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Association Between Histological Type of Tumour Growth and Patient Survival in T2-T3 Lymph Node-Negative Rectal Cancer Treated with Sphincter-Preserving Total Mesorectal Excision

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Abstract For rectal cancer patients without nodal metastases the identification of unfavourable factors can be helpful for the better selection for adjuvant therapy and multimodality treatment. The aim of this study was to evaluate the impact of clinico-histological parameters on prognosis in node-negative rectal cancer patients. One hundred and thirty-nine consecutive node negative rectal cancer patients with complete five-year follow-up were studied prospectively. All of them underwent curative anterior resection with total mesorectal excision technique. Seventy-eight patients with tumour penetration beyond the bowel wall received neo-adjuvant short-course radiation (25 Gy) followed by surgery within 1 week and postoperative chemotherapy with 5-fluorouracil and folinic acid in six cycles or adjuvant radiochemotherapy: irradiation (50.4 Gy) combined with chemotherapy (as above). Cancer-specific survival was calculated according to the Kaplan-Meier method. Variables significant in univariate analysis by log-rank test ($P<0.05$) entered the Cox proportional hazard model. Survival was decreased for males, older patients (>60 years) with extraperitoneal, poorly differentiated cancers, tumours with mucinous histology and with the absence of lymphocytic infiltration but with the lack of statistical importance. Prognosis was significantly improved for patients with T2 tumours versus T3 ($P<0.01$) and with cancers with expanding growth comparing to diffusely infiltrating ones ($P<0.01$). In multivariate analysis these parameters significantly and independently influenced survival ($P<0.01$ and $P<0.05$, respectively). Diffusely infiltrating growth of tumour can reflect the more aggressive cancer behaviour and unfavourable course of disease despite the optimised local control. Apart from the extent of tumour penetration the type of invasive margin can be an additional parameter helpful for the optimal treatment planning and better patient selection for postoperative chemotherapy.

Keywords Anterior resection · Invasive margin character · Lymphocytic infiltration · Rectal cancer
Total mesorectal excision

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Introduction

Surgery is still the mainstay of the management of rectal carcinoma. Since the introduction of total mesorectal excision (TME) technique by Heald et al. in the eighties

[1] this method has become widely adopted. It results in optimal local control being nowadays the operative treatment of choice [2]. In spite of TME the long-term prognosis for patients with positive nodal status remains poor because of the high risk of distant metastases [3]. They can benefit from combined-modality therapy [4]. On the contrary excellent oncological results achieved in series with surgery alone were reported [5]. Therefore, the need of adjuvant treatment for node-negative rectal cancer operated on according to TME principles is sometimes questioned. In the era of TME a more individual approach for adjuvant therapy with consideration of numerous predictive factors is postulated [6].

The purpose of this study was to evaluate the impact of clinical and histological parameters on prognosis in node-negative rectal cancer patients after TME anterior resection.

Materials and Methods

Patients

At the 2nd Department of Surgical Oncology at Lower Silesian Oncology Centre two hundred and twenty-seven consecutive patients with histologically confirmed node-negative rectal cancer underwent an anterior resection with TME technique from January 1998 to December 2003. One hundred and sixty-eight of them (UICC stage I, n=83; UICC stage II, n=85) entered the study fulfilling the inclusion criteria: primary tumour localised maximally 12 cm from the anal verge, absence of distant metastases, lack of intraoperative bowel perforation, absence of macroscopic infiltration of adjacent organs, distal and radial margins microscopically free of cancer infiltration (R0 resection). In each case the minimum distance between the tumour edge and the circumferential margin was larger than 1 mm, mean radial clearance was 4.3±2.1 mm. The minimum number of harvested and examined lymph nodes was 12, mean was 14.1±1.7. Fifteen patients with pT1 tumour were not considered because of the lack of the invasion in the muscular wall in this stage, and consequently, the lack of possibility to characterise adequately the pattern of the leading edge of lesion. Fourteen patients without any evidence of recurrence died due to other than cancer causes during the follow-up. They were also excluded from the analysis. Thus, the group of one-hundred and thirty-nine patients remained to undergo the analysis.

Surgical Treatment

All patients underwent elective surgery with preoperative bowel preparation by means of 4 L of polyethylene glycol solution one day before surgery. Resection of the rectum was strictly performed according to the TME principles with sharp dissection under direct vision of the plane between the parietal and visceral pelvic fascia to the levators level. The rectum was mobilised to the pelvic floor, lateral ligaments were divided as laterally as possible. Special effort was made to identify and preserve the hypogastric plexuses and the pelvic nerves. End to end anastomosis was constructed using double-stapling technique with Proximate TLH transverse device and Proximate ILS circular intraluminal one (Ethicon Endo-Surgery Europe, Norderstedt, Germany). Bowel wash-out was performed using 2% povidone iodine solution. In each case a macroscopic assessment of the plane of surgical excision was done and the mesorectal fascial plane was confirmed: the mesorectal surface was smooth with only minor irregularities such that no defect was deeper than 5 mm, the mesorectum itself was of good bulk posteriorly and anteriorly and there was no coning near the tumour.

Adjuvant Therapy

For 78 patients with UICC stage II tumours (pT3) adjuvant therapy was given. Seventeen patients with cancer penetration beyond the muscularis propria imaged preoperatively in MRI or endorectal ultrasound received preoperative five-day radiation 25 Gy (5×5 Gy) and postoperative chemotherapy with 5-fluorouracil (325 mg/m^2) and folinic acid (20 mg/m^2) in six courses. In no case a complete pathologic response (ypT0) was found. For sixty-one patients with cancer foci in mesorectum diagnosed in postoperative pathologic examination combined adjuvant radiochemotherapy (5-fluorouracil ± folinic acid and 50.4 Gy radiation: 25×1.8 Gy±5.4 Gy boost) was administered.

Follow-up

Time of the follow-up was 5 years. It was scheduled every three months during the first postoperative year and every six months thereafter. Physical examination, blood tests, serum markers, barium enema, endoscopy, chest radiograph and abdominal ultrasound were done. In every supposition of cancer recurrence more precise investigation using endorectal sonography, CT or radioisotope scanning was performed.

Clinical Factors

For each patient age and gender were recorded. There were sixty-five females and seventy-four males. Patient age ranged from 34 to 89 years, median was 60, mean was 60.7. Therefore, a level of 60 years as a cut-off point for age analysis was stated. Site of the primary tumour was

categorised in two groups: >7 cm and ≤7 cm from the anal verge for separate consideration of the intra- and extraperitoneal tumours.

Pathological Features and Microscopic Evaluation

Microscopic analysis was carried out using formalin-fixed, paraffin-embedded tissue sections routinely hematoxylin-eosin stained and assessed at a x 200 and 400 magnification. According to the extent of direct tumour spread patients were classified to pT3 or pT2 group (penetration beyond the muscularis propria or not, respectively). Adenocarcinomas with mucin histology (more than 50% of the tumour volume composed extracellular mucin pools) were distinctly evaluated from non-mucinous ones. Patients were divided into two groups depending on differentiation grade: well/moderately and poorly differentiated. The character of invasive margin (expanding or infiltrating) and lymphocytic infiltration (conspicuous or little/absent) were assessed strictly according to criteria originally described by Jass et al. [7], as follows below. Tumour margin was defined as the transition zone between the periphery of the tumour and normal rectal tissue. Considering the character of invasive margin the term infiltrating applied to the subset with extensive, irregular and diffuse dissection of normal tissues by the tumour (Fig. 1). Lesions invading with a broad front and a smooth-pushing border were defined as to have an expanding margin (Fig. 2) [8]. Conspicuous lymphocytic infiltration (Figs. 1 and 2) of the tumour was recognised when the loose inflammatory lamina including lymphocytes at the deepest point of tumour penetration was present. Venous invasion was diagnosed when tumour was present within an extramural endothelium-lined space that was either surrounded by a rim of muscle or contained red blood cells. Involvement of submucosal veins was not evaluated. A positive diagnosis of perineural invasion was made when cancer cells were found to exist inside the perineurium.

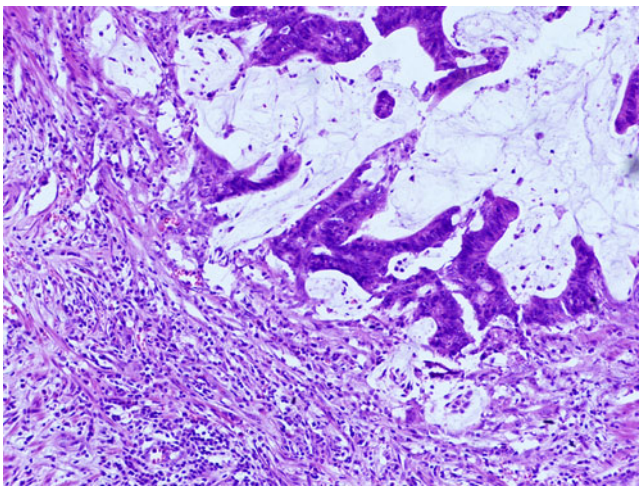

Fig. 1 Rectal adenocarcinoma with mucinous histology G3. Invasive diffusely infiltrating tumour margin with conspicuous lymphocytic infiltration. H&E, 100x

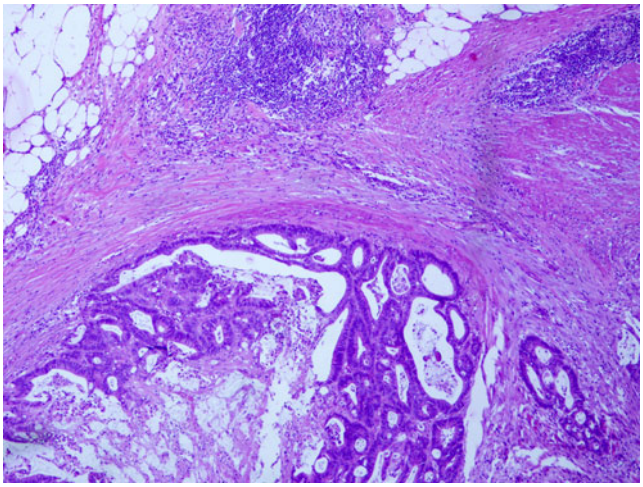

Fig. 2 Rectal adenocarcinoma with mucinous histology G2. Invasive circumscribed expanding tumour margin with conspicuous follicular lymphocytic infiltration. H&E, 40x

Statistical Analysis

The data were collected in a prospective manner. All clinical and pathological variables were considered in univariate analysis. To examine the impact of individual parameters on long-term outcome the analysis of observed five-year cancer-specific survival was used. One hundred and fifty-three pT2-3 patients with complete follow-up entered the survival analysis. Patients who died due to any other cause with the lack of cancer recurrence (14 from a total series) were excluded from the analysis. Patients who died because of rectal cancer were stated as non-survivors. Survival was calculated according to the Kaplan-Meier method. Survival curves were compared by the log-rank test using $P<0.05$ as significance limit. Variables significant in univariate analysis were entered into Cox's proportional hazard regression model to evaluate them in multivariate analysis as independent factors.

Results

Five-year cancer-specific survival rate was 82.3%. Prognosis was improved for females, younger patients (≤60 years) with intraperitoneal (>7 cm from the anal verge) cancers, well or moderate differentiated tumours without mucinous histology and with the presence of lymphocytic infiltration but with the lack of statistical importance. Venous invasion and perineural invasion were found in 23% ($n=32$) and

17% (n=24) of patients, respectively. None of these features was associated with patient outcome. Five-year cancer-specific survival was significantly decreased in patients with pT3 tumours versus pT2: 63.1±9.2 vs 97.2±3.8 (P≤0.01, odds ratio 19.483 [95%CI 3.983–129.514], relative risk 14.537 [95%CI 1.952–92.734]). Prognosis was poorer for patients with diffusely infiltrating cancers comparing to those with expanding growth: 65.2±5.8 vs 96.1±4.7 (P≤0.01, odds ratio 9.538 [95%CI 1.673–39.627], relative risk 7.21 [95%CI 1.478–23.915]). Taking the tumour penetration and growth pattern into account, the differences in outcomes remained significant when preoperatively irradiated patients were excluded from the analysis and only patients who underwent surgery with or without postoperative radiochemotherapy were studied: pT2 vs pT3, 97.2±3.8 vs 60.8±7.3, P≤0.01; expanding vs infiltrating margin, 96.8±6.1 vs 64.7±7.3, P≤0.05. Results are shown in Table 1. In multivariate analysis using Cox regression hazard model these both parameters significantly and independently influenced survival: tumour penetration, P 0.0219, relative risk 12.845; margin pattern, P 0.0372, relative risk 5.386. Data are presented in Table 2.

Discussion

Traditional Dukes', Astler-Coller's, and TNM staging of rectal cancer is based on the extent of primary tumour penetration and lymph modes metastases. In our group of patients direct tumour spread was a statistically important prognostic factor. Similarly, in our previous study of all the patients treated in the years 1998–1999 with curative TME anterior resection, long-term survival was significantly decreased when tumour penetration through the bowel wall was present [9]. However, T3 tumours compose a large group of surgically treated lesions, even if only node-negative cancers are considered. Obviously, a prognostic heterogeneity amongst these tumours must be present. Some investigations were carried out to subdivide T3 tumours according to the depth of tumour invasion beyond the outer border of the muscularis propria. Significantly improved prognosis was reported for patients with tumours with the less extensive infiltration when cut-off value of 4 mm [10], 5 mm [11], and 6 mm [12] beyond the muscularis propria layer was used. Further studies are needed for defining the optimal cut-off value for the extent of invasion to identify the shallow and deep invasion subgroups precisely. Yoshida and co-authors found in multivariate analysis the distance of tumour invasion beyond the muscularis propria to be the most important prognostic factor. Patients with invasion equal or deeper than 4 mm had a significantly poorer prognosis both for cancer-related survival and disease-free survival. The prognostic importance of tumour infiltration pattern was also observed. Interestingly, they noticed that the depth of invasion less than 4 mm was significantly more common in cancers with expanding growth [13].

The prognostic importance of patient age remains the matter of debate [14]. Site of primary rectal tumour should not be related to survival if the surgery is of good quality and the criteria of TME are met. Significance of mucinous carcinoma is disputable. It is believed by some to be linked with adverse outcome only if it occurs in specific subset of patients, e.g. those younger than 45 years. On the other hand, when it is associated with microsatellite instability, it is prognostically favourable [15]. Moreover, there is also some evidence that neoadjuvant therapy may result mucin lakes in the tumour, inducing a mucinous phenotype difficult to interpret [16]. Prognostic importance of venous invasion is still controversial. Difficult assessment and poor reproducibility are postulated by some to be reasons of discrepant results. Special techniques needed to increase the ease and accuracy of evaluation are labour intensive, time

Table 1 Prognostic value of clinico-pathological factors in univariate analysis

Parameter	N	Cancer-specific survival	P (log-rank)	
Patient's age	≤ 60 years	58	85.1±4.8	>0.05
	> 60 years	81	78.3±7.1	
Patient gender	female	65	82.4±6.6	>0.05
	male	74	77.2±5.9	
Tumour location	> 7 cm	92	85.8±7.4	>0.05
	≤ 7 cm	47	79.6±4.2	
Differentiation grade	I/II	96	83.6±8.0	>0.05
	III	43	77.1±9.8	
Mucinous histology	absent	125	86.7±8.1	>0.05
	present	14	79.6±5.2	
Tumour direct penetration	pT2	61	97.2±3.8	<0.01
	pT3	78	63.1±9.2	
Venous invasion	absent	107	83.5±8.2	>0.05
	present	32	78.1±7.9	
Perineural invasion	absent	115	82.5±9.5	>0.05
	present	24	76.9±6.8	
Invasive margin character	expanding	78	96.1±4.7	<0.01
	infiltrating	61	65.2±8.5	
Lymphocytic infiltration	present	57	85.9±7.5	>0.05
	absent	82	79.2±6.8	

Table 2 Multivariate analysis using Cox proportional hazard with P=0.0005 as significance level of regression model

Parameter	P	Relative risk
Invasive margin character	0.0372	5.386
Tumour direct penetration	0.0219	12.845

consuming, expensive and not routinely performed. At present existing standards for the pathologic assessment of this feature are not widely accepted, and pathology sampling practices may vary widely on both individual and institutional levels [15]. Other authors claim that the prognostic significance of venous invasion is well established since guidelines to optimise its identification have been given [17]. Perineural invasion is another intensively investigated pathologic feature but it is not routinely assessed because its independent association with prognosis remains unclear [15]. It is one of the microscopic criteria by which the infiltrating growth can be recognised [15]. Interestingly, we noticed this pattern of tumour border in all the patients for whom the perineural invasion was diagnosed. However, this pathologic feature was not a significant indicator of poor prognosis in our group, in contrast to invasive margin.

We found the histological configuration of the tumour at the advancing edge (tumour border) prognostically important. Survival rate of patients with carcinomas growing with diffuse infiltration was significantly lower than the ones with expanding growth. Poorer cancer-specific survival was observed despite the fact that over than 50% of these patients ($n=32$) have had early stage pT2 tumours (UICC I). Prognostic significance of growth pattern (pushing vs infiltrating margin) was also noticed in other studies [18–21]. In some recent series this factor independently influenced long-term survival [13, 22, 23], risk of cancer-related death [24] and disease-free interval before metastases [24]. These findings can be partially explained by the strong relationship between presence of tumour pushing margin and low risk of lymph nodes metastases [25]. Moreover, node-negative malignancies with infiltrating margin are more likely to develop systemic dissemination [26].

Tumour growth pattern (next to the number of positive lymph nodes, cancer direct spread and the presence of lymphocytic infiltration) has been included into new classification of rectal cancer originally described by Jass and co-workers [16]. Some studies confirmed significant association of this grouping with recurrence risk [27] and survival rates [28, 29]. Other authors observed the prognostic value of Jass' classification only for node-negative tumours [26, 30]. On the contrary, in studies by Fisher and co-investigators [31] and Deans and co-authors [32] Jass' grouping was an invalid classification because it was significantly related to Dukes system and did not improve the traditional staging in prognosis prediction.

Accurate evaluation of tumour growth pattern is difficult, thus, should be performed by a pathologist with adequate experience [32, 33]. Nowadays the treatment of rectal cancer becomes centralised in high-volume institutions with special interest in this malignancy. It is believed that then an excellent intra- and inter-observer agreement in pathological assessment can be achieved [33, 34].

In the era of TME surgery in operative management of rectal cancer improved outcomes can be achieved. When the surgery is optimised the need of radiation and chemotherapy may be considered more individually to avoid unnecessary side effects. For the optimal treatment planning a lot of factors have to be taken into account. Thus, additional histological features can be useful for identifying patients with an increased risk of oncological relapse and systemic dissemination. The main conclusion of this study is that diffusely infiltrating growth of tumour can reflect more aggressive cancer behaviour and predict an unfavourable course of disease despite of optimal local clearance obtained with TME technique. Therefore, the type of invasive margin can be a pathologic parameter helpful for the better patient selection for adjuvant chemotherapy. Potential benefit from more aggressive treatment for them should be analysed in further studies.

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