ORIGINAL ARTICLE

Prognostic value of tumour regression grading and depth of neoplastic infiltration within the perirectal fat after combined neoadjuvant chemo-radiotherapy and surgery for rectal cancer

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Objective: To evaluate histological variables correlated with pathological response to chemo-radiotherapy protocols for rectal cancer and with local recurrence and survival.

Methods: From 1994 to 2003, 58 patients with rectal cancer were enrolled in a non-randomised study based on standardised treatment with radiotherapy, 5-fluorouracil, and surgical resection, followed by histological examination, including tumour regression grading and depth of neoplastic infiltration within the perirectal fat. All patients were followed up. Mean (SD) length of follow up was 55.3 (28.1) months, range 5 to 108.

Results: No case was found with no regression (grade 0). Tumour regression was defined as grade 1 in 24.5% of cases, grade 2 in 58.5%, grade 3 in 7.5%, and grade 4 (complete regression) in 9.5%. Neoplastic infiltration of >4 mm within the perirectal fat was found in 25.6% of cases in grade 1, 55.8% in grade 2, 2.7% in grade 3, and 11.6% in grade 4. In 80% cases of pT4 depth of neoplastic infiltration within the perirectal fat was >4 mm (100% were pN+), and the same spread was also found in 53.4% of pT2 and 86.2% of pT3. Pathological response was associated with regression grade ($p=0.006$) and depth of neoplastic infiltration within the perirectal fat ($p=0.04$). Tumour regression grading was an independent variable for pT ($p=0.0002$), pN status ($p=0.00004$), pathological staging ($p=0.000001$), and local recurrence ($p=0.003$).

Conclusions: Involvement of the lateral resection margins correlates with a poor prognosis and indicates the likelihood of local recurrence of rectal cancer. Tumour regression grading and the depth of neoplastic infiltration within the perirectal fat are important prognostic factors that need to be evaluated routinely.

The objective of neoadjuvant chemo-radiotherapy (CRT) regimens is a downstaging or downsizing of an advanced rectal tumour to increase the rate of curative resection and reduce locoregional failure, which is 2–3% in irradiated patients and 10–12% in non-irradiated patients.^{1–4} A reliable method of assessing response to adjuvant treatment is required to help standardise the assessment of new forms of multimodal therapy.

Mandard *et al* reported the grading of oesophageal tumour regression after preoperative chemoradiotherapy.⁵ Their method was based on a quantitative and reproducible analysis of tumour size, lymph node status, oesophageal wall involvement, and tumour regression grading. All these variables were highly correlated with survival.

A similar analysis of tumour response to neoadjuvant CRT was used by Dworak *et al*, who showed that while preoperative neoadjuvant treatment could not eliminate all tumour cells, it was useful for tumour downstaging and led to improved surgical results.⁶

Further studies revealed that tumour regression is a predictive factor for local recurrence and influences survival from rectal cancer after preoperative combined neoadjuvant treatment.^{7–10} The main factor responsible of local recurrence is yet to be defined.

Since Heald *et al*¹⁰ first reported evidence of isolated tumour deposits in the mesorectum, other investigators have shown that residual microscopic foci of tumour within the pelvis, resulting from inadequate excision of the mesorectum, were the cause of local recurrence.^{11–14}

The technique of total mesorectal excision (TME) places great importance on the anterior and posterior planes of resection, but little information is available about the lateral resection margins, which are known to be important determinants of loco-regional recurrence.^{12 13 15 16}

Surgery for rectal cancer has almost gone full circle.^{12 13 17} First there was a 5 cm rule, which was reduced to 2 cm because it was shown that intramural spread was confined within this border except in the most advanced disease.¹⁸ Currently, in order to reduce the high morbidity of TME (16–17% leak rate),^{19 20} it has been suggested that a modified form of TME—subtotal mesorectal excision (SME) (mesorectal excision limited to 5 cm in a caudal direction)—be applied to all but the very lowest tumours, and that the 5 cm rule be reintroduced for all other rectal resections, but again there is no indication of the extent of the lateral resection margins. Recent evidence has resulted in the safe distance from the distal edge of tumour being reduced from 5 to 1–3 cm, but in view of the depth of neoplastic infiltration within the perirectal fat some doubts remain about the necessary extent of radical resection.^{12 13}

Our aim in this study was to evaluate histological variables correlated with the pathological response to CRT protocols, and independent predictors of local recurrence and survival. In particular we focused our attention on the pathological grading of tumour regression and the depth of neoplastic infiltration into the perirectal fat.

Abbreviations: CRT, chemo-radiotherapy; TME, total mesorectal excision

METHODS

Patients

Between 1994 and 2003, 58 patients with a primary diagnosis of rectal cancer were studied at our department and enrolled in a neoadjuvant protocol of CRT followed by surgery.

Inclusion criteria for the study were as follows: pathological diagnosis of carcinoma of the rectum (from fine needle aspiration biopsy (FNAB) at proctoscopy); radiological and endoscopic diagnosis of locally advanced adenocarcinoma of the rectum (we included clinical stages II and III, T2–T3 UICC, or B–C Astler-Coller); no metastases recorded at the start of the treatment protocol; no diagnosis of other tumours or previous CRT; no relevant comorbidity; WBC $>4000/\text{mm}^3$, Hb >12 g/dl, platelet count $>100\,000/\text{mm}^3$, and Karnofsky performance status >60 .

All procedures involved in the protocol were approved by our ethics committee. All patients were informed by a surgeon and a radiotherapist involved in the study about all the procedures and foreseeable side effects of the protocol, and about the aim of the study. Signed informed consent was obtained from all the patients.

The protocol is a single centre, non-randomised study based on radiotherapy (2 Gy/day for five days a week given for 4.3 weeks; 23 fractions in all; total dose 46 Gy) plus a 30 day infusion of 5-fluorouracil (5-FU), 300 mg/m² per day, followed by surgical resection.

The study population included 35 male patients (60.4%) and 23 female patients (39.6%); their mean (SD) age was 59.98 (10.76) years, range 25 to 77. All patients had rectal adenocarcinoma.

The diagnosis was made on the basis of clinical evaluation (digital rectal examination), proctoscopy, and biopsy of the lesion. The stage was defined on the basis of abdominopelvic computed tomography (CT) and endorectal ultrasonography.

Protocol schedule

All patients were treated with the standard course of CRT, at the end of which every patient underwent to clinical examination—including digital rectal examination, proctoscopy, and abdominopelvic CT—to define the clinical response to the CRT.

Surgical resection was undertaken in all patients three weeks after the end of CRT. It is our policy during the surgical procedure to dissect the lateral mesorectal tissue sufficiently to obtain a 1.5 cm margin.

Pathology

Surgical specimens were fixed in buffered 10% formalin for 24 hours. After macroscopic examination, samples were taken from the resection margins and multiple samples from flat, atrophic, fibrous, elevated, or ulcerated areas on the rectal wall. The deepest grossly evident infiltration level in the rectal wall and fatty tissue suspect of containing abnormal areas were also sampled, together with the sphincter muscles and anal skin from the abdominoperineal resection specimens. According to the macroscopic features, various techniques for sampling tumour tissue were applied:

- macroscopically no visible tumour: the whole suspect (mostly fibrotic) area was sliced (5–8 mm thick slices) and paraffin embedded
- macroscopically obvious tumour: at a minimum, four paraffin blocks were sampled, with an additional fragment from the surrounding area.
- Lymph nodes were dissected and embedded according to the TNM lymph node groups.

The minimum requirement for tumour diagnosis was the presence of vital tumour cells or cell groups.

The histological sections were reviewed by two pathologists (AC, ID) and the regression grade was quantified. Regression grading was undertaken using the grading system for tumour regression proposed by Dworak *et al*⁶ as follows:

- Grade 0: no regression;
- Grade 1: dominant tumour mass with obvious fibrosis and/or vasculopathy;
- Grade 2: dominantly fibrotic changes with few tumour cells or groups (easy to find);
- Grade 3: very few (difficult to find microscopically) tumour cells in fibrotic tissue with or without mucous substance;
- Grade 4: no tumour cells, only a fibrotic mass (total regression or response).

Evaluation of the depth of neoplastic infiltration in the perirectal fat was undertaken as follows: gross rectal specimens, once fixed and ink marked on the resection margin, were sliced transversely at 3–4 mm intervals to select blocks from areas with a suspect, clearly neoplastic, or fibrotic appearance. On histological sections we measured the whole thickness of neoplasia, the distance between the deepest point of infiltration and the corresponding inked margin, and the distance between the outer limit of muscularis propria and the deepest point of infiltration. Considering that lateral extent of the mesorectum is at least 1.5 cm, the inner mesorectum measures about 5 mm. In the light of tissue shrinkage following radiotherapy we chose 4 mm as the border of the inner lateral mesorectum. The inner lateral

Table 1 pTNM and pathological stage after chemo-radiotherapy

PTNM stage	
T	
0	12.1%
1	3.5%
2	25.8%
3	50.0%
4	8.6%
N	
0	65.5%
1	17.2%
2	5.2%
3	3.5%
M	
0	84.5%
+	6.9%
Pathological stage	
0	8.6%
I	24.1%
II	31.1%
III	20.7%
IV	6.9%

Table 2 Tumour regression grade according to tumour location

Location	Grade 1	Grade 2	Grade 3	Grade 4
Recto-sigmoid junction	27.3%	54.6%	9.1%	9.1%
Upper rectum	14.3%	71.4%	14.3%	–
Middle rectum	26.0%	54.0%	8.0%	12.0%
Lower rectum	33.3%	59.9%	–	6.8%

mesorectum was therefore defined as <4 mm, and the middle and outer as >4 mm. Lesions that spanned the border were considered as belonging to the middle-outer mesorectum (>4 mm).

Follow up

All patients enrolled in the study were followed up three times during the first year after resection and twice in subsequent years. Follow up consisted of clinical examination, blood examination, liver function tests, serum levels of α -fetoprotein and carcinoembryonic antigen, and CT or ultrasound examination, or both, of the abdomen and pelvis. Mean (SD) length of follow up was 55.25 (28.06) months (range 5 to 108).

Statistical analysis

Data are expressed as mean (SD). Survival was calculated using the Kaplan–Meier method, with differences estimated by the log-rank test. Multivariate analysis (MANOVA) was used to assess independent variables. Significant difference was defined as $p < 0.05$. Data processing was undertaken using Statistica®, version 5.97 for Windows.

RESULTS

The tumour was localised to the recto-sigmoid junction in 18.9% of patients, in the upper rectum in 12.1%, in the

middle rectum in 43.1%, and in the inferior rectum in 25.9%; thus intraperitoneal tumours formed 31% of the cases and extraperitoneal tumours, 69%.

The surgical procedures undertaken were: 43 anterior resections (74.2%), one Hartmann procedure (1.7%), nine abdominoperineal resections (15.5%), and five anterior resections plus stoma (8.6%).

The pTNM classification and the pathological staging are given in table 1.

CRT morbidity

The overall incidence of morbidity during and at the end of CRT was 79.3%. We recorded the following (some combined in the same patient): 1.3% debility, 1.3% nausea, 3.9% haematological toxicity (first and second grade), 18.5% genitourinary toxicity (first and second grade), 52.6% gastrointestinal toxicity (first and second grade), 22.4% dermatological toxicity (first and second grade); third grade haematological, genitourinary, gastrointestinal, and dermatological toxicity were recorded rarely.

Pathological results

The stage 0 rate was 8.6%. Clinical stage II became pathological stage I in 43.2% of cases, while the downstaging rate for clinical stage III was 30%. pT0 was obtained by 50% of cT2 cases and 11% of cT3 cases, while no cT0 cases showed

Table 3 Results of multivariate analysis

Factor	Variable	p Value
Overall survival	Tumour site	0.003
	Distal margin	0.04
	Grading	0.04
Disease-free survival	Tumour site	0.005
	pN	0.006
	Liver metastases	0.007
	Other metastases	0.0003
Regression grading	Pathological response	0.04
PT	Distal margin	0.00001
	Grading	0.01
	Depth of neoplastic infiltration within the perirectal fat	0.0005
	Regression grade	0.0002
PN	Margin	0.000002
	Grading	0.0001
	Depth of neoplastic infiltration within the perirectal fat	0.000001
	Regression grade	0.00004
	Pathological response	0.0031
Depth of neoplastic infiltration within the perirectal fat	pT	0.013
	Pathological staging	0.049
Pathological staging	Distal margin	0.008
	Grading	0.001
	Depth of neoplastic infiltration within the perirectal fat	0.002
	Regression grade	0.000001
	Lymph node status	0.000001
	Pathological response	0.000001
Pathological response	Depth of neoplastic infiltration within the perirectal fat	0.04
	Regression grade	0.006
	Lymph node status	0.015
Local recurrence	Lymph node status	0.002
	Regression grading	0.003
Lung metastases	Tumour site	0.04
Liver metastases	Distal margin	0.02
Other metastases	Distal margin	0.01

pN, pathological lymph node staging; pT, pathological tumour infiltration staging.

Table 4 pN status according to the tumour regression grading

Grade	pN0	pN1	pN2	pN3
1	17.6%	40	33.3%	50
2	64.7%	40	33.3%	50
3	5.9%	10	33.3%	0
4	11.8%	10	0	0

pN, pathological lymph node staging.

progression during CRT. pN0 was obtained by 37.5% of cN1 cases and by 25% of cN2 cases, while 83.8% of cN0 had no progression during CRT. Overall, 15.5% of patients recorded no progression of lymph node disease, while 8.6% had nodal disease progression during CRT. As commonly defined by pathological analysis, a complete response was obtained in 8.6% of cases, a partial response in 44.8%, stationary disease in 34.5%, and progressive disease was present in 12.1%.

Complete regression was obtained in the middle and lower rectum (80%) and in the recto-sigmoid junction (20%), while partial regression was obtained in the recto-sigmoid junction and upper rectum (57.1%) and in the middle rectum (42.9%). No case was found with no regression (grade 0). Tumour regression was defined as grade 1 in 24.5% of cases, grade 2 in 58.5% of cases, grade 3 in 7.5%, and grade 4 (complete regression) in 9.5%. Of cases of complete tumour regression (grade 4), 60% were also defined as complete pathological response (pCR), while 20% were defined partial response (pPR) and 20% as stationary disease (pSD). Progressive disease was not shown by the tumour regression grading. The tumour regression grades according to location are given in table 2.

A depth of neoplastic infiltration within the perirectal fat <4 mm was found in 20.4% of cases, and >4 mm in 79.6%, of which 23% were in the recto-sigmoid junction, 12.8% in the upper rectum, 41.2% in the middle rectum, and 23% in the lower rectum. According to grading regression, a depth of neoplastic infiltration within the perirectal fat >4 mm was found 25.6% of cases in grade 1, 55.8% in grade 2, 7% in grade 3, and 11.6% in grade 4. The pathological response depended on: regression grading ($p=0.006$), depth of neoplastic infiltration within the perirectal fat ($p=0.04$) and lymph node status ($p=0.01$). Independent variables related to pT were: distal margin ($p=0.00001$), grading ($p=0.01$), depth of neoplastic infiltration within the perirectal fat ($p=0.0005$), tumour regression grading ($p=0.0003$), and pN status ($p=0.01$). Pathological staging depended on: the distal margin ($p=0.008$), the depth of neoplastic infiltration within the perirectal fat ($p=0.002$), regression grade ($p=0.000001$), grading ($p=0.001$), pathologic response ($p=0.000001$), and lymph node status ($p=0.000001$) (table 3).

Figure 1 Disease-free survival: pN0 v pN+ ($p<0.02$). **Figure 2** Overall survival: intraperitoneal v extraperitoneal N0 tumours ($p<0.05$).

Nodal disease

The mean number of lymph nodes was eight per surgical specimen. At the clinical staging, before the neoadjuvant CRT, 63.8% of patients were classified as N0, 27.6% as N1, 6.9% as N2, and 3.5% as N3. At the pathological staging, after surgery, 65.5% of patients were classified as N0, 17.2% as N1, 5.2% as N2, and 3.5% as N3. The distribution of pN0 tumours was: 63.6% of tumours of the recto-sigmoid junction, 42.8% of tumours of the upper rectum (55.5% of which were intraperitoneal tumours), 76% of tumours of the middle rectum, and 60% of tumours of the lower rectum (70% of which were extraperitoneal). The distribution of pN+ tumours was: 27.3% of tumours of the recto-sigmoid junction, 28.6% of tumours of the upper rectum (27.7% intraperitoneal), 24% of tumours of the medium rectum, and 26.6% of tumours of the lower rectum (25% extraperitoneal). Of seven patients with no evidence of residual tumour in the bowel wall, six had no nodal disease, while only one was staged pN2. Nodal dissemination was not observed in the two pT1 cases. Of the 15 pT2 cases, 20% were pN+, and of the 39 pT3 tumours, 28% were pN+. We analysed pN status according to tumour regression grading and the results are given in table 4. Prognostic factors for pN+ were: grading ($p=0.0001$), tumour regression grading ($p=0.00004$), depth of neoplastic infiltration in the perirectal fat ($p=0.0000001$), distal margin ($p=0.000002$), and pathological response ($p=0.003$) (table 3).

Depth of neoplastic infiltration in the perirectal fat

Depth of infiltration into the perirectal fatty tissue was divided into cases with more than or less than 4 mm: infiltration <4 mm was found in 20.4% of cases, and >4 mm in 79.6%. A depth of infiltration <4 mm was found in 46.6%

Figure 3 Overall survival: intraperitoneal v extraperitoneal N+ tumours ($p<0.05$).

of pT2 (of which 71.4% were pN0), and in 3.4% of pT3. The depth of infiltration was >4 mm in 80% of pT4 cases (100% of which were pN+), in 53.4% of pT2 cases (of which 87.5% were pN0), and in 86.2% of pT3 cases (of which 60% were pN0 and 40% were pN+). On multivariate analysis, the depth of neoplastic infiltration in the perirectal fat was independently related to pT ($p = 0.013$) and pathological staging ($p = 0.049$) (table 3).

Local recurrence and metastases

The overall local recurrence rate was 13.8%, with 62.5% identified in pN+ (50% N1–2 and 12.5% N3). Liver metastases were diagnosed in 15.5% of patients, 45.5% associated with pN+ (only N1–2); lung metastases were diagnosed in 10.3% of patients, 66.7% of which were in pN+ cases (50% N1–2 and 16.7% N3). “Other site” metastases were found in 8.6% of patients (60% in pN1–2). Prognostic factors for local recurrence were tumour regression grading ($p = 0.003$) and lymph node status ($p = 0.002$). Prognostic factors for distant metastases were site of tumour for lung metastases ($p = 0.04$), distal margin for liver metastases ($p = 0.02$), and distal margin for other metastases ($p = 0.01$) (table 3).

Disease-free survival and overall survival

There was no postoperative mortality. With a mean (SD) follow up of 55.3 (28.1) months (range 5 to 108), five year disease-free survival and overall survival were 69.9% and 71.1%, respectively, while nine year disease-free survival and overall survival were 69.9% and 68.3%. There was a significant difference on univariate analysis in five year disease-free survival between cN0 and cN+ (83.2% v 51.7%; $p = 0.005$); between pN0 and pN+ (82.3% v 49.8%, $p < 0.02$); between intraperitoneal and extraperitoneal N0 tumours (100% v 66.9%; $p = 0.04$); and between intraperitoneal and extraperitoneal N+ tumours (80% v 17.1%; $p < 0.05$) (fig 1, table 6). On univariate analysis, a significant difference in overall five year survival was found between intraperitoneal and extraperitoneal N0 tumours (100% v 69.8%; $p < 0.05$), and between intraperitoneal and extraperitoneal N+ tumours (80% v 50.9%; $p < 0.05$) (table 6, figs 2 and 3).

Independent variables associated with disease-free survival were: site of tumour ($p = 0.005$), pN status ($p = 0.006$), liver metastases ($p = 0.007$), and other metastases ($p = 0.0003$). Independent variables associated with overall survival were: site of tumour ($p = 0.003$), distal margin ($p = 0.04$), and grading ($p = 0.04$) (table 3).

DISCUSSION

The accuracy of imaging post-neoadjuvant restaging (Wheeler⁹) and of pathological analysis of surgical resection specimens represent two main steps in rectal cancer management. Local recurrence and distant metastases after surgery are still major predictive factors for survival in rectal cancer treated with neoadjuvant CRT. The objective of neoadjuvant

regimens is a downstaging or downsizing of advanced tumours to increase the rate of curative resection and reduce local recurrences (which occur in 2–3% of irradiated patients v 10–12% of non-irradiated patients^{1–21–23}). Some investigators have reported the absence of neoadjuvant treatment as an independent prognostic factor in reduced overall survival as well as local recurrence.¹⁴

If the surgeon's objective is the radical resection of the tumour, the goal of neoadjuvant CRT should be complete regression of the tumour. The effect of radiation on the rectal wall, lymph nodes, and perirectal adipose tissue, and its relation to neoplastic growth is the in the pathologist's charge, not only to identify regression of the morphological features of malignancy, but also to follow non-neoplastic tissue changes. A separate task is the pTNM staging after multimodal treatment, which gives a picture of the presence and extent of neoplastic growth.

Both residual tumour evaluation methods—grading the tumour regression and defining the effects of adjuvant therapy—offer the surgeon an estimate of how radical the surgery was and helps to define the prognosis for the patient. Assessment of the extent of the histological response to preoperative therapy can allow one to distinguish between residual disease and signs of previous neoplastic infiltration with regressive changes. However, different tumours and different patients have different response, and it is mandatory to undertake a careful comparative analysis between cases. In particular, the gross features and cytology of neoplastic cells provide useful data, the former in relation to pathological sampling, the latter in differentiating viable cells and regressive changes. Mandard's assessment scheme⁵—apart from the gross evaluation of persistent tumour growth—underlines the importance of vacuolated, eosinophilic neoplastic cells, with or without nuclear pyknosis on the one hand, and ghost cells with a granulomatous reaction, the latter being included in the “complete regression” group.

In our series, the numbers of lymph nodes retrieved was relatively small (8) compared with that recommended in other surgical series (12),²⁴ but we do not believe this to be a weak point of the study because, as reported elsewhere,^{25–28} CRT for advanced rectal cancer results in a significant decrease in the numbers of lymph nodes detected within the tumour bearing specimen. Because the number of lymph nodes identified within an excised specimen in patients undergoing resection of a rectal cancer is positively correlated with the size of the tumour, the downsizing/downstaging obtained by CRT would lower the number of lymph node identified by the pathologist.²⁶ Although neoadjuvant CRT downstages rectal cancer, it results in a significantly reduced yield of lymph nodes, which are also smaller than those in non-irradiated controls.²⁷ As reported by Wijesuriya *et al*,²⁷ the median nodal harvest was 4 (range 0 to 12) in the neoadjuvant CRT group versus 9 (range 1 to 19) in controls

Table 5 Local recurrence and metastases according to pN

N	Local recurrence (n, %)		Liver metastases (n, %)		Lung metastases (n, %)		Other metastases (n, %)	
0	3	37.5%	5	55.5%	2	33.3%	2	40%
1–2	4	50%	4	45.5%	3	50%	3	60%
3	1	12.5%	–	–	1	16.7%	–	–
N0	3	37.5%	5	55.5%	2	33.3%	2	40%
N+	5	62.5%	4	45.5%	4	66.7%	3	60%
Total	8 (13.8%)		9 (15.5%)		6 (10.3%)		5 (8.6%)	

pN, pathological lymph node staging.

Table 6 Disease-free survival and overall survival rate: prognostic factors, univariate analysis

Survival	5 Year	7 Year	9 Year	p Value
Disease-free survival				
Lymph node status				
Clinical				0.005
N0	83.2%	83.2%	83.2%	
N+	51.7%	34.5%	34.5%	
Pathological				<0.02
N0	82.3%	82.3%	82.3%	
N+	49.8%	49.8%	39.9%	
Localisation				0.04
N0				
Intraperitoneal	100%	100%	100%	
Extraperitoneal	66.9%	66.9%	0%	
N+				<0.05
Intraperitoneal	80%	80%	80%	
Extraperitoneal	17.1%	17.1%	0%	
Overall survival				
Localisation				
N0				<0.05
Intraperitoneal	100%	100%	100%	
Extraperitoneal	69.8%	69.8%	0%	
N+				<0.05
Intraperitoneal	80%	80%	80%	
Extraperitoneal	50.9%	25.5%	0%	

($p = 0.001$). The median size of the largest lymph node was 5 mm (range 2 to 12) in the CRT group versus 9 mm (range 4 to 15) in the control group ($p = 0.004$).²⁷ Finally, two previous papers from preoperatively treated patients showed a median of 16⁶ and a mean number of 9.3⁹ nodes, which confirms the variability of collected nodes within homogeneously examined surgical specimens.

There is no general and consistent agreement on the minimum number of nodes to be examined in this setting. During the past decade this was a matter of debate. Nevertheless, a report from Goldstein²⁹ suggested that "there is no minimum number that reliably or accurately stages all patients." All palpable nodes should be retrieved, irrespective of their dimensions and total number.

In our series 60% of cases of complete tumour regression were also defined as having a complete pathological response (pCR), while 20% were defined as having a partial response (pPR); nevertheless, it may be very difficult to compare the results on the basis of the pathological response, given the subjective component and the absence of quantitative indices.

In our series no case with no regression (grade 0) was found, and CRT obtained tumour regression of grade 3 in 7.5% of cases (very few tumour cells, which were hard to find microscopically within fibrous tissue, with or without a mucinous component), and complete regression (grade 4) in 9.5% of patients; thus 17% of the patients had a very good response to neoadjuvant therapy.

On multivariate analysis independent variables associated with disease-free survival were the site of the tumour ($p = 0.005$), pN status ($p = 0.006$), presence of liver metastases ($p = 0.007$), and presence of other metastases ($p = 0.0003$); independent variables associated with overall survival were the site of the tumour ($p = 0.003$), the distal margin ($p = 0.04$), and the grading ($p = 0.04$).

Because grading of tumour regression is an independent variable for pT ($p = 0.0002$), pN status ($p = 0.00004$), pathological staging ($p = 0.000001$), and local recurrence ($p = 0.003$), it is also indirectly associated with overall and disease-free survival. Different regression grading systems have been proposed for oesophageal cancer⁵ and rectal

cancer.^{6 7 9 30 31} All of these give useful indications, but there are some differences in the grading definitions. The greater number of groups in the proposals of Mandard⁵ and Dworak⁶, with similar definitions in both systems, differs from the Wheeler rectal cancer regression grade,⁹ which relies on a good or a poor response with a third intermediate group, and is based on variable fibrosis and microscopic or gross evidence of neoplasm.

Despite the worthy aim of the latter system of reducing as far as possible the subjective component in the grading process, in all the systems we find attributes such as "little", "marked", "dominantly", "difficult to find", which allow for subjective opinion. While a simple grouping of categories has its advantages, nevertheless, given the accuracy require, it is important also to consider different variables and combinations. For practical purposes, the best grade of regression is strongly associate with a better overall and disease-free survival, with low rates of local recurrence and distant metastatisation.^{7 30-33}

Pathological response were associated with regression grade ($p = 0.006$) and the depth of neoplastic infiltration in the perirectal fat ($p = 0.04$). This is to be expected as regression grade and depth of neoplastic infiltration within the perirectal fat are two pathological features that are used to evaluate the response to CRT. It is noteworthy on the other hand that regression grade and depth of neoplastic infiltration within the perirectal fat are quantitative and comparable pathological features.

Most series report lymph node involvement as the most important predictor of local recurrence and survival, even in neoadjuvant chemoradiation. Other predictive factors are pT and pathological staging.³⁴⁻³⁷

The depth of neoplastic infiltration in the perirectal fat represents the phenomenon of neoplastic growth within the linear course of the rectum, penetrating the extraperitoneal perirectal fat in a lateral direction, perpendicular to the rectal wall. This variable was associated with a worse prognosis in our series and can be expressed as the maximum depth of infiltration within the perirectal fat, measured on histological sections. If the resection margin (radial margin) shows neoplastic, direct infiltration, than surgery was not radical;

Take home messages

- Tumour regression grading and the depth of neoplastic infiltration within the perirectal fat are important prognostic factors in rectal cancer.
- These indices should be evaluated after combined neoadjuvant radiochemotherapy and surgery, to plan the optimum therapeutic strategy in the individual case.

on the other hand, if radial margin is free, the distance—measured on histological sections—from deepest neoplastic infiltration of perirectal fat and the radial margin is an important prognostic index: if the distance is ≤ 1 mm, then the circumferential margin is involved (College of American Pathologists, revised January 2005). Moreover, in the new version of TNM from the American Joint Committee on Cancer (AJCC)³⁸ and the International Union Against Cancer (UICC),³⁹ an optional expansion of T3 is considered, as follows: pT3a, minimal invasion: <1 mm beyond the border of the muscularis propria; pT3b, slight invasion: 1–5 mm beyond the border of the muscularis propria.⁴⁰ Although it is not required as a mandatory part of pT3 assessment, measurement of the depth of extramural soft tissue invasion may be justified, because data indicate that the deeper a tumour invades into the perimuscular tissues the worse the prognosis.^{40–41} Extramural extension exceeding 5 mm appears to be the critical cut off point linked to adverse outcome.^{40–41} This adverse prognostic association is observed whether or not regional lymph node metastasis is present.^{40–41} Extramural extension of the tumour within lymphatics or veins does not count as local spread of tumour as defined by T3, but extramural tumour nodules discontinuous from the primary tumour mass that are irregular in shape are included in the T category as pT3.^{40–41}

We measured on histological sections the whole thickness of neoplasia, the distance from the deepest point of infiltration to the corresponding inked margin, and the distance from outer limit of muscularis propria to the deepest point of infiltration. The latter values, representing infiltration depth within the perirectal fat, fall within the range 1–5 mm of pT3b and cluster, in this series, around 4 mm (<4 and ≥ 4) with a better distribution than around 5 mm (inner mesorectum), possibly because of tissue shrinkage caused by neoadjuvant treatment. Nevertheless, the value of 5 mm obviously maintains its general cut off role. As a result, in our series we simply recorded the best distribution of pT3 cases.

In our series, independent variables for depth of neoplastic infiltration within the perirectal fat were pT ($p = 0.013$) and pathological staging ($p = 0.049$); according published reports, the depth of neoplastic infiltration within the perirectal fat plays an important role as a predictor for local recurrence and survival. Depth of neoplastic infiltration within the perirectal fat (more than or less than 4 mm) is reported as an important prognostic factor both in pN0 and pN+, and correlations with lymph node metastases is recorded. Involvement of the lateral resection margins correlates with a bad prognosis and is a prognostic factor for local recurrence.^{12–42–43}

These results led us to consider tumour regression grading and the depth of neoplastic infiltration within the perirectal fat as new important prognostic factors for rectal cancer that should be evaluated, after combined neoadjuvant CRT and surgery, in order to plan better therapeutic strategies based on a quantitative evaluation of the response to neoadjuvant treatment and the extent of tumour involvement.

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