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Circumferential Margin Involvement Is the Crucial Prognostic Factor after Multimodality Treatment in Patients with Locally Advanced Rectal Carcinoma

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Abstract

Purpose: After preoperative (radio)chemotherapy, histologic determinants for prognostification have changed. It is unclear which variables, including assessment of tumor regression, are the best indicators for local recurrence and survival.

Experimental Design: A series of 201 patients with locally advanced rectal cancer (cT3/T4, M0) presenting with an involved or at least threatened circumferential margin (CRM) on preoperative imaging (<2 mm) were evaluated using standard histopathologic variables and four different histologic regression systems. All patients received neoadjuvant radiochemotherapy or radiotherapy. The prognostic value of all factors was tested with univariate survival analysis of time to local recurrence and overall survival.

Results: Local recurrence occurred in only 8% of the patients with a free CRM compared with 43% in case of CRM involvement ($P < 0.0001$). None of the four regression systems were associated with prognosis, not even when corrected for CRM status. However, we did observe a higher degree of tumor regression after radiochemotherapy compared with radiotherapy ($P < 0.001$). Absence of tumor regression was associated with increasing invasion depth and a positive CRM ($P = 0.02$ and 0.03, respectively).

Conclusions: Assessment of CRM involvement is the most important pathologic variable after radiochemotherapy. Although tumor regression increases the chance on a free CRM, in cases with positive resection margins prognosis is poor irrespective of the degree of therapy-induced regression.

For patients with locally advanced rectum carcinoma (LARC), surgery alone is often not curative. In case of cT4 tumors or a threatened circumferential margin (CRM; <2 mm on preoperative imaging) in cT3 tumors, long-term neoadjuvant radio(chemo)therapy is required. This will result in downstaging and increased local control (1–4). The histopathology of specimens obtained after this kind of preoperative therapy is markedly different compared with untreated cases. Various stages of histologic tumor regression may be present, often resulting in changed morphology.

Histologic changes after the radiochemotherapy regimen range from absence of any treatment effect to a complete response with no residual tumor identified. One of the first systems for grading histologic regression focused on patients with esophageal carcinoma who were treated with radiochemotherapy (5). Their results showed that, after multivariate analysis, only grading of tumor regression was a significant predictor for disease-free survival (5). Subsequently, this system was modified by Dworak et al. (6) for grading regression in the rectum. Currently, several different methodologies for measuring the degree of histologic tumor regression after radiochemotherapy in rectal cancer have been described (6–10) but none has become universally accepted. Reproducibility seems to be a key factor.

The objective of our study is to evaluate which factors determine outcome in patients with LARC after radiochemotherapy, focusing on the contribution of histologic tumor regression grading and the CRM. Tumor regression after radiochemotherapy was measured using four different methodologies and evaluated for prognostic effect with respect to overall survival and local recurrence. CRM was evaluated according to Quirke et al. (11, 12). Additionally, prognostic implications of clinicopathologic and histologic variables were determined.

Materials and Methods

Patient selection. The patient population consisted of a consecutive series of patients with LARC with biopsy-proven adenocarcinoma. All

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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patients received multimodality treatment at the Catharina hospital between 1994 and 2005 (13). Patients were referred from all over the Netherlands based on the assumption of the referring surgeon that a free CRM was unlikely to be obtained without neoadjuvant treatment. Until August 2005, 201 patients with stage cT4 or cT3 and a predicted CRM of <2 mm have been treated. Confirmation of the tumor proximity to the CRM and the absence of distant metastasis (M0) on magnetic resonance imaging were crucial for enrollment. Median follow-up was 22.8 months (range, 0-124 months). Approval for the study was given by the local ethical committee of the Catharina hospital.

Therapy. Preoperatively, patients received different treatment regimens, considered state of the art at the time of treatment. Long-term radiotherapy (n = 74) involved a total dose of 50.4 Gy in 1.8 Gy fractions, five times a week. Two radiochemotherapy schedules have been used. The MAYO schedule, hereafter mentioned as interrupted schedule (n = 102), comprises concurrent radiotherapy and chemotherapy: a total irradiation dose of 50.4 Gy, 1.8 Gy per fraction during 5 weeks synchronously with 5-fluorouracil (350 mg/m^2) and leucovorin (20 mg/m^2) in irradiation weeks 1 and 5. The radiation scheme of the continuous radiochemotherapy regimen (n = 25) compromises 45 Gy in fractions of 1.8 Gy during 5 weeks. On every radiation day, 820 mg/m^2 capecitabine was administered twice and 50 mg/m^2 oxaliplatin was given at the first irradiation day of each week.

Surgery. The objective in both cT3 and cT4 tumors was to obtain a radical resection (negative CRM). Especially in cT4 tumors, the CRM encompassed surrounding structures (i.e., prostate vesicle, vaginal wall, pelvic floor, uterus, and sacrum). The CRM was considered negative if the outer margin of the en bloc specimen was negative.

In case of all treatment schedules, surgery was done 6 to 8 weeks after the last radiation date. All patients underwent resection by experienced and designated colorectal surgeons (H.J.T. Rutten and G.A.P. Nieuwenhuijzen) who routinely do total mesorectal excision surgery. The extended surgical procedures used were abdominoperineal resection (n = 98), low anterior resection (n = 91), abdominotranssacral resection (n = 9), and exenteration (n = 3). The total mesorectal excision principle was adhered to in all cases, even in extended resections (14).

Histopathologic assessment. Surgical specimens were assessed according to the protocol of Quirke et al. (11, 12). The most important issue is assessment of the CRM. To determine the CRM, the lateral resection margin of the fresh specimen was inked and subsequently the specimen was fixed in formalin for 48 h. Blocks of the tumor in relation to the inked CRM were collected. Measurements of the margin were done microscopically. A specimen with tumor ≤1 mm from the inked margin was considered as having a positive CRM. Classification of tumors was done using the WHO guidelines; a tumor was considered mucinous when the proportion of the mucinous component was ≥50%. Tumors were graded according to histologic differentiation into well, moderately, and poorly differentiated based on the poorest differentiated part of the tumor excluding the invasive front (15). Growth patterns were assessed as circumscribed or infiltrating (16). Evaluation of the tumor biopsies included assessment of tumor type and differentiation grade.

Histologic regression grading. Histologic therapy-induced tumor regression was assessed according to four different grading systems described by Dworak et al. (6), Scott et al. (7), Bouzourene et al. (8) and Rödel et al. (9). All four regression systems semiquantitatively asses the relative proportion of residual tumor to stromal fibrosis. The following descriptions characterized the different regression grades of the regression systems used: Dworak grade 0: no regression detectable, grade 1: dominant tumor mass with obvious fibrosis and/or vasculopathy, grade 2: dominantly fibrotic changes with few tumor cells or groups (easy to find), grade 3: very few (difficult to find microscopically) tumor cells in fibrotic tissue with or without mucin, and grade 4: no tumor cells, only fibrotic mass or mucin; Scott minimal: less than 1/3 tumor regression, moderate: 1/3 to 2/3 tumor regression, good: more than 2/3 regression, and maximal: no primary tumor remaining; Bouzourene tumor regression grade (TRG) 5: tumor shows no signs of regression, TRG 4: residual tumor cells outgrowing the fibrosis, TRG 3: more tumor cells than TRG 2 but fibrosis still predominates, TRG 2: rare residual cancer cells scattered throughout the fibrosis, and TRG 1: absence of residual cancer and fibrosis extending through the different layers of the rectal wall; and Rödel 0: no regression or <25% of tumor mass, Rödel 1: 25% to >50% tumor regression, and Rödel 2: complete regression.

When no tumor could be found macroscopically, sufficient tumor blocks were sampled to establish a complete response. In the present series, 21 patients had a complete response. The mean number of block

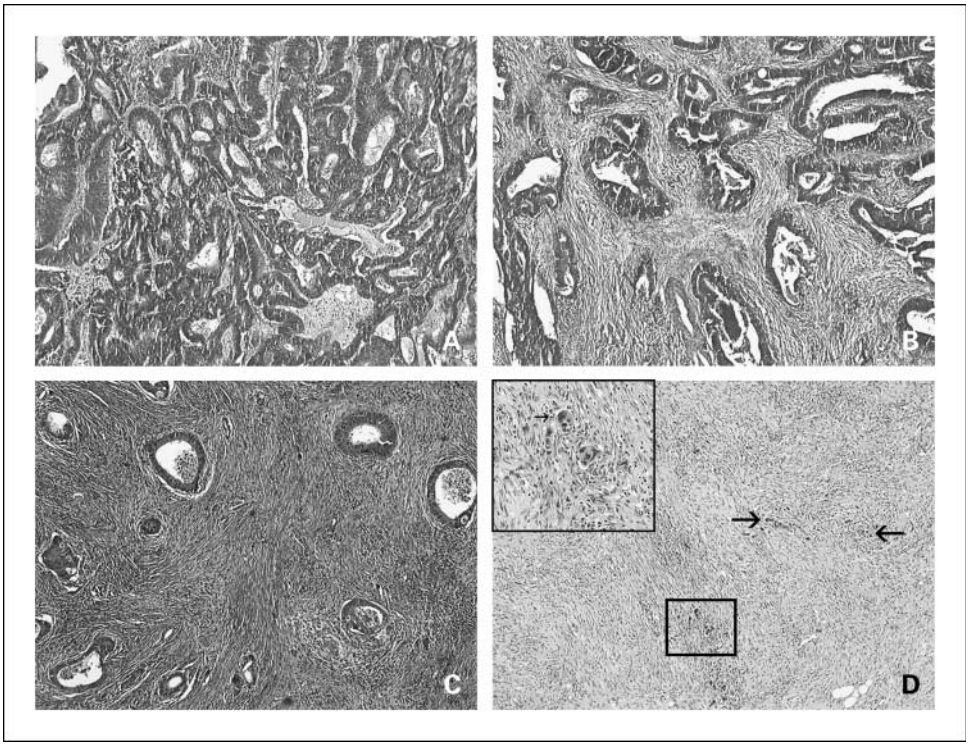

Fig. 1. Representative slides stained with H&E of different degrees of tumor regression observed after radio(chemo)therapy in patients with LARC. *A,* no sign of regressive changes, the fibrosis present is probably intrinsic to tumor development. Original magnification, ×50. *B,* marked fibrosis but large masses of vital tumor can still be observed. Original magnification, ×50. *C,* predominately fibrotic changes with smaller tumor masses. Original magnification, ×50. *D,* extensive tumor regression with few small clusters of tumor cells (*arrow*) scattered through the fibrotic area. Original magnification, ×50. The boxed area in *D* is depicted with a higher magnification as an insertion in this panel. Three small clusters of tumor cells can be appreciated. Original magnification, ×400.

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Table 1. Correlations between tumor regression and pretreatment and posttreatment factors

	Factor type	Factor	Category	Rödel 0, *n* (%)	Rödel 1, *n* (%)	Rödel 2, *n* (%)	*P*
Pretreatment	Clinicopathologic	Therapy	Radiotherapy	53 (72)	15 (20)	6 (8)	<0.001
			Radiochemotherapy	48 (38)	64 (50)	15 (12)	
		Clinical T stage	cT3	38 (46)	32 (38)	13 (16)	0.12
			cT4	63 (53)	47 (40)	8 (7)	
		Differentiation grade	Good/moderate	56 (58)	31 (32)	10 (10)	0.03
			Poor/undifferentiated	20 (36)	29 (52)	7 (12)	
Posttreatment*		ypT stage	ypT1, T2	5 (29)	12 (71)		0.02
			ypT3, T4	96 (59)	66 (41)		
		ypN stage	ypN0	65 (50)	49 (37)	17 (13)	0.38
			ypN1	24 (51)	19 (40)	4 (9)	
			ypN2	12 (52)	11 (48)	0 (0)	
		ypTNM	Stage I	5 (36)	9 (64)		0.16
			Stage II	51 (62)	31 (38)		
			Stage III	36 (55)	30 (45)		
		CRM	Negative	71 (52)	66 (48)		0.03
			Positive	30 (70)	13 (30)		
	Histologic	Type	Adenocarcinoma	75 (55)	62 (45)		0.50
			Mucinous	19 (53)	17 (47)		
		Differentiation grade	Good/moderate	62 (56)	49 (44)		0.40
			Poor/undifferentiated	39 (59)	27 (41)		
		Growth pattern	Circumscript	17 (68)	8 (32)		0.20
			Diffuse	83 (56)	64 (44)		
		Lymphoid reaction	None/few	56 (55)	45 (45)		0.20
			Moderate	32 (57)	24 (43)		
			Extensive	12 (80)	3 (20)		
		Eosinophilic infiltrate	None	71 (55)	57 (44)		0.19
			Moderate/extensive	29 (64)	16 (36)		
		Lymphangio invasion	No	94 (58)	69 (42)		0.54
			Yes	6 (54)	5 (45)		
		Calcification	No	76 (60)	50 (40)		0.18
			Yes	25 (51)	24 (49)		
		Tumor necrosis	No	24 (32)	52 (68)		<0.001
			Yes	76 (78)	22 (22)		

Abbreviations: ypT, pathologic T stage; ypN, pathologic N stage; ypTNM, pathologic tumor stage.
*After a complete response (Rödel 2), posttreatment factors could not be assessed.

samples collected from the fibrotic area was 9 (median, 7; range, 3-22). Figure 1 shows representative examples of different degrees of tumor regression. These microscopic images were digitalized using a Zeiss Axioskop 2 Plus microscope with a Sony 950P camera attached to it. Images were digitized using 5× or 40× Plan-Neofluar objectives (Carl Zeiss MicroImaging). A cellular mucin was considered as absence of residual tumor. The degree of tumor regression was determined semiquantitatively by two pathologists (J.H.J.M. van Krieken and I. Tan-Go) who were blinded for patients' clinical outcome. In addition, the amount of necrosis and the presence of calcifications were scored as alternative variables for regression.

Statistical analysis. Data were analyzed with the Statistical Package for the Social Sciences package (Statistical Product and Service Solutions 11.0 for Windows, SPSS, Inc.). Univariate survival analyses of time to death were done using the Kaplan-Meier method with the time of surgery as the entry date. Differences in observed survival between groups were tested for statistical significance using log-rank tests. χ^2 tests were used to determine correlations between categorized variables. Multivariate analysis was done using the Cox proportional hazards regression model [backward elimination (conditional)]. P values of ≤ 0.05 were considered as statistically significant.

Results

Pretreatment patient characteristics. The majority of the patients were male (61%); median age was 63 years (range, 35-86 years). Clinical T stage was cT4 in 59% and cT3 in 41% of the patients. Slightly more CRM involvement was present in cT4 tumors (25% versus 17%; $P = 0.13$). There was no difference in outcome between cT3 and cT4 tumors (local recurrence, 12% versus 19%, $P = 0.31$; metastases, 22% versus 29%, $P = 0.50$; overall 5-year survival, 58% versus 47%, $P = 0.22$). Because there was no significant difference in outcome, we combine both groups for further analysis.

Correlations between pretreatment factors and regression. The three-tier Rödel system was used to show correlations between the degree of regression and pretreatment and posttreatment factors (Table 1). The Rödel system consists of the lowest amount of categories and therefore avoids subgroups containing small numbers of patients. Furthermore, this system showed significant correlation within the framework of a randomized trial (9).

A strong association between treatment regimen and tumor regression was found (Table 1). The degree of tumor regression was significantly higher after radiochemotherapy (12% Rödel 2) compared with radiotherapy (8% Rödel 2; $P < 0.001$). Regression was more pronounced in tumors showing poor differentiation in their pretreatment biopsy ($P = 0.03$). Tumor regression was not influenced by preoperative cT stage.

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Posttreatment clinicopathologic and histologic factors and prognosis. CRM involvement, lymph node status, and tumor stage were strongly associated with both local recurrence and overall survival (Table 2). CRM involvement was the strongest predictor of local recurrence (43% versus 8%, at 24 months; $P < 0.001$) and overall survival (58% versus 80%; $P = 0.004$; Fig. 2).

Local recurrence rates increased and overall survival rates decreased with the number of lymph nodes involved ($P = 0.001$). Mucinous histology and poor differentiation were both associated with poor prognosis. Although lymphangio invasion was not associated with local recurrence, it did predict poor overall survival (40% versus 75%; $P = 0.04$). All the analyses were repeated for the different treatment regimens, but this did not reveal different results. However, the groups are too small for firm conclusions per regimen.

Multivariate analysis of clinicopathologic factors. Because histologic factors could not be determined in patients with a complete response, multivariate analysis for local recurrence and overall survival was done for clinicopathologic factors (category pathologic T stage, pathologic N stage, and CRM) and the Rödel system only. A hazard ratio (HR) of 1 was attributed to the most favorable category. In case of local recurrence, the CRM was the only factor significantly associated with this event (HR, 4.44; 95% confidence interval, 1.83-10.81; $P = 0.001$). With respect to overall survival, both N status and CRM were selected by the Cox regression model using conditional backward elimination: pathologic N stage I: HR, 1.75, 95% confidence interval, 1.01-3.05, $P = 0.046$; pathologic N stage II: HR, 2.60, 95% confidence interval, 1.28-5.27, $P = 0.008$; and CRM: HR, 1.68, 95% confidence interval, 0.99-2.85, $P = 0.054$.

Correlations between posttreatment factors and regression. Histologic posttreatment factors could only be correlated to Rödel 0 and 1 because no tumor cells were left after complete tumor regression (Rödel 2). However, after complete tumor regression at the site of the primary tumor, positive lymph nodes were still found in 4 (19%) of 21 complete responders (Table 1). In two cases, no lymph nodes were found. More extensive histologic tumor regression was present in ypT_1 and ypT_2 tumors as could be expected. As a consequence, an involved CRM was observed more than twice as often in patients with limited regression (Rödel 0) compared with patients with Rödel 1 (70% versus 30%; $P = 0.03$). No significant correlations were found between regression and histologic posttreatment factors. Tumor necrosis, which might be considered as an alternative variable for regression, was inversely related to the degree of regression. In 78% of the tumors with minimal (<25% of the tumor mass) or no regressive changes, necrotic areas were observed. After more extensive regression, these areas were identified in only 22 (22%) of tumor specimens.

Regression grading and prognosis. Surprisingly, none of the regression systems analyzed were found to be significantly associated with local recurrence. In addition, no correlation with overall survival was found (Table 3). We repeated the analysis correcting for CRM status because this factor was found

Table 2. Univariate analysis of posttreatment pathologic variables in relation to local recurrence and overall survival

Factor type	Factor	Category	*n* (%)	% LR at 24 mo	*P*	% alive at 24 mo	*P*
Clinicopathologic	ypT stage	ypT0-ypT2	38 (19)	3	0.061	89	0.04
		ypT3, ypT4	162 (81)	19		72	
	ypN stage	ypN0	131 (65)	9	0.001	81	0.001
		ypN1	47 (23)	25		69	
		ypN2	23 (12)	46		40	
	ypTNM	No residual tumor	14 (7)	0	0.022	100	0.004
		Stage I	19 (9)	0		81	
		Stage II	97 (49)	13		82	
		Stage III	70 (35)	29		59	
	CRM	Negative	158 (79)	8	<0.001	80	0.004
		Positive	43 (21)	43		58	
Histologic	Type	Adenocarcinoma	137 (79)	13	0.04	75	0.02
		Mucinous	37 (21)	32		60	
	Differentiation grade	Good/moderate	111 (63)	12	0.04	80	0.05
		Poor	66 (37)	26		61	
	Growth pattern	Circumscript	25 (14)	17	0.90	59	0.35
		Diffuse	147 (86)	18		76	
	Lymphoid reaction	None/few	101 (58)	22	0.23	67	0.19
		Moderate	56 (32)	15		75	
		Extensive	16 (9)	8		94	
	Eosinophilic infiltrate	None	129 (74)	19	0.85	73	0.33
		Moderate/extensive	45 (26)	17		77	
	Lymphangio invasion	No	163 (94)	16	0.51	75	0.04
		Yes	11 (6)	30		40	
	Calcification	No	126 (71)	19	0.58	75	0.56
		Yes	51 (29)	13		67	
	Tumor necrosis	No	76 (43)	6	0.02	68	0.76
		Yes	99 (57)	22		75	

NOTE: Patients with missing data were excluded from local recurrence and overall survival analysis.
Abbreviation: LR, local recurrence.

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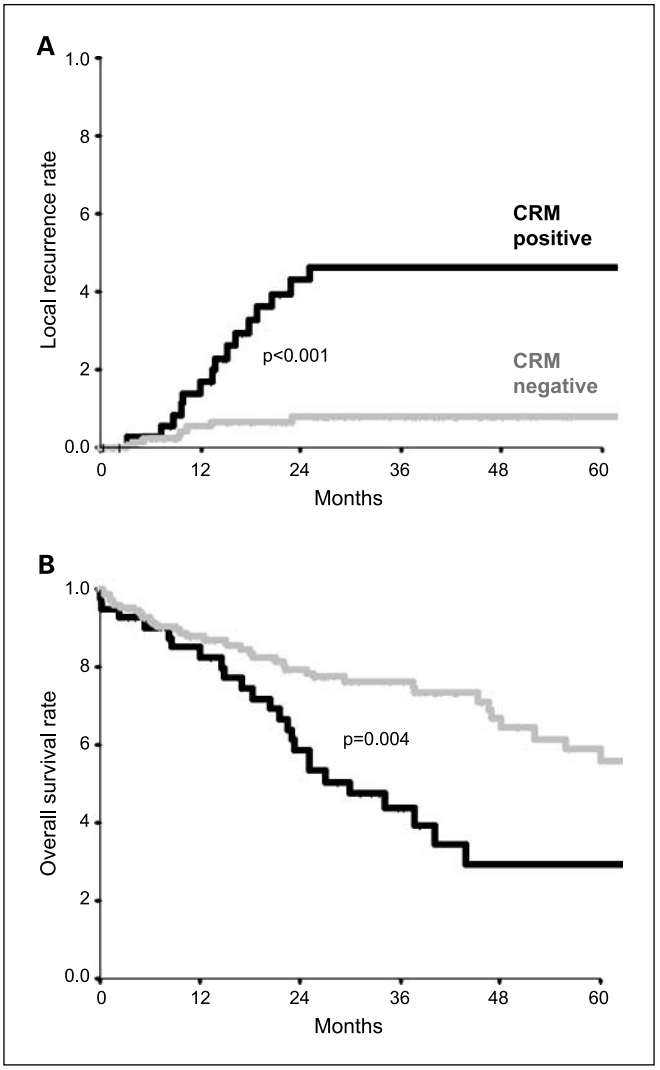

Fig. 2. Kaplan Meier curves of CRM involvement in relation to local recurrence (*A*) and overall survival (*B*). Black lines, patients with an involved margin; gray lines, patients with a free CRM.

the most potent predictor of prognosis. In the CRM-negative cases (n = 158), again grading of tumor regression lacked prognostic implications for local recurrence and overall survival.

Reproducibility of regression grading. To test reproducibility of our results and to determine the interobserver variability, tumor regression was also assessed by a second pathologist in all cases (n = 201). Analysis with the data obtained by the second pathologist confirmed our initial finding (data not shown). To express interobserver variability, measurements of agreement were indicated in κ values for each system (Supplementary Data). κ values for the regression system as a whole and for two successive categories within a system were calculated. Categories defining minimal regressive changes are more difficult to discriminate from one another (κ values ranging from 0.29 to 0.47) than categories defining extensive to complete regression (κ values ranging from 0.77 to 0.85). All four systems showed that that κ values improve as the amount of residual tumor decreases.

Discussion

Our study shows the great importance of optimal surgery for patients with LARC who are treated with radiochemotherapy. Although it has been suggested that neoadjuvant therapy could compensate for poor surgery, we show that radical excision (free CRMs) is essential for local control (Fig. 2). The prognostic value of standard clinicopathologic factors, such as CRM, lymph node status, and tumor stage, is superior to grading therapy-induced tumor regression in patients with LARC (Table 1). This finding was also confirmed in a multivariate model. CRM involvement is a very important risk factor for local recurrence that is vastly influenced by treatment factors (neoadjuvant therapy and surgery). Evaluation of the CRM could therefore be considered as an early alternative end point for future randomized trials comparing different treatment regiments for patients with LARC (17). The most advantageous treatment strategy for this specific subset of patients requires a multidisciplinary approach. This implies not only high-quality surgery and neoadjuvant therapy but also optimal imaging to identify patients who will benefit from this strategy and accurate pathologic assessment of CRM involvement (11, 12) for evaluating successfulness of the strategy.

The degree of tumor regression was found to be correlated with the neoadjuvant treatment regimen used. Tumor regression was found to be more extensive after radiochemotherapy compared with long-term radiotherapy, which is in accordance with literature (18, 19). We were not able to show any prognostic effect of regression scoring, irrespective of stratification for CRM involvement. However, tumor regression is important because the chance to obtain a negative CRM is increased after extensive tumor regression (Table 1).

The percentage of patients with a positive margin (21%) was relatively low taking into account that the inclusion criterion was a threatened CRM. This finding agrees with reports by Mawdsley et al. (17) and Glynne-Jones et al. (20) who found that 20% of the patients with LARC had a positive CRM after radiochemotherapy. LARC was defined by these authors as borderline resectable or irresectable disease; patients underwent curative surgery after neoadjuvant chemoradiation. Univariate analyses done in the present study confirmed the importance of the CRM for both overall survival (P = 0.004) and local recurrence (P < 0.001). Multivariate analysis of clinicopathologic factors confirmed the importance of CRM involvement for the prediction of local recurrence (HR, 4.44; 95% confidence interval, 1.83-10.81; P = 0.001). Several other studies report similar results about the importance of the CRM as a predictor for outcome after neoadjuvant treatment (17, 21–24).

Numerous studies investigated the prognostic value of tumor response after neoadjuvant therapy in rectal cancer, without consistent results. Similar to our study, no correlation was found in three different studies (19, 25, 26) with a total number of 385 patients. On the other hand, tumor regression was associated with local recurrence (389 patients; refs. 4, 8, 27, 28), overall survival (247 patients; refs. 8, 29), or disease-free survival (270 patients; refs. 4, 8, 9, 29).

However, reproducibility of tumor regression assessment leaves room for improvement (Supplementary Data). Measurements on the variance between two successive categories within each system showed that κ values improve as the amount of

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Table 3. Univariate analysis of regression systems in relation to local recurrence and overall survival

System	Total population (*N* = 201)					CRM-negative patients only (*n* = 158)				
	n (%)	% LR at 24 mo	*P*	% alive at 24 mo	*P*	*n* (%)	% LR at 24 mo	*P*	% alive at 24 mo	*P*
Dworak										
Grade 0	18 (9)	30	0.21	59	0.13	13 (8)	10	0.61	74	0.31
Grade 1	83 (41)	18		75		59 (38)	7		85	
Grade 2	57 (28)	19		70		48 (30)	12		66	
Grade 3	22 (11)	0		75		17 (11)	0		78	
Grade 4	21 (11)	5		94		21 (13)	5		94	
Scott										
Minimal	48 (42)	19	0.26	68	0.29	59 (37)	10	0.11	79	0.15
Moderate	35 (18)	17		73		27 (17)	13		74	
Good	61 (30)	9		76		51 (33)	0		77	
Maximal	21 (10)	5		94		21 (13)	5		94	
Bouzourene										
TRG 5	17 (9)	31	0.19	57	0.29	12 (8)	11	0.53	72	0.66
TRG 4	82 (41)	18		72		58 (36)	7		81	
TRG 3	58 (29)	19		77		47 (30)	13		76	
TRG 2	23 (11)	0		66		20 (13)	0		69	
TRG 1	21 (10)	5		94		21 (13)	5		94	
Rödel										
0	101 (50)	20	0.44	74	0.15	71 (45)	8	0.95	86	0.15
1	79 (39)	14		69		66 (42)	9		68	
2	20 (11)	5		94		21 (13)	5		94	

residual tumor decreases. Distinguishing absence (Fig. 1A) from little regressive signs (Fig. 1B) was reproduced poorly, probably because formation of fibrosis is also an intrinsic characteristic of tumor development. Discriminating intrinsic tumor fibrosis from therapy-induced fibrosis based on morphology is difficult. A complete tumor response, on the other hand, which is the only clearly definable degree of tumor regression, largely depends on tissue processing and sampling, which are often responsible for discrepancies in literature about the rate of complete responders (9, 29). A possible way to standardize the criteria for a complete response could be as follows: sample five sites of the tumor area, and if no tumor is present in these blocks, the whole area suggestive for disease should be embedded in paraffin blocks. If still no tumor is present, H&E slides will be obtained from each block at three levels. If no tumor was found after this procedure, a complete response was established (30). The lack of clear definitions with respect to the morphologic aspects of therapy-induced fibrosis and a complete tumor response explains both interobserver and interstudy variance.

The term locally advanced is also not clearly defined. Definitions range from patients who received long-term neoadjuvant radiotherapy or radiochemotherapy to patients with positive lymph nodes, advanced cT3 or cT4, or patients with a threatened CRM. Three of the nine studies investigating the prognostic value of grading tumor regression stated to have analyzed patients with LARC (8, 25, 28). The percentages of stage cT4 in these three reports range from 12% (25) to 32% (28) and are relatively low compared with the percentage of cT4 in the present population (59%). Moreover, none of the reports on LARC selected patients based on a threatened CRM (a predicted CRM on magnetic resonance imaging of <2 mm). These unique pretreatment characteristics distinguish the present population from other reports about patients with LARC. These differences in patient selection can also explain the inconsistency, about the prognostic implications of tumor regression, between the findings described in the present study and those described by others.

Our data indicate that tumor response to neoadjuvant long-term radiotherapy or radiochemotherapy results in tumor shrinkage (Fig. 3, *arrow*) rather than fragmentation of the tumor (Fig. 3, *dotted arrow*). The scenario of tumor fragmentation implicates that the degree of tumor regression is not informative for depth of infiltration (e.g., vital tumor cells may still be scattered throughout the whole fibrotic area and reach

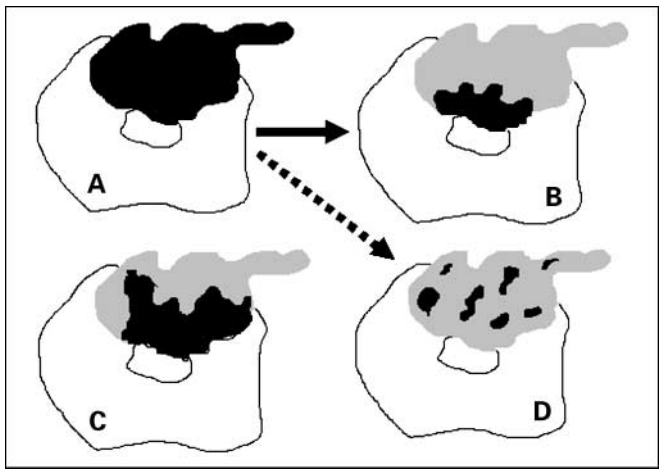

Fig. 3. Schematic representation of the relation between the degrees of tumor regression and CRM involvement. Black, areas with vital tumor cells; gray, fibrotic areas. *A,* pretreatment situation: the tumor is locally advanced (cT4) and the CRM is threatened. The contours of this pretreatment stage are also depicted in *B, C*, and *D*. *B* and *D,* after neoadjuvant long-course radiotherapy or radiochemotherapy, two different scenarios about tumor regression are sketched. *B,* "tumor shrinkage" scenario in which the infiltration depth is less extensive than in the pretreatment situation. *D,* "tumor fragmentation" scenario that implies scattered tumor cells throughout the whole fibrotic area, reaching the initial infiltration depth. However, if the CRM is still positive after tumor shrinkage (*C*), patient's outcome will still be poor irrespective of the degree of tumor regression.

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the pretreatment level of tumor invasion). Tumor fragmentation after neoadjuvant treatment still results in CRM involvement. However, our data suggest that tumor shrinkage is the main event after neoadjuvant therapy, resulting in negative CRM, which was obtained in ~80% of patients with a clinically threatened margin. Moreover, our data revealed that patients with a negative CRM experienced significantly less local recurrence compared with patients with a positive CRM. This indicates that the fibrotic area that is depicted in gray in Fig. 3B is sterile, pleading for the scenario of tumor shrinkage. However, if the CRM is involved, patient prognosis remains poor despite elaborate histologic regression after neoadjuvant treatment (Fig. 3C).

In case of overall survival, lymph node status revealed to have strong prognostic implications. Because treatment of patients with LARC consists of intensified local treatment aimed on the primary tumor, locoregional tumor spread resulting in positive lymph nodes (and as a consequence of decreased overall survival) is essentially not affected by this treatment. This was illustrated by our finding that positive lymph nodes can still be found after complete regression of the primary tumor mass (Table 1), which can explain why these patients can still develop metastasis.

In this study, which investigates a unique population of patients with LARC that had a threatened CRM and a high percentage of cT4, we have shown that assessment of the CRM is the most important pathologic factor after radiochemotherapy. Extensive tumor regression, resulting in tumor shrinkage, is essential for obtaining a free CRM, but incomplete resection implies a poor prognosis irrespective of the degree of these regressive changes.

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