Virchows Arch (2015) 466:517–523
DOI 10.1007/s00428-015-1723-x

ORIGINAL ARTICLE

Prognostic value of tumor shrinkage versus fragmentation following radiochemotherapy and surgery for rectal cancer

Monirath Hav · Louis Libbrecht · Karen Geboes · Liesbeth Ferdinande · Tom Boterberg · Wim Ceelen · Piet Pattyn · Claude Cuvelier

Received: 27 June 2014 / Revised: 4 January 2015 / Accepted: 20 January 2015 / Published online: 19 February 2015

Abstract Most patients with rectal cancer receive neoadjuvant radiochemotherapy (RCT), causing a variable decrease in tumor mass. We evaluated the prognostic impact of pathologic parameters reflecting tumor response to RCT, either directly or indirectly. Seventy-six rectal cancer patients receiving neoadjuvant RCT between 2006 and 2009 were included. We studied the association between disease-free survival (DFS) and the "classical" clinicopathologic features as well as tumor deposits, circumferential resection margin (CRM), Dworak regression grade, and tumor and nodal downstaging. Patients with tumor downstaging had a longer DFS (p=0.05), indicating a more favorable prognosis when regression was accompanied by a decrease in tumor infiltrative depth, referred to as tumor shrinkage. Moreover, tumor downstaging was significantly associated with larger CRM and nodal downstaging (p=0.02), suggesting that shrinkage of the primary tumor was associated with a decreased nodal tumor load. Higher Dworak grade did not correlate with tumor downstaging, nor with higher CRM or prolonged DFS. This implies that tumor mass decrease was sometimes due to fragmentation rather than shrinkage of the primary tumor. Lastly, the presence of tumor deposits was clearly associated with reduced DFS (p=0.01). Assessment of tumor shrinkage after RCT via tumor downstaging and CRM is a good way of predicting DFS in rectal cancer, and shrinkage of the primary tumor is associated with a decreased nodal tumor load. Assessing regression based on the amount of tumor in relation to stromal fibrosis does not accurately discern tumor fragmentation from tumor shrinkage, which is most likely the reason why Dworak grade had less prognostic relevance.

Keywords Rectal cancer · Neoadjuvant · Chemoradiation · Shrinkage · Fragmentation · Tumor response

M. Hav (✉)
Department of Pathology, Calmette Hospital, #3, Monivong Boulevard, Phnom Penh, Cambodia
e-mail: hav_monirath@yahoo.com

M. Hav · L. Libbrecht · L. Ferdinande · C. Cuvelier
Department of Pathology, Ghent University Hospital, Ghent, Belgium

K. Geboes
Department of Gastrointestinal Oncology, Ghent University Hospital, Ghent, Belgium

T. Boterberg
Department of Radiation Oncology, Ghent University Hospital, Ghent, Belgium

W. Ceelen · P. Pattyn
Department of Gastrointestinal Surgery, Ghent University Hospital, Ghent, Belgium

Introduction

Evidence exists from literature that preoperative radiation with or without chemotherapy followed by total mesorectal excision is the standard treatment for patients with locally advanced rectal cancer [1–3]. Decrease in tumor burden after radiochemotherapy (RCT) regimen ranges from absence of any treatment effect to a pathologic complete response (pCR). Various systems have been developed specifically for the assessment of residual tumor burden, and they can be classified into two main categories, namely stage based and cellular based. The former focuses on the stage shift in the treated specimen and includes tumor (T) and nodal (N) downstaging, while the latter is based on the amount of residual viable tumor in relation to remodeling fibrous tissue in the rectal wall [4–12]. A number of studies have consistently demonstrated the prognostic relevance of downstaging,

whereas that of cellular-response grading has been an issue of much discussion [4, 7–9, 12–15]. Besides the abovementioned grading methods, other features such as circumferential resection margin (CRM) and extramural tumor deposit (TD) may indirectly reflect tumor response to RCT. The CRM status, i.e., positive or negative, has repeatedly been demonstrated to be the best predictor for local recurrence and disease-free survival (DFS) after preoperative RCT and surgery, and one would expect that a considerable decrease in tumor burden would be associated with a larger CRM [16–18]. Although the association of TD with unfavorable prognosis has been confirmed in many colorectal cancer studies, none of them looked at the prognostic value of TD after preoperative RCT [19–23]. Nagtegaal and Quirke [24] and Quirke et al. [25] regarded the presence of residual "microfoci" or tumor deposits as a good response to preoperative RCT; they speculated that the presence of TDs in this setting might have resulted from fragmentation of the advanced primary tumor (cT≥3), creating separate tumor nodules of various sizes and shapes in the mesorectum. To date, it remains unclear which pathologic features associated with tumor load constitute the most reliable predictors of response to neoadjuvant therapy [17, 26–28].

The aim of this study was to evaluate both the prognostic impact of and the associations among all pathologic parameters reflecting residual tumor burden after preoperative RCT, either directly or indirectly. To the best of our knowledge, this is the first study which examines the prognostic value of all these parameters in a neoadjuvant setting within one study population.

Materials and methods

Patients

This study consisted of 76 consecutive patients with locally advanced (cT3–4 or/and cN+), histologically proven adenocarcinoma of the rectum registered between November 2005 and August 2009 at the Ghent University Hospital. Patients with tumors limited to the wall (cT2) of the distal third of the rectum, i.e., lower margin<6 cm from the anal verge, were also included in the study. No patient had metastatic disease at the time of surgery. Rectal tumors were defined as those of which the distal edge was seen at 15 cm or less from the anal verge. Preoperative staging of the tumors was done using endoscopic ultrasound, CT, and/or MRI.

Preoperative chemoradiation therapy regimen

Of the 76 patients who all received preoperative radiotherapy, 72 also received preoperative chemotherapy. The radiotherapy regimen consisted of 45 Gy in 25 fractions of 1.8 Gy with 18 or 25 MV photons. Patients were treated once daily, 5 days a week for 5 weeks. Three patients with a T3N0 tumor higher than 7 cm from the anal verge received 39 Gy in 13 fractions of 3 Gy, without chemotherapy. One patient received 25 Gy in five consecutive fractions of 5 Gy for a T3N0 tumor. Concerning chemotherapy, 68 of the 72 patients received 5-fluorouracil (5FU) either as a bolus injection in week 1 and week 5 of the radiotherapy or as a continuous infusion, and the remaining four patients were included in a study protocol in which they received cetuximab in combination with radiotherapy or 5FU and oxaliplatin prior to chemoradiotherapy with 5FU.

Surgery

Rectal excision was performed between 6 and 8 weeks after the end of preoperative RCT. The inferior mesenteric vessels were divided at 1 cm from their origin, and the splenic colonic flexure was routinely mobilized. The rectum was defined as follows: low rectum (0 to 5 cm from the anal verge), mid rectum (5 to 10 cm), and high rectum (10 to 15 cm). In mid and lower third tumors, a nerve sparing total mesorectal excision was performed (TME) consisting of sharp dissection between the visceral and parietal layers of the mesorectal envelope up to the level of the pelvic floor musculature [29]. Depending on invasion or proximity of the sphincter apparatus, a rectal amputation, a circular end-to-end stapled anastomosis, or a pull through with colo-anal, anastomosis was performed.

Pathologic assessment

Macroscopic examination

Unopened, unpinned, fresh specimens were sent to the Pathology Department of Ghent University Hospital for examination following the guidelines by Quirke et al. [30]. The quality of the mesorectum was assessed. Then, the non-peritonealized areas of the specimens were inked, and the specimens were measured and cut open along the anterior aspect from the top, leaving the bowel intact at a level just above the peritoneal reflection. After placing loose, formalin-soaked gauze wicks into the unopened segment of the rectum, we left the specimens in formalin for at least 72 h. After fixation, the unopened segment was sliced transversely at 4 to 5 mm intervals in order to identify the area of deepest invasion. Suspicious lesions were sampled in five blocks, one of which included the transition zone from the tumor to normal rectal mucosa, and the other contained the deepest invasion of the tumor where it was closest to the CRM. When no obvious tumor was found grossly, the entire scarred area was embedded. Finally, a rigorous search of regional lymph nodes and other suspicious mesorectal nodules was performed.

Microscopic examination

Histologic sections were reviewed simultaneously by two observers (MH and LL). Histopathologic assessments included ypTNM stage according to the 5th edition of the tumor node metastasis (TNM) classification system [31], tumor differentiation grade according to the WHO classification [32], CRM according to Quirke [30], and lymphovascular (LVI) and perineural invasion (PNI). We did not take into account acellular mucin pools in assessing ypT stage. In other words, when no viable tumor cells but only acellular mucin pools were found after rigorous microscopic examination, the cases were classified as ypT0. The CRM was measured in millimeters from the outermost part of the tumor to the lateral resection margin. The evaluation of extramural tumor deposits was done in two separate ways because of the disparity among TNM5 [31], TNM6, and TNM7 [33] regarding their classification. Quirke recently criticized the changes in TD classification and suggested that the new classifications were not better than the ones used in the TNM5, particularly in terms of reproducibility [34]. In this study, TDs were defined as groups of extramural tumor cells which, regardless of size and shape, were discontinuous from the main tumor mass and not organized in recognized lymph node structures (i.e., presence of lymphoid follicles or subcapsular sinuses) or vascular spaces. The first method of TD evaluation in this series was based on the 3-mm rule proposed in the TNM5 [35]. That is, all extramural discontinuous tumor nodules from the primary tumor were included in the ypT category if they measured ≤3 mm, while those measuring >3 mm were considered as invaded lymph nodes (ypN category). In the second method, TDs were included neither in the ypT nor in the ypN categories, and their presence, size, and number were recorded separately. Lymph node ratio (LNR) was also calculated and defined as the ratio of metastatic to totally examined lymph nodes. T downstaging was defined as ypT<cT, and N downstaging was recorded when cN+ became ypN0. Cases clinically staged as N0 were not included in the assessment of N downstaging. The degree of tumor regression was recorded based on Dworak's regression system as follows [7]:

- Grade 0: no regression
- Grade 1: dominant tumor mass with obvious fibrosis
- Grade 2: dominantly fibrotic changes with a few tumor cells or groups (easy to find)
- Grade 3: very few (difficult to find microscopically) tumor cells in fibrotic tissue with or without mucous substance
- Grade 4: no viable tumor cells (complete response)

Statistical analysis

The correlation of selected clinical and pathological variables with disease-free survival was calculated using univariate Cox regression analysis, calculating the hazard ratio, and associated 95 % confidence intervals. Variables analyzed were as follows: cT, cN, ypT (tumor limited to the rectum wall, ypT0–2 versus advanced tumor stage, ypT3–4), ypN, differentiation grade (good and moderate versus poor and mucinous), PNI, LVI, CRM, T downstaging, N downstaging, LNR, TDs, and Dworak regression grading. Continuous variables were expressed as mean±SD. To determine the best cutoff value for CRM, the p value from a log-rank test comparing CRM< cutoff with CRM≥cutoff was calculated for every possible cutoff value between 1 and 5 mm. StatView 5.0.1 statistical software system (SAS Institute, Inc., Cary, NC) was used for all analyses. Differences were considered statistically significant at $p<0.05$.

Results

Clinicopathologic findings

The cohort consisted of 54 (71 %) male and 22 (29 %) female patients with a mean age of 61±12. The pathologic characteristics of the 76 patients are summarized in the table. Seventy-two (95 %) patients received concomitant chemotherapy in addition to preoperative radiotherapy. The preoperative RCT induced T downstaging in 46 (61 %) of the 76 patients and N downstaging in 40 (71 %) of the 57 cN+ patients. The mean number of lymph nodes retrieved was 8±5. CRM assessment was not feasible in five cases, all of which had tumor above the peritoneal reflection. During a median follow-up period of 20 months (range, 1–42 months), 19 (25 %) patients developed relapse, with 15 cases of distant metastatic disease only, 1 case of local recurrence with synchronous distant metastasis, and 2 cases of local recurrence only. Six patients died of the disease. Because local recurrence and rectal cancer-related death were rare, it was not possible to study their association with pathologic variables.

Correlation of clinicopathologic features with survival

Clinicopathologic parameters and their correlation with DFS are detailed in Table 1. An increase in CRM correlated significantly with prolonged DFS ($p=0.03$), with 4 mm as the best cutoff value in predicting distant metastasis ($p=0.01$). Although not statistically significant, patients with advanced pathologic tumor stage (ypT3–4) or regional lymph node metastasis had a shorter DFS ($p=0.09$ and $p=0.08$, respectively). Furthermore, assessment of LNR showed prognostic relevance, with lower ratio observed in cases with longer DFS ($p=0.03$). Patients with T downstaging had a longer DFS ($p=0.05$, Fig. 1a). On the other hand, Dworak regression grade, even after grouped into responders (Dworak grades

Table 1 Clinicopathologic characteristics of the tumors in the study cohort and their correlation with disease-free survival

Characteristics	*n* (%)	HR	CI		*p*	Characteristics	*n* (%)	HR	CI		*p*
			LL	UL					LL	UL	
Clinical stage	76					PNI	59				
cT2	15 (19.7)	0.72	0.16	3.20	0.66	Present	4 (6.8)	2.78	0.78	9.87	0.11
cT3	57 (75.0)	1.00				Absent	55 (92.2)	1.00			
cT4	4 (5.3)	2.70	0.60	12.3	0.19						
Pathologic T stage	76					Clinical N stage	76				
ypT0–ypT2	49.(64.4)	1.00	–	–	–	cN0	19 (25.0)	1.00	–	–	–
ypT3–ypT4	27 (35.6)	2.37	0.86	6.55	0.09	cN+	57 (75.0)	1.33	0.38	4.66	0.66
Pathologic T stage	76					Dworak regression grade	76				
(TD≤3 mm as ypT3)						Dworak 0	–				
ypT0–ypT2	46 (60.5)	1.00	–	–	–	Dworak 1	3 (3.9)	0.90	0.12	7.04	0.92
ypT3–ypT4	30 (39.5)	2.89	1.006	8.36	0.049	Dworak 2	45 (59.2)	1.00	–	–	–
						Dworak 3	11 (14.5)	1.24	0.34	4.48	0.74
						Dworak 4	17 (22.4)	0.21	0.03	1.6	0.13
T downstaging	76					Dworak regression grade	76				
Present	46.(60.5)	1.00	–	–	–	Dworak 0–2 (nonresponders)	48 (63.15)	1.00	–	–	–
Absent	30 (39.5)	2.88	1.00	8.30	0.05	Dworak 3–4 (responders)	28 (36.85)	0.56	0.18	1.72	0.33
Pathologic N stage	76					Differentiation	59				
ypN0	56 (73.7)	1.00	–	–	–	Good and moderate	47 (79.7)	1.00	–	–	–
ypN+	20 (26.3)	2.40	0.89	6.42	0.08	Poor (including mucinous)	12 (20.3)	1.005	0.28	3.56	0.94
Pathologic N stage	76					CRM cutoff	59				
(TD>3 mm as ypN)						<4 mm	14 (23.7)	3.56	1.28	9.91	0.015
ypN0	54.(71.1)	1.00	–	–	–	≥4 mm	45 (76.3)	1.00	–	–	–
ypN+	22 (28.9)	3.04	1.13	8.18	0.028						
Mean LNR±SD	0.08±0.19	8.70	1.17	64.6	0.034	Mean CRM±SD	8.5±6.2	0.87	0.77	0.99	0.036
LVI	59					TDs	76				
Present	5 (8.5)	0.54	0.07	4.12	0.55	Present	10 (13.2)	4.07	1.39	11.95	0.011
Absent	54 (91.5)	1.00				Absent	66 (86.8)	1.00	–	–	–

HR hazard ratio, *CI* 95 % confidence interval, *LL* lower limit, *LNR* lymph node ratio, *CRM* circumferential resection margin, *LVI* lymphovascular invasion, *PNI* perineural invasion, *TDs* tumor deposits

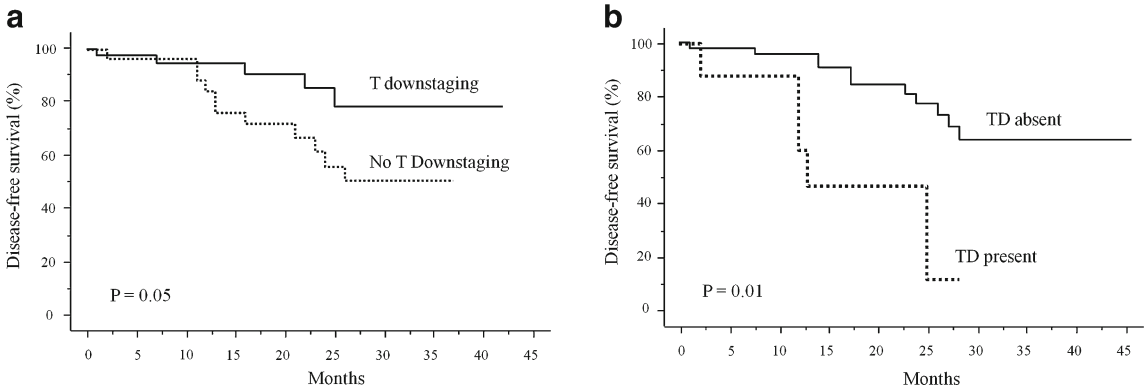

Fig. 1 Actuarial survival curves of tumor downstaging (**a**) and tumor deposits (**b**) and their influence on disease-free survival

3–4) and nonresponders (Dworak grades 0–2), showed no prognostic value in our study (p=0.33). The presence of TDs was associated with unfavorable prognosis (p=0.01, Fig. 1b).

Correlation of T downstaging with other tumor response grading parameters

Given the significant impact of T downstaging on DFS, the association of the former with the other tumor response grading parameters was further analyzed. T downstaging was significantly associated with N downstaging (p=0.02), large CRM (p=0.02), and lower LNR (p=0.04) but not with higher Dworak grade (0-1-2 versus 3). Moreover, higher Dworak grade was not associated with an increase in CRM (p=0.88).

Discussion

The standard treatment of locally advanced rectal cancer is TME following preoperative RCT, the latter resulting in a very heterogeneous tumor response [1–3]. Inconsistent findings exist regarding the impact of pathologic features reflecting residual tumor burden on therapy response [4, 17, 26, 28].

Rullier et al. studied the impact of different methods of assessing tumor response to RCT and concluded that T downstaging, and not other regression grading systems, could influence survival independently [4]. Parallel with this finding, we showed that patients with either ypT <3 or T downstaging had a longer DFS, indicating a more favorable prognosis when regression is accompanied by a decrease in maximum infiltrative depth, referred to as tumor shrinkage. A wide CRM (cutoff=4 mm) was associated with T downstaging and a longer DFS, which indirectly supports

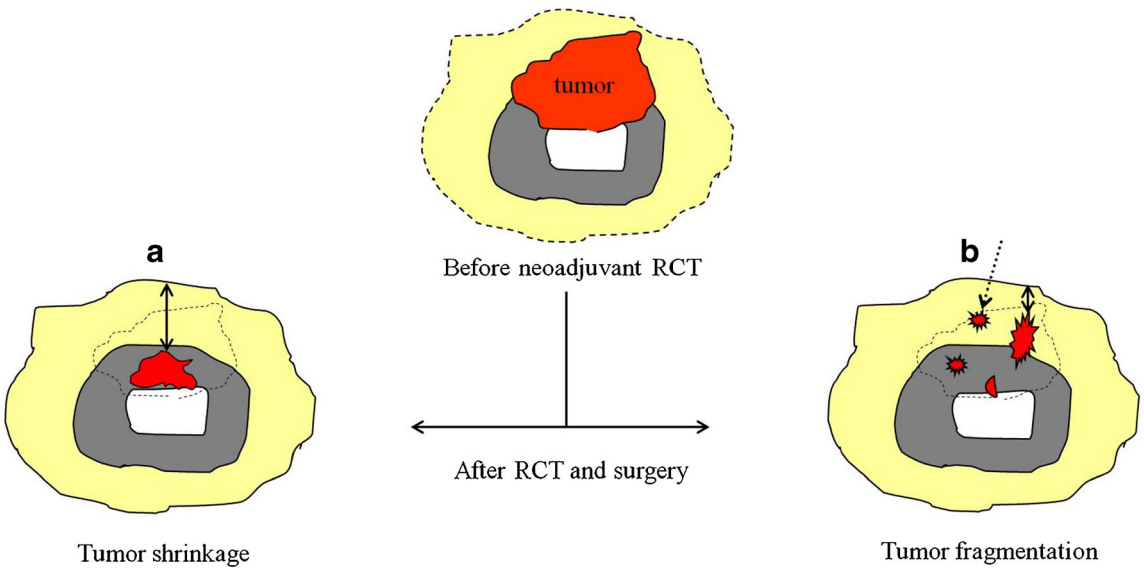

Fig. 2 Two possible forms of response to preoperative RCT. Preoperative RCT can result in either shrinkage or fragmentation of the primary tumor. Tumor shrinkage (**a**) reflects good response to RCT and is associated with favorable prognosis since it is associated with larger CRM (*long*, *two-headed arrow*). If response to RCT takes the form of tumor fragmentation (**b**), the patient may have unfavorable prognosis, not only because tumor fragmentation can generate mesorectal tumor deposits (*dotted arrow*) but also because the CRM might still be at risk (*short*, *two-headed arrow*)

the hypothesis that tumor shrinkage reflects a good response to neoadjuvant therapy since tumor shrinkage increases CRM. LNR was much lower in patients with T downstaging and correlated inversely with CRM. Moreover, N downstaging correlated significantly with T downstaging. Taken together, these findings suggest that shrinkage of the primary tumor is associated with a decrease of tumor load in the lymph nodes. This study confirmed the previously reported close association between T downstaging and prolonged DFS [4–6].

While some studies showed that tumor regression measured with Dworak grading system could predict patient outcome, other studies did not confirm this finding [4, 7, 8, 15, 36]. An increase in Dworak grade is generally assumed to refer to a decrease in tumor mass. In our study, however, we did not find any correlation between Dworak regression grade and T downstaging. In addition, higher Dworak grade was not associated with a large CRM. This implies that an increase in Dworak grade or a decrease in tumor mass is sometimes associated with tumor fragmentation rather than shrinkage (Fig. 2). For example, residual small tumor nests might be scattered throughout the rectal wall and/or mesorectum. In a study looking at the prognostic value of tumor regression after RCT, Nagtegaal et al. showed that tumor shrinkage was more common than tumor fragmentation [18]. The fact that prolonged DFS was observed in cases with T downstaging but not in those with higher Dworak grade implies that tumor fragmentation could weaken the prognostic significance of this cellular-based response grading.

TDs can represent different types of tumor involvement of the mesorectal fat, i.e., extreme, destructive form of lymph node, extramural vascular, or neural invasion. In the neoadjuvant setting, TDs could also result from fragmentation of the locally advanced primary tumor (cT≥3), leaving isolated mesorectal islands of carcinoma cells unconnected to the primary tumor. In this context, Nagtegaal and Quirke and Quirke et al. considered the presence of TDs as a sign of good response to RCT [24, 25]. In contrast, two other studies by Ratto et al. who looked at the prognostic impact of TDs in rectal cancer specimen after neoadjuvant RCT showed that this feature was associated with reduced disease-free and overall survival [37, 38]. In agreement with the latter studies, we found that the presence of TDs was clearly associated with poorer prognosis and that patients with TDs had a much smaller CRM compared to those without TDs. Moreover, it appeared that classifying TDs into T or N categories according to the 3-mm rule was prognostically relevant, despite the fact that the size criterion was based on unpublished data [24, 34].

To summarize, response to preoperative RCT can take the form of either tumor shrinkage or tumor fragmentation. In our study, the patients had better prognosis when RCT induced tumor shrinkage, characterized by T downstaging. One of the good prognostic factors associated with tumor shrinkage is obviously the increased chance of achieving large pathologic CRM, while in the case of tumor fragmentation, CRM might still be at risk despite a high Dworak grade. In addition, tumor fragmentation constitutes a new origin of TDs, and their morphologic features were indistinguishable from those of TDs of other origins.

In conclusion, we have shown that assessment of tumor shrinkage after neoadjuvant therapy via T downstaging and CRM has prognostic relevance. Shrinkage of the primary tumor is associated with a decreased nodal tumor load. The presence of TDs was clearly associated with poor prognosis and might reflect tumor fragmentation. Assessing regression based on the amount of tumor in relation to stromal fibrosis does not accurately discern tumor fragmentation from tumor shrinkage, which is most likely the reason why Dworak regression grading had less prognostic relevance.

Conflicts of interest None of the authors has conflicts of interest to declare.

References

1. Ceelen W et al (2009) Preoperative chemoradiation versus radiation alone for stage II and III resectable rectal cancer: a systematic review and meta-analysis. Int J Cancer 124(12):2966–72
2. Kapiteijn E et al (2001) Preoperative radiotherapy combined with total mesorectal excision for resectable rectal cancer. N Engl J Med 345(9):638–46
3. van Gijn, W., et al., Preoperative radiotherapy combined with total mesorectal excision for resectable rectal cancer: 12-year follow-up of the multicentre, randomised controlled TME trial. Lancet Oncol, 2011
4. Rullier A et al (2010) Impact of tumor response on survival after radiochemotherapy in locally advanced rectal carcinoma. Am J Surg Pathol 34(4):562–8
5. Moureau-Zabotto, L., et al., Predictive factors of tumor response after neoadjuvant chemoradiation for locally advanced rectal cancer. Int J Radiat Oncol Biol Phys, 2010
6. Valentini V et al (2002) Does downstaging predict improved outcome after preoperative chemoradiation for extraperitoneal locally advanced rectal cancer? A long-term analysis of 165 patients. Int J Radiat Oncol Biol Phys 53(3):664–74
7. Dworak O, Keilholz L, Hoffmann A (1997) Pathological features of rectal cancer after preoperative radiochemotherapy. Int J Color Dis 12(1):19–23
8. Rödel C et al (2005) Prognostic significance of tumor regression after preoperative chemoradiotherapy for rectal cancer. J Clin Oncol 23(34):8688–96
9. Mandard AM et al (1994) Pathologic assessment of tumor regression after preoperative chemoradiotherapy of esophageal carcinoma clinicopathologic correlations. Cancer 73(11):2680–6
10. Wheeler J et al (2004) Preoperative chemoradiotherapy and total mesorectal excision surgery for locally advanced rectal cancer: correlation with rectal cancer regression grade. Dis Colon Rectum 47(12):2025–31
11. Ruo L et al (2002) Long-term prognostic significance of extent of rectal cancer response to preoperative radiation and chemotherapy. Ann Surg 236(1):75–81

12. Bouzourene H et al (2002) Importance of tumor regression assessment in predicting the outcome in patients with locally advanced rectal carcinoma who are treated with preoperative radiotherapy. Cancer 94(4):1121–30
13. Debucquoy A et al (2009) Molecular and clinico-pathological markers in rectal cancer: a tissue micro-array study. Int J Color Dis 24(2):129–38
14. Debucquoy A et al (2008) Morphological features and molecular markers in rectal cancer from 95 patients included in the European Organisation for Research and Treatment of Cancer 22921 trial: prognostic value and effects of preoperative radio (chemo) therapy. Eur J Cancer 44(6):791–7
15. Topova L et al (2011) Prognostic value of tumor response to neoadjuvant therapy in rectal carcinoma. Dis Colon Rectum 54(4):401–11
16. Glynne-Jones R, Mawdsley S, Novell JR (2006) The clinical significance of the circumferential resection margin following preoperative pelvic chemo-radiotherapy in rectal cancer: why we need a common language. Color Dis 8(9):800–7
17. Nagtegaal I, Quirke P (2008) What is the role for the circumferential margin in the modern treatment of rectal cancer? J Clin Oncol 26(2): 303–12
18. Gosens M et al (2007) Circumferential margin involvement is the crucial prognostic factor after multimodality treatment in patients with locally advanced rectal carcinoma. Clin Cancer Res 13(22 Pt 1):6617–23
19. Ueno H, Mochizuki H (1997) Clinical significance of extrabowel skipped cancer infiltration in rectal cancer. Surg Today 27(7):617–22
20. Ueno H, Mochizuki H, Tamakuma S (1998) Prognostic significance of extranodal microscopic foci discontinuous with primary lesion in rectal cancer. Dis Colon Rectum 41(1):55–61
21. Prabhudesai A et al (2003) Impact of microscopic extranodal tumor deposits on the outcome of patients with rectal cancer. Dis Colon Rectum 46(11):1531–7
22. Puppa G et al (2007) Pathological assessment of pericolonic tumor deposits in advanced colonic carcinoma: relevance to prognosis and tumor staging. Mod Pathol 20(8):843–55
23. Puppa G et al (2009) Tumor deposits are encountered in advanced colorectal cancer and other adenocarcinomas: an expanded classification with implications for colorectal cancer staging system including a unifying concept of in-transit metastases. Mod Pathol 22(3): 410–5
24. Nagtegaal ID, Quirke P (2007) Colorectal tumour deposits in the mesorectum and pericolon; a critical review. Histopathology 51(2): 141–9
25. Quirke P et al (2007) The future of the TNM staging system in colorectal cancer: time for a debate? Lancet Oncol 8(7): 651–7
26. Lindebjerg J et al (2009) The prognostic value of lymph node metastases and tumour regression grade in rectal cancer patients treated with long-course preoperative chemoradiotherapy. Color Dis 11(3): 264–9
27. Shia J et al (2011) Significance of acellular mucin pools in rectal carcinoma after neoadjuvant chemoradiotherapy. Am J Surg Pathol 35(1):127–34
28. Quah HM et al (2008) Pathologic stage is most prognostic of disease-free survival in locally advanced rectal cancer patients after preoperative chemoradiation. Cancer 113(1):57–64
29. Heald RJ, Ryall RD (1986) Recurrence and survival after total mesorectal excision for rectal cancer. Lancet 1(8496):1479–82
30. Quirke P et al (1986) Local recurrence of rectal adenocarcinoma due to inadequate surgical resection. Histopathological study of lateral tumour spread and surgical excision. Lancet 2(8514): 996–9
31. Sobin LH, Fleming ID (1997) TNM classification of malignant tumors, fifth edition. Union Internationale Contre le Cancer and the American Joint Committee on Cancer. Cancer 80(9):1803–4
32. Hamilton, S., B. Vogelstein, and S. Kudo, Carcinoma of the colon and rectum., in World Health Organization Classification of Tumors-Pathology and Genetics of Tumors of the Digestive System., S. Hamilton and L. Aaltonen, Editors. 2000, IARC Press: Lyon. p. 105–119
33. Sobin, L., M. Gospodarowicz, and C. Wittekind, *TNM Classification of Malignant Tumors*. 7th ed, ed. L. Sobin, M. Gospodarowicz, and C. Wittekind. 2009, New York: Wiley-Blackwell
34. Quirke P et al (2010) Evidence-based medicine: the time has come to set standards for staging. J Pathol 221(4):357–60
35. Ssobin LH, Ch W (1997) TNM classification of malignant tumors. Wiley, New York
36. Losi L et al (2006) Prognostic value of Dworak grade of regression (GR) in patients with rectal carcinoma treated with preoperative radiochemotherapy. Int J Color Dis 21(7):645–51
37. Ratto C et al (2002) Mesorectal microfoci adversely affect the prognosis of patients with rectal cancer. Dis Colon Rectum 45(6):733–42, discussion 742–3
38. Ratto C et al (2007) Neoplastic mesorectal microfoci (MMF) following neoadjuvant chemoradiotherapy: clinical and prognostic implications. Ann Surg Oncol 14(2):853–61