

Predictive value of lymphocytic infiltration and character of invasive margin following total mesorectal excision with sphincter preservation for the high-risk carcinoma of the rectum

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Abstract

Purpose: To assess the prognostic significance of clinico-pathological factors, especially histological parameters of new Jass classification, following sphincter-sparing total mesorectal excision (TME) for high-risk rectal cancer.

Material and methods: Forty-five consecutive patients treated with curative intent in 1998-1999 due to rectal cancer in Dukes stage B and C were studied prospectively. All of them underwent anterior resection with TME technique. Prognostic value was evaluated by the impact on five-year recurrence-free survival (RFS) in uni- and multivariate analysis. Only factors significant in univariate analysis entered the multivariate regression model. P value <0.05 was stated as a significance limit.

Results: Regarding traditional clinico-pathological factors patient age, tumor site, differentiation grade, mucinous histology and the extent of direct tumor penetration did not significantly affect survival rates. Only the lymph nodes status was associated with prognosis with statistical importance (negative vs positive, RFS: 53.8±10.0% vs 26.3±10.4%, respectively). Considering the additional parameters of Jass classification the character of invasive margin of the tumor did not reveal the important predictive value although the lymphocytic tumor infiltration was significantly related to patient outcome (presence vs absence, RFS: 63.6±15.2% vs 37.5±8.7%, respectively). In multivariate analysis the only one statistically important and independent predictive parameter was the lymph nodes status.

Conclusions: Lymph nodes metastases remain the most important prognostic factor after anterior resection with TME for Dukes B and C rectal cancer. From variables included into

Jass classification the absence of lymphocytic infiltration of the tumor can be helpful to identify patients with enhanced risk of oncological relapse.

Key words: rectal cancer, anterior resection, total mesorectal excision, lymphocytic infiltration, invasive margin character.

Introduction

Surgery remains the mainstay of treatment for rectal carcinoma. With the development of screening programs, diagnostic tools and stapling devices an anterior resection with sphincter preservation became the preferred option for the most of cases [1]. Moreover, since the introduction of the method of total mesorectal excision (TME) an optimal local control resulting in improved survival can be achieved [2]. In spite of the sub-specialisation, surgical training and advances in operating technique [3] a lot of patients with regional stage of disease have a high risk of cancer recurrence and may benefit from combined-modality therapy [4]. Thus, in the era of TME more individual approach for adjuvant treatment with consideration of numerous predictive factors is postulated [4,5].

In 1987 Jass and co-workers introduced a new prognostic system for rectal cancer considering four variables: tumor penetration through the bowel wall (transmural =1, limited to the bowel wall =0), lymph-node metastases (>4 involved nodes =2, 1-4=1, negative nodes =0), invasive margin character (infiltrating =1, expanding =0) and peritumoral lymphocytic infiltration (absent =1, present =0). The scores are summated for tumor grouping with the final score: I =0-1, II =2, III =3, IV =4-5 [6]. Authors claimed that new classification was simple to use and was superior to Dukes staging because it placed twice as many patients into groups and provided a more confident prediction of clinical outcome. The independent value of that scoring was noticed in multivariate analysis by some [7-9]. Moreover, prognostic significance of Jass classification even for patients being

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Received 24.10.2006 Accepted 08.01.2007

at the same Dukes stage was observed [10-13]. However, other authors reported that traditional Dukes system was of greater prognostic value and was more reproducible than Jass' classification [14-16]. In their opinion the continued use of Dukes staging is, therefore, warranted for prognostic and therapeutic decisions and should be recommended for routine clinical practice [16].

The aim of this study was to estimate the prognostic value of clinical and pathological factors, especially additional histological parameters of Jass classification following anterior resection with TME for rectal cancer patients with high risk of oncological relapse (Dukes stage B/C).

Material and methods

Patients

At the 2nd Department of Surgical Oncology at Lower Silesian Oncology Center seventy-seven consecutive patients with histologically confirmed rectal cancer underwent an anterior resection with sphincter preservation from January 1998 to December 1999. Forty-five of them entered the study fulfilling the inclusion criteria: primary tumor 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). Time of the follow-up was five years. The data were collected in a prospective manner. Written informed consent was obtained from all the patients.

Surgical treatment

All patients underwent elective surgery with preoperative bowel preparation by means of 4L of polyethylene glycol solution one day before surgery. Prophylactic antibiotics were administered at the anaesthesia induction. 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. 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.

Adjuvant therapy

Fifteen patients received preoperative five-day radiation 25 Gy (5 x 5 Gy) and postoperative chemotherapy with 5-fluorouracil (325 mg/m²) and folinic acid (20 mg/m²) in six courses. For thirty patients combined adjuvant radiochemotherapy (5-fluorouracil + folinic acid and 50.4 Gy radiation: 25 x 1.8 Gy + 5.4 Gy boost) was administered.

Follow-up

Follow-up 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, computed tomography or radioisotope scanning was performed.

Clinical factors

For each patient age and gender were recorded. There were twenty-two females and twenty-three males. Patient age ranged from 37 to 88 years, mean was 60.3, median was 60. Therefore, a level of 60 years as a cut-off point for age analysis was stated. Site of the primary tumor was categorised in two groups: >7 cm and ≤7 cm from the anal verge for separate consideration of the intra- and extraperitoneal tumors.

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. Stage parameters were analyzed considering the extent of direct tumor spread (beyond the bowel wall or not) and regional lymph nodes status (presence or absence of metastases). Patients were divided into two groups depending on differentiation grade: well/moderately and poorly differentiated. Adenocarcinomas with mucin histology (more than 50% of the tumor volume composed of mucin) were distinctly evaluated from non-mucinous ones. 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. [6], as following below. Tumor margin was defined as the transition zone between the periphery of the tumor and normal rectal tissue. Considering the character of invasive margin tumors were divided into three categories: circumscribed, intermediate and diffusely infiltrating and then the circumscribed and intermediate were defined as expanding. Conspicuous lymphocytic infiltration of the tumor was recognized when the loose inflammatory lamina including lymphocytes at the deepest point of tumor penetration was present. *Tab. 1* shows patients characteristics and factors analyzed in this study.

Statistical analysis

Statistical analysis was performed using software package StatisticaTM ver. 5.0. All clinical and pathological variables were considered in univariate analysis. To examine the impact of individual parameters on long-term outcome, five-year recurrence-free survival analysis was used. Recurrence-free survival was calculated according to the Kaplan-Meier method. Survivals were compared by the F Cox test using P<0.05 as significance limit. Variables significant in univariate analysis were entered into Cox's proportional hazards regression model to evaluate them in multivariate analysis as independent factors.

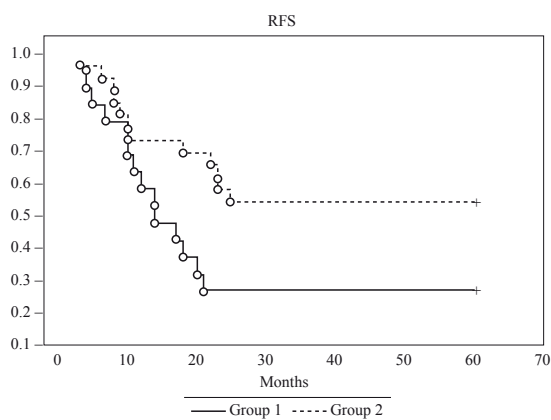
Results

Postoperative mortality was not noticed. Survival rates were enhanced for the female patients not older than 60 years, with well or moderately differentiated cancers sited >7 cm from the anal verge and without tumor penetration beyond the

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

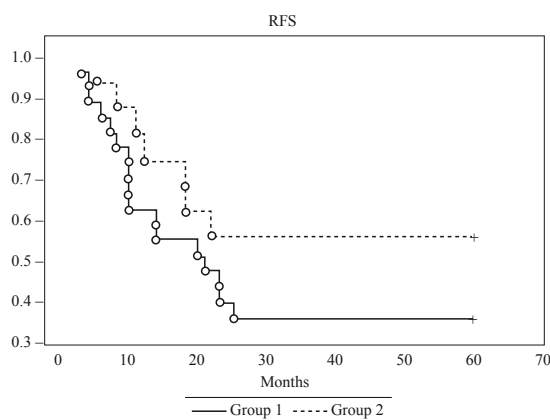
Parameter		n	Survival	P
Patient age	≤ 60 years	19	52.6±11.8	0.260
	> 60 years	26	34.6±9.5	
Patient gender	Female	22	45.5±10.9	0.367
	Male	23	39.1±10.4	
Tumor location	> 7 cm	9	55.6±17.6	0.287
	≤ 7 cm	36	38.9±8.2	
Differentiation grade	I/II	26	44.0±10.1	0.376
	III	19	42.1±11.6	
Mucine secretion	Absent	38	42.9±20.2	0.864
	Present	7	42.1±8.1	
Lymph-node status	Negative	26	53.8±10.0	0.025
	Positive	19	26.3±10.4	
Penetration beyond the bowel wall	Absent	6	43.6±8.0	0.702
	Present	39	33.3±21.1	
Invasive margin character	Expanding	18	56.3±12.8	0.094
	Infiltrating	27	37.0±9.5	
Lymphocytic infiltration	Present	13	63.6±15.2	0.034
	Absent	32	37.5±8.7	

Figure 1. Impact of lymph-node metastases on survival



RFS: recurrence-free survival; Group 1: lymph-node metastases positive; Group 2: negative

Figure 2. Impact of invasive margin character on survival



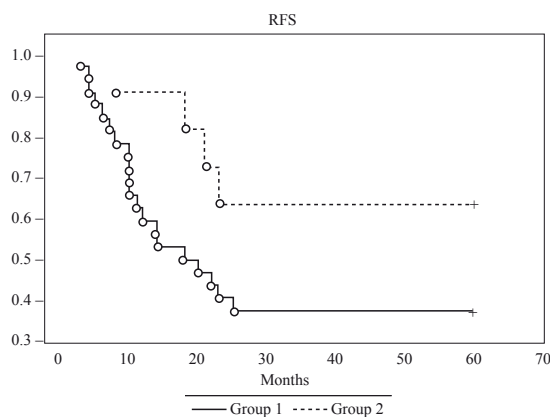
RFS: recurrence-free survival; Group 1: infiltrative margin; Group 2: expanding margin

bowel wall (Tab. 1). However, the differences were not significant. Survival was decreased for node-positive patients and for patients without lymphocytic tumor infiltration with statistical significance (Fig. 3 and Fig. 1). Considering additional parameters included into Jass classification an improved survival was observed in cases with expanding vs infiltrating tumor margin although with the lack of importance (Fig. 2). Multivariate analysis in Cox' proportional hazard regression model revealed the lymph nodes status to be the most important and the only one independent prognostic factor (Tab. 2).

Discussion

Dukes and TNM staging of rectal cancer are based on the extent of primary tumor penetration and lymph nodes metastases. In our group of patients the predictive value of the lymph nodes status was more important than the direct tumor spread

Figure 3. Impact of lymphocytic infiltration on survival



RFS: recurrence-free survival; Group 1: absence of lymphocytic infiltration; Group 2: presence of lymphocytic infiltration

Table 2. Multivariate analysis with Cox regression proportional hazard model

Parameter	P	Rr	Odds Ratio	95% CI
Lymph-node status	0.045229	2.362942	3.305622	0.835239-13.08265
Lymphocytic infiltration	0.106113	2.229606	2.912445	0.631508-13.43186

Rr – relative risk; CI – confidence interval

which was not significant probably because of analysis of only Dukes B and C cases. Among these patients the absence of tumor penetration beyond the bowel wall with the presence of lymph nodes metastases (Astler-Coller C1 stage; node-negative cases should be regarded as A stage) is a very rare situation. This rarity resulted in statistically non-adequate comparison of six vs thirty-nine patients in our study.

Considering other clinico-pathological factors and histological parameters included in Jass classification we found the statistically important predictive value of conspicuous lymphocytic infiltration of the tumor. That histological feature is well-known to be more frequently associated with early-stage rectal cancers than with those with lymph-node or distant metastases [17]. Tumor stage is a multiparametric variable summarizing some histological features. Jass and co-investigators added two biological parameters to Dukes system: the character of invasive margin which reflects cancer aggressiveness in local spreading and the lymphocytic infiltration of the tumor which pictures patient immune response.

In our study the absence of lymphocytic infiltration significantly influenced decreased recurrence-free survival but it was not independent factor probably because of too small patients number for adequate multivariate analysis. Its independent prognostic value was reported by others [18-21]. Also in the most recent papers low tumor lymphocytic infiltration predicted increased recurrence rate and poor long-term cancer-specific survival [22-26]. In some opinions possible discrepancies of results may be caused by difficult assessment and poor reproducibility for this feature [27]. However, pathological assessment can be significantly improved through the provision of recommended guidelines and gaining the experience [28].

Lymphocytic infiltration is a marker of an effective cell-mediated immune response against the tumor [29]. Some authors suggest the relationship between host immune reaction and the production of angiogenic promoters and thus, with stimulation of tumor vascularity [30]. As the consequence the favourable effect of peritumoral lymphocytic infiltration may interfere with the negative effect of a large vascular surface area within the tumor [31]. On the other hand, host immune response observed as conspicuous lymphocytic infiltration, may prevent metastasis formation [32] and growth of liver metastases [33]. It is more common in the microsatellite instability-high cancers with favourable prognosis [34]. The extent of lymphocytic infiltration correlates with bcl-2 expression, which is known to suppress programmed cell death (apoptosis) [35].

Immune response against rectal cancer is multipathway and multistep mechanism involving different types of lymphocytes, NK cells, dendritic cells and macrophages [27,36,37]. However, the possibility of immunomodulation in cancer patients is intensively investigated [38]. Interferon-alpha can induce cyto-

toxic T lymphocytes and may elicit long-lasting tumor-specific immunity while interleukin-12 seems to stimulate non-specific killing [39]. Lymphocytic infiltration of the tumor may be suppressed by pentoxifylline [40] and significantly enhanced by preoperative short course of histamine H2 receptor antagonist [41,42]. Those findings are the initial observations but they may be promising perspective for the regulation of immune response against cancer of the rectum.

With the development of combined-modality treatment and the prospect of immunotherapy and gene therapy the identification of markers of cancer behaviour is increasingly needed. The importance of our results is limited by the relatively small sample size. Moreover, neoadjuvant radiotherapy used in one-third of our patients might influence the accuracy of postoperative microscopic evaluation. Despite these disadvantages our results suggest that the lack of conspicuous lymphocytic infiltration of the tumor is related to poor recurrence-free survival of rectal cancer patients. Dismal prognosis was noticed in spite of TME technique and optimal surgical local control. Therefore, lymphocytic infiltration may give additional predictive information for optimal management and follow-up. Potential benefit from more aggressive treatment for these patients should be evaluated in further studies including controlled randomized trials.

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