.58 p2=0.27 sig.level = 0.05 power = 0.8 alternative = two.sided
Total number needed 2n=266 patients	Total number needed 2n=78 patients

Table 2. Sample size calculation for pCR and tumor downstaging.

		Total number of patients (%)
Mean age (y) ±SD		59 ±12
	Men	309 (66)
	Women	160 (34)
Pre treatment T-stage	T1	1 (0.02)
	T2	55 (12)
	T3	381 (81)
	T4	32 (7)
Pre treatment N-stage	N0	190 (41)
	N1	260 (55)
	N2	19 (4)
Percent of circumferential extent	Mean % ±SD	60 ±25
Pretreatment mean height from the anal verge	Mean in cm ±SD	6.7 ±3
Mean CEA level (ng/mL) ±SD		8.3 ±15.5
Smoker	Yes	109 (23)
	No	360(77)
Tumor differentiation	Poor	47 (10)
	Moderate	381 (81)
	High	37 (8)
	Mucinous	46 (10%)
	Signet rings features	8 (2%)
Type of surgery	Low Anterior Resection	280 (60%)
	Abdominoperineal Resection	171 (36%)
	Hartmann's operation	16 (3%)
	Total procto colectomy	2 (0.04%)
Staging Method	Endorectal ultrasound	423 (90%)
	Pelvic CT scan	24 (5%)
	Pelvic MRI	46 (10%)
	PET CT	5 (1%)
Mean duration between completion of CRT and surgery (number of weeks)		8.7 ±7.4
Average radiation dose (Gy) ±SD		50±3

Table 3. Characteristics of the study population

Tumor response to CRT	n
Pathologic complete response	96 (20%)
Microscopic residual disease	84 (18%)
Lower T-stage	265 (59%)
Lower N-stage	181 (40%)
Mean pre-treatment tumor size in cm (±SD)	4.6 ±1.9
Mean post-treatment tumor size in cm (±SD)	1.8 ±1.8

Table 4. Tumor response to CRT defined by the endpoints pathologic complete response, tumor downstaging, and tumor size reduction.

		Complete response (%) [95% C.I] (n=96)	No complete response (%) [95% C.I] (n=373)	P
Gender	Male	64 (67%)	245 (66%)	0.86
	Female	32(33%)	128 (34%)	
Age (y)		58 [56-60]	59 [58-60]	0.70
Pretreatment CEA ng/ml		3.4 [2.4-4.4]	9.6 [7.3-11.9]	0.008
Smoker	Yes	20 (21%)	89 (24%)	0.53
	No	76 (79%)	284 (76%)	
Pretreatment tumor height (cm)		6.9 [6.3-7.5]	6.7 [6.4-7.0]	0.41
Percent of circumferential extent		56 [51-60]	61 [58-64]	0.09
Pretreatment tumor size (cm)		4.2 [3.9-4.5]	4.7 [4.5-4.9]	0.02
Duration CRT-surgery (weeks)		8.3 [7.3-9.3]	8.8 [8.0-9.6]	0.58
Pre treatment T-stage	1	0 (0%)	1 (0.3%)	0.57
	2	15 (16%)	40 (11%)	
	3	75 (78%)	306 (82%)	
	4	6 (6%)	26 (7%)	
Pre treatment N-stage	0	40 (42%)	150 (40%)	0.76
	1	51 (53%)	209 (56%)	
	2	5 (5%)	14 (4%)	
Radiation dose (cGy)	≥5040	76 (79%)	287 (77%)	0.43
	<5040	9 (9%)	46 (12%)	
	Missing data	11 (12%)	40 (11%)	
Chemotherapy	I.V continuous 5-FU	64 (66%)	277 (74%)	0.60
	I.V bolus 5-FU	10 (11%)	45 (12%)	
	Oral 5-FU (Xeloda)	10 (11%)	30 (8%)	
	5-FU + oxaliplatin	5 (5%)	12 (3%)	
	5FU+irinotecan	1 (1%)	0 (0%)	
	5FU+mitomycin	1 (1%)	0 (0%)	
	Missing data	5 (5%)	9 (3%)	
Interrupted or break in CRT	Yes	5 (5%)	45 (12%)	0.05
	No	91 (95%)	328 (88%)	

Table 5. Predictive factors of pCR.

		Complete response (%)	No complete response (%)	p-value	OR [95% CI]
Pre treatment CEA	**≤ 5 ng/mL**	45 (47%)	134 (36%)	0.017	0.86 [0.77-0.97]
	>5 ng/mL	11 (11%)	77 (21%)		
	Missing	40 (42%)	162 (43%)		
Smoking	**Yes**	20 (21%)	89 (24%)	0.53	0.97 [0.88-1.07]
	No	76 (79%)	284(76%)		
Pretreatment tumor size	**≤ 5cm**	73 (76%)	256 (69%)	0.10	0.92 [0.83-1.02]
	>5cm	17 (17%)	96 (26%)		
	Missing	6 (7%)	21 (5%)		
Interrupted or break in CRT	**Yes**	5 (5%)	45 (12%)	0.05	1.01 [0.90-1.13]
	No	91(95%)	328(88%)		

Table 6a.

Multivariate analysis		**Complete response**	**No complete response**	**p-value**	**OR [95% CI]**
Pre treatment CEA	**≤ 5 ng/mL**	42 (26%)	121 (74%)	0.03	0.87 [0.76-0.98]
	>5 ng/mL	10 (12%)	73 (88%)		
Pretreatment tumor size	**≤ 5cm**	43(83%)	147 (76%)	0.43	0.95 [0.84-1.08]
	>5cm	9 (17%)	47 (24%)		
Interrupted or break in CRT	**Yes**	5 (9%)	24 (12%)	0.49	0.96 [0.85-1.08]
	No	47 (91%)	170 (88%)		

Table 6b

Table 6.Uni- (6a) and multivariate analyses (6b) of predictors of pCR. In the multivariate analysis adjustment for break/interruption in CRT, pre-treatment CEA and tumor size are performed. A total of 246 patients were available for the multivariate analysis.

		Tumor downstaging (%) [95% C.I]	No tumor downstaging (%) [95% C.I]	P
Age (years)		59 [57-61]	59 [57-60]	0.90
Pretreatment CEA (ng/ml)		7.3 [4.8-9.7]	10.0 [7.1-12.9]	0.41
Smoker	Yes	58 (21%)	51 (27%)	0.14
	No	220 (79%)	139 (73%)	
Pretreatment tumor height (cm)		6.6 [6.3-6.9]	6.9 [6.5-7.4]	0.22
Percent of circumferential extent		59 [55-62]	63 [57-69]	0.08
Pre-treatment tumor size (cm)		4.5 [4.0-4.9]	4.9 [3.0-6.7]	0.03
Duration CRT-op (weeks)		9.1 [0.7-17.4]	8.3 [4.8-11.7]	0.24
Gender	Male	187 (67%)	121 (64%)	0.45
	Female	91 (33%)	69 (36%)	
Pre treatment T-stage	1	0 (0%)	1 (1%)	0.00001
	2	22(8%)	33 (17%)	
	3	226(81%)	155 (81%)	
	4	30 (11%)	2 (1%)	
Pre treatment N-stage	0	114 (41%)	76 (40%)	0.38
	1	149 (54%)	110(57%)	
	2	14 (5%)	5 (3%)	

Table 7. Predictive factors for tumor downstaging. The total number of patients with available data for tumor downstaging n=468.

		Tumor size reduction ≥75% (%)[95% C.I] n=184	Tumor size reduction <75% (%)[95% C.I] n=224	P
Age (years)		57 [49-66]	59 [56-63]	0.14
Pretreatment CEA (ng/ml)		6.6 [2.8-10.4	9.9 [0-22.8]	0.11
Smoker	Yes	40 (22%)	52 (23%)	0.72
	No	144 (78%)	172 (77%)	
Pretreatment tumor height (cm)		6.9 [5.0-8.9]	6.6 [4.3-8.9]	0.27
Percent of circumferential extent		58 [38-78]	61 [55-68]	0.18
Pre-treatment tumor size (cm)		4.5 [4.0-5.0]	4.7 [2.9-6.5]	0.16
Duration CRT-op (weeks)		8.8 [1.5-16]	9.0 [3.4-15]	0.76
Gender	Male	118 (64%)	144 (64%)	0.97
	Female	66 (36%)	80 (36%)	
Pre treatment T-stage	1	1 (1%)	0 (0%)	0.19
	2	27 (14%)	22 (10%)	
	3	147 (80%)	184 (82%)	
	4	9 (5%)	18 (8%)	
Pre treatment N-stage	0	70 (38%)	98 (44%)	0.08
	1	101(55%)	120 (53%)	
	2	13 (7%)	6 (3%)	

Table 8. Predictive factors for tumor size reduction ≥75%. The total number of patients with available data for tumor size reduction ≥75%/<75% n=408

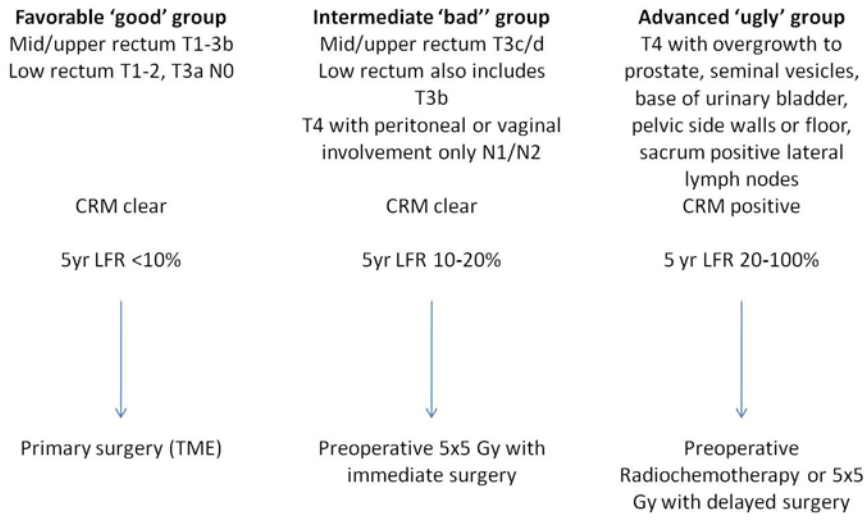

Figure 1.Diagram of a stratified approach for treating rectal cancer. *From L.Blomqvist and B. Glimelius (Blomqvist and Glimelius, 2008)*

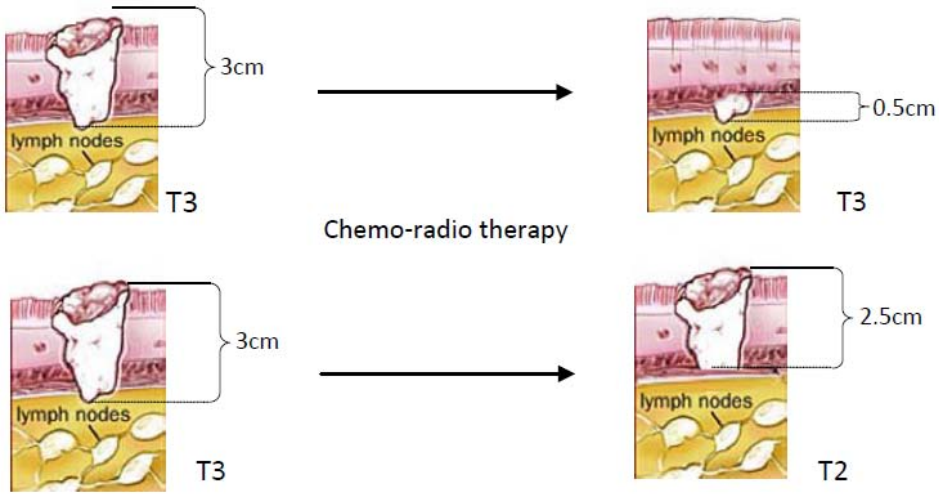

Figure 2a and 2b.
Schematic illustration of the potential risk of misclassifying tumor response to CRT when using tumor downstaging as an endpoint.
2a) “Poor” response according to T downstaging (T3 to T3), but good tumor shrinkage (3cm to 0.5 cm). 2b) “Good” response according to T downstaging (T3 to T2), but poor tumor shrinkage (3cm to 2.5 cm)

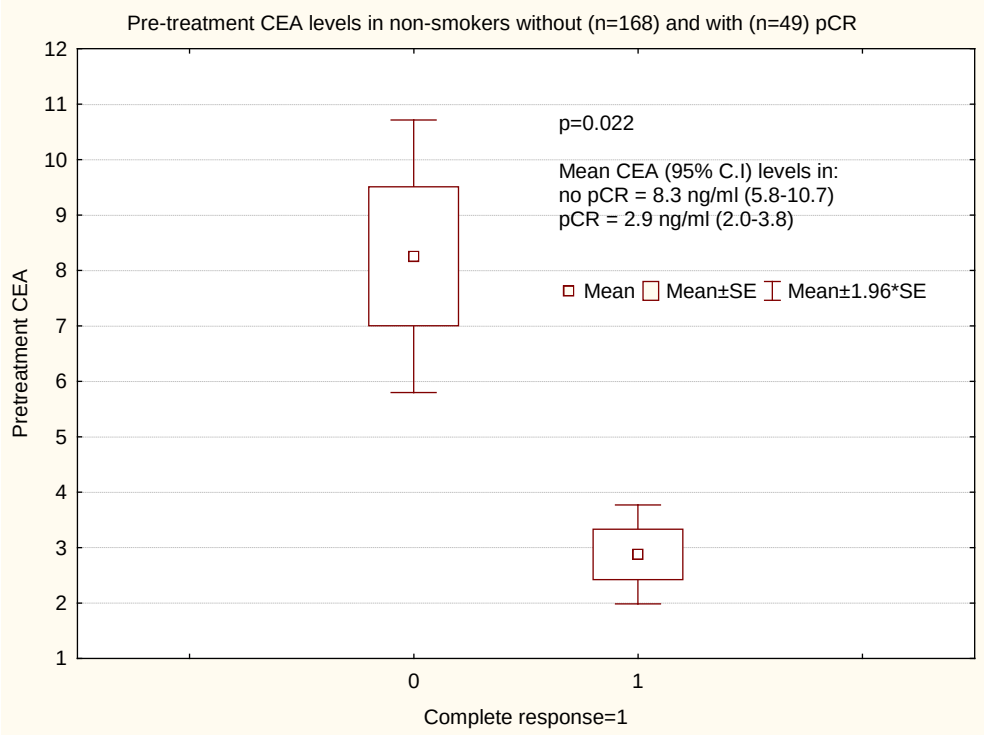

Figure 3a

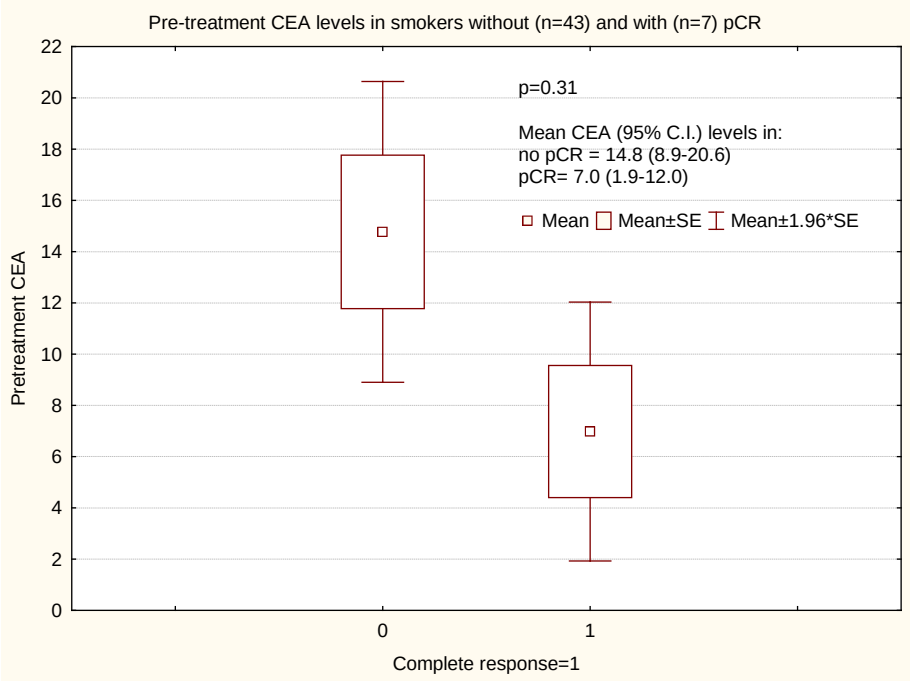

Figure 3b

Figure 3. Pretreatment CEA levels in patients with and without pCR stratified by smoking status, non-smokers (3a) smokers (3b). Circles are outliers with values between 1.5 and 3 box lengths from the upper/lower edge of the box plot. Extreme values (> 3 box lengths from the upper edge of the box plot) are not shown in the graph but included in the analy