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Degree of tumor regression after preoperative chemo-radiotherapy in locally advanced rectal cancer—Preliminary results

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ABSTRACT

Aim: The aim of this investigation is to determine the degree of tumor regression by histopathological evaluation of surgical specimen after neoadjuvant chemo-radiotherapy for patients with stage IIIB rectal cancer.

Background: The standard therapy for rectal carcinoma is surgical, however, preoperative radiochemotherapy will play an increasing role especially in locally advanced disease. To estimate the prognosis and the effect of radiochemotherapy the postradiochemotherapeutical pathological features are important to assess.

Materials and methods: Ten patients with cT3–4, cN1 stage rectal cancer received preoperative chemo-radiotherapy. A total tumor dose of 50 Gy was applied to all patients, with a daily fraction of 2 Gy, 5 times a week, with concomitant Capecitabine 1650 mg/m^2. A pathomorphologic assessment of the therapeutic response of the residual tumor volumes and estimation of tumor control were performed using Dworak's system of tumor regression grading (TRD) from no regression (0) to a complete tumor control (4).

Results: Dworak's TRD for the examined patients is as follows: in 20% of the patients no tumor regression was observed – Grade 0, in 30% – Grade 1, in 20% – Grade 2 and in 30% a complete tumor regression was achieved – Grade 4. Four of the patients (40%) presented with borderline resectable tumors before the neoadjuvant chemo-radiotherapy. Nine of the patients (90%) underwent radical surgery. In one case (10%) a radical surgery was not possible. One patient (10%) developed severe radiation enteritis in both the early and late postoperative period, with her tumor regression evaluated as Grade 4.

Conclusion: Accurate evaluation of local tumor control using Dworak's tumor regression grading scale after preoperative chemo-radiotherapy gives the basis for a larger investigation and search for a correlation with the prognosis of the disease and individual choice of adjuvant treatment.

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1. Introduction

Neoadjuvant treatment is the standard of care for locally advanced rectal carcinoma. The use of combined preoperative chemo-radiotherapy in the management of patients with locally advanced rectal carcinoma with stage cT3–4, cN1 and M0 has been shown to improve the yearly relapse-free survival with 46% compared to a group treated with surgery alone.[1] Histological investigation of the primary tumor at diagnosis and of the surgical specimen is a key for assessing the therapeutic response to the chemo-radiotherapy and surgical treatment.

The predominant colloid changes observed after preoperative radiotherapy have been first described by Dworak et al.[2] The author recommends specific standards for pathomorphologic workup of the specimens after neoadjuvant treatment and the degrees of tumor response for rectal carcinoma. Tumor regression grading (TRG) described by Dworak begins with 0 indicating no tumor regression and rises up to 4 referring to total tumor regression:

Grade 0: No tumor regression;
Grade 1: Dominant tumor mass with obvious fibrosis and/or vasculopathy;
Grade 2: Dominantly fibrotic changes with few tumor cells or groups;
Grade 3: Very few (difficult to find microscopically) tumor cells in fibrotic tissue;
Grade 4: No tumor cells, only fibrotic mass (total tumor regression or response).

A simpler version of the grading can be used in clinical practice:

(1) Weak pathomorphologic response: no tumor regression: dominant fibrotic changes with few tumor cells or groups of tumor cells (Dworak 0–2).
(2) Excellent pathomorphologic response: good tumor regression: very few (difficult to find microscopically) tumor cells in fibrotic tissue with or without mucin or total tumor regression (Dworak 3–4).

Dworak's grading system for tumor regression is mainly used in Europe. It has been modified by Ryan in 2005[3] so that full tumor response is defined as 0:

- 0 – full tumor regression, no visible tumor cells;
- 1 – moderate tumor regression, very few or small groups of tumor cells;
- minimal tumor regression, residual tumor in fibrotic tissue;
- no tumor regression, residual tumor without any signs of destroyed tumor cells.

Current recommendations of the NCCN Rectal Cancer Guidelines Panel 2011[4] follow the American system of Ryan for evaluating the degree of tumor regression after neoadjuvant treatment.

Results of clinical investigations using Dworak's scale have shown that TRG as a measure of the response to preoperative treatment correlates very precisely with the observed relapse-free and overall survival.[5] Shia et al.[6] consider that the degree of reduction of preoperative T and N stage and limphovascular invasion statistically correlate with relapse-free survival ($p = 0.002$). Additional morphological features have been found to correlate with the reduction of relapse-free survival, independent of other risk factors: type of stromal response, inflammation infiltrates ($p = 0.001$) and lack of superficial ulcerations ($p = 0.026$). These morphologic characteristics are subject to additional investigations and analysis. Different authors report that full pathomorphologic remission correlates with an improved 3-year survival rate.[7–9]

That is why, we thought it useful to fully investigate the histopathologic changes observed after treatment using Dworak's scale for tumor regression grading (TRG).

2. Background

Residual tumor after surgical therapy of rectal cancer is one of the most important prognostic factors. Therefore, the major goal of therapy is a curative resection. Large tumors with invasion of adjacent structures (e.g. pelvic wall, urinary bladder, prostate) may be, however, not completely resectable. Radiochemotherapy has been described as a useful tool for the reduction of tumor mass.

3. Materials and methods

In the period between 2008 and 2010, 10 patients with locally advanced rectal carcinoma stage cT3, cT4 and cN1 were treated at the Radiotherapy Clinique. Rectoscopy, endorectal ultrasonography and CT of the abdomen and pelvis were used to stage the patients preoperatively. All the patients received preoperative chemo-radiotherapy to a total dose of 50 Gy, 2 Gy a day, five times a week, with a concomitant daily oral intake of Capecitabine 1650 mg/m^2 (an average of 6 tablets at night). The treatment planning was done using computer tomography after bowl contrasting with an oral agent and the patient scanned in prone position. Clinical target volume (CTV) included the primary tumor and the regional pelvic lymph nodes—upper, middle and lower rectal, lower mesenterial, obturator, internal iliac up to the bifurcation of arteria iliaca communis, mesorectal, lateral sacral, presacral, and sacral promontorial. External iliac lymph nodes were included only in patients with T4 tumors infiltrating neighboring organs—the bladder, prostate, female genitalia. In patients with lower situated tumors, below linea dentata, the inguinal lymph nodes were also included. Planned target volume (PTV)—included the CTV with 1 cm margin. Patients were irradiated using three or four field technique and the dose in the small intestines was not higher than the tolerated 40–45 Gy. All patients were operable and only one surgery ended with positive margins—R1 resection.

Evaluation of the degree of tumor regression was performed and the following pathomorphologic changes of the surgical specimens and regional lymph nodes were investigated in detail: size of the tumor, tumor invasion, grade of tumor differentiation (G), lymphovascular invasion (LVI), state of resection margins and response to the preopera-

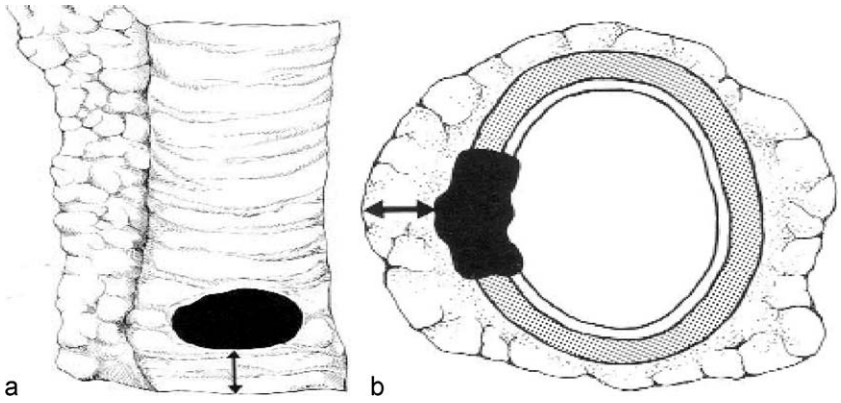

Fig. 1 – Defining the distal border of the tumor (a) and the circumferential border—invasion of the tumor (b).

tive treatment—Dworak's TRG. The basic rules followed by the pathomorphologists working with the surgical specimens were:

After surgery the pathomorphologic material is sent for investigation together with the medical history (anamnesis), the cTNM stage, type of surgery performed, type of preoperative treatment (chemo-radiotherapy or radiotherapy alone).

The surgical specimen is examined by the pathologist who determines tumor topography (Fig. 1), evaluates the quality of mesorectal excision and examines the periphery, the proximal and distal resection margins.

The description of the examining pathologist must include: histological type of the tumor: adenocarcinoma, colloid carcinoma, squamous cell carcinoma; the degree of malignancy: well differentiated G1, moderately differentiated G2, low differentiated G3 and undifferentiated G4; LVI; lateral, distal and proximal resection margins; number of regional lymph nodes examined; number of metastatic lymph nodes; pathomorphologic response grade.

Lymph node dissection is performed to thoroughly examine the meso of the rectum. As many lymph nodes as possible should be submitted for microscopic investigation to determine the postoperative stage of the disease. Meticulous microscopic examination of the lymph nodes identified during gross examination, as well as all tissue suspicious for metastasis should be performed. Even if there is no residual tumor in the rectal wall, there may be residual malignancy in the lymph nodes and therefore they should be thoroughly examined.

Staging of rectal cancer was performed according to the UICC system (1993)[10], with the "y" symbol identifying the cases following initial chemo-radiotherapy and differentiating them from the cases with no initial treatment. Determination of the depth of invasion and lymph node status were used for estimation of the effectiveness of preoperative chemo-radiotherapy. Clinical T staging was defined with usT when endorectal sonography was used for the initial diagnosis and with ctT when CT was used. Similarly, the status of the lymph nodes was defined as usN and ctN. Histologic evaluation of the surgical specimen following neoadjuvant treatment adds the symbol "y" for the primary tumor ypT and the regional lymph nodes ypN.

4. Results

Histologic changes following radiotherapy or chemo-radiotherapy were characterized not only by the existence or absence of tumor invasion in depth of the rectal wall, ypT, but also by the type of necrosis, fibrosis, specific vascular and cellular changes, not recognized in the tumors without neoadjuvant treatment, defined by Dworak's TRG.

Table 1 – .

	Histological type	G	usT	ctT	yR	ypT	GTR Dworak	usN	ctN	ypN	cTHM	pTNM	LVI
1	Adenoca.	2	3	3	0	2	1	1	1	0	IIIB	I	No
2	Adenoca.	3	4	4	1	4	0	0	1	0	IIIB	IB	Yes
3	Adenoca.	3	3	3	0	0	4	1	1	0	IIIB	0	No
4	Mucinous adenoca.	2	3	2	0	2	2	1	1	1	IIIB	ETA	No
5	Mucinous adenoca.	3	3	3	0	0	4	1	1	1	IIIB	0	No
6	Adenoca.	2	0	4	0	3	2	0	1	0	IIIB	IIA	Yes
7	Adenoca.	2	2	3	0	0	4	1	1	0	IIIA	0	No
3	Adenoca.	3	4	4	0	4	0	1	1	0	IIIB	IB	Yes
9	Adenoca.	2	3	3	0	2	1	0	1	0	IIIB	I	No
10	Adenoca.	3	0	4	0	3	1	0	1	0	IIIB	IIA	No

Histological type, grade, depth of infiltration and lymph node status by endosonography, CT and the pathologist (Tus, Tct, pT; Nus, Nct, pN). Clinical stages before radiochemotherapy and UICC stages after surgery. Presence (local and distant) or absence (yR0) of residual tumor after surgery and pathological examination and lymph vascular invasion (LVI).

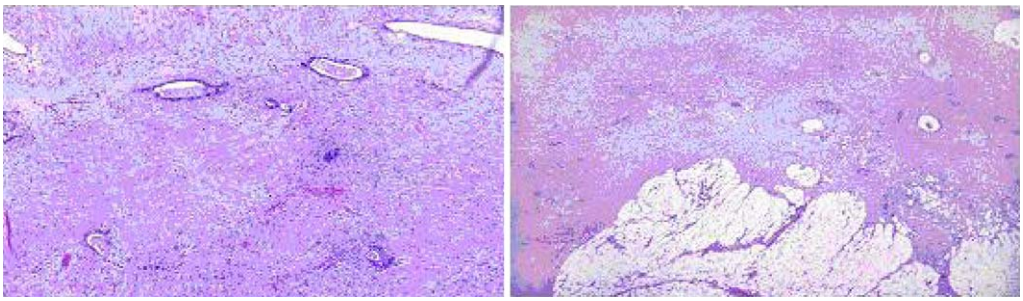

Fig. 2 – Morphologic features after radiochemotherapy with residual tumor cells.

The histologic type of the tumor for all patients is presented in Table 1, with 80% of the patients having adenocarcinoma and the rest, mucinous adenocarcinoma. The degree of malignancy of the tumor (G) showed that 50% of the cases were undifferentiated carcinoma G3 and 50% were moderately differentiated carcinoma G2. The depth of tumor infiltration, assessed by endorectal ultrasonography, usT, fully correlated with the ctT defined by CT.

Four of the patients (40%) were borderline operable before the preoperative chemo-radiotherapy. In 90% of the cases, radical surgery was managed and in only one patient positive margins were found—R1 resection. Four patients (40%) underwent Miles's rectal amputation, the other 60%, had sphincter-preserving surgeries—frontal or lower resection.

Histological verification of the surgical specimens and evaluation of the depth of tumor infiltration after the neoadjuvant treatment and surgery showed tumor regression in 80% of the patients. In 3 patients (30%), all with pretreatment usT3, full tumor control was achieved—ypT0. In 50%[5] of the patients, partial tumor regression was achieved, three regressed from usT3 to ypT2 and two from usT4 to ypT3. No change was observed in 2 patients (20%) with usT4.

According to Dworak's definition of tumor regression as the lack of tumor cells and with only fibrous changes present (total tumor regression), full response to treatment, TRG 4 and ypT0, was achieved in 30% of the patients. In 50% of the cases, partial regression was evaluated as Grade 1 and 2 with an additional examination of tumor features including necrosis, fibrosis, specific vascular and cellular changes. Tumor regression grading assessed tumor changes more precisely and did not correlate with tumor changes evaluated only by the depth of invasion defined as ypT. Eighty percent of the patients with pretreatment usN1 and ctN1 regressed to ypNo and in 20% with usN1 or ctN1 no regression of the metastatic changes in the lymph nodes was achieved—ypN1.

Following neoadjuvant treatment, full correlation between TRG 4, full tumor regression and the stage of disease evaluated as stage 0 was found, because in all cases with ypT0, full control was also achieved in the regional lymph nodes ypN0. In all the cases without tumor response TRG 0, ypN1 was observed.

All the patients included in the study were diagnosed as stage III. The following results from the combined chemo-radiotherapy and surgical treatment were assessed: 30% of the cases regressed to stage 0, 20% to stage I, 40% to stage II and only 10% remained as stage III. Limphovascular invasion was found in three patients (30%), and full tumor regression was not achieved in any of those cases and, vise versa, in all patients with full tumor regression no LVI was observed.

One of the patients (10%) developed severe enterocolitis in the early and late postoperative period, which was the reason for wound healing by secondary intention and prolonged hospital stay. No abnormal postoperative complications were reported in the rest of the cases.

In all specimens, in the tumor area, superficial or deep ulceration was found, as well as fibrosis of the submucosa,

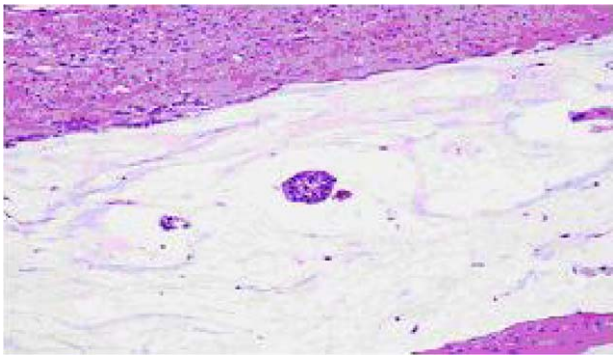

Fig. 3 – Morphologic characteristics after radiochemotherapy with isolated tumor cell in the area of colloid changes.

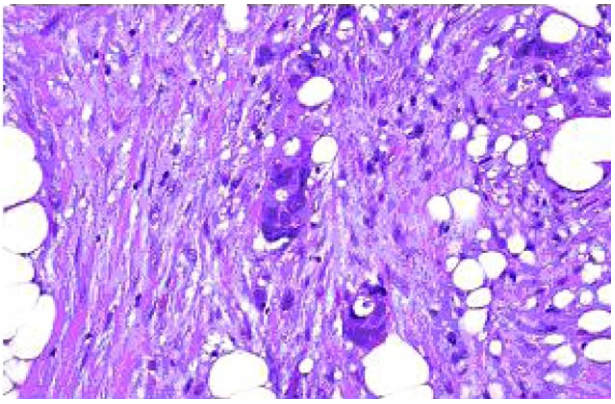

Fig. 4 – Solitary malignant cell deep in the rectal wall.

muscle layer and parirectal adipose tissue. Blood vessels exhibited radiogenic changes in the form of thickening and fibrosis of the intima and media, together with thrombotic obliteration. Vital and necrotic tumor cells were found in the lymph nodes, with no normal lymph node structure found. Changes in the lymph node capsule were seen in the form of fibrosis, especially in the sinus.

The use of preoperative chemo-radiotherapy modifies the histologic appearance of rectal cancer (Fig. 2), showing the replacement of neoplastic glands by hyaline fibrosis, lack of tumor necrosis, increased hyperchromasia and nuclear atypia in the present tumor cells. After neoadjuvant treatment tumor cells are replaced by fibrous or fibroinflammatory tissue. In the irradiated tissues, hemosiderin-laden (siderophagus) and the so-called "colloid response" or "mucin lakes" are observed.

Fibrotic changes are also found in the lymph nodes after treatment. Diffuse fibrotic changes and sparse lymphoid cells or focal fibrotic changes with mucinous substance are identified. Radiation changes are observed in both the capsule and the lymph node sinus.

5. Discussion and conclusion

Currently, there is no standard for pathologic workup of surgical specimen after neoadjuvant treatment. In the case of a visible tumor, a standard pathologic protocol for handling the material is used, but if there is no visible tumor some authors suggest embedding the whole suspicious area and performing a thorough search for residual tumor cells. Some authors using this technique report finding vital tumor cells in all cases after a preoperative treatment.[2] Hiotis et al.[11] and Shia et al.[12] achieved a full histologically proven response (lack of tumor cells) in 10–14% of the patients who underwent neoadjuvant treatment, which shows that it is possible to fully eliminate tumor cells. The high percentage of tumor control in our study (30%) is connected with the small number of patients included. Picciochi et al.[13] did not detect any tumor cells in 15% of studied cases after neoadjuvant treatment. If no tumor cells are identified on the initial material, it is recommended to perform further investigation of the whole specimen with additional step sectioning and searching for tumor cell foci. In our study, full tumor control for the primary tumor correlates with tumor control in the regional lymph nodes. In case of suspicion for tumor persistence, imunohistochemical investigation should be performed with antibodies for cytokeratin.

Some additional characteristics provided by the pathologist correlate with the prognosis of the disease. For example, vascular invasion of the rectal wall is an unfavorable parameter corresponding to a high risk for development of liver metastases.[14] In our investigation, in 2 out of 3 patients with LVI, there was no tumor regression after the chemo-radiotherapy. Ulceration of the peritoneum due to tumor cells is a poor prognostic sign. Accurate pathomorphologic evaluation of the circumferential resection margins can predict the risk of local relapse.

The observed colloid changes in resection specimen should not to be confused by the pathologist with colloid rectal carcinoma. This changes are less basophilic in comparison with the non-cured colloid carcinoma. The presence of mucin lakes dictates a meticulous examination for residual tumor cells (Fig. 3).

If such cells are identified, it is possible to find more of them scattered in other parts of the rectal wall or perirectal tissue. In the case of full regression of superficial lesions, neoplastic cells can be found deep in the rectal wall (Fig. 4).

The residual malignant cells can acquire eosinophilic cytoplasm or undergo oncocytic differentiation. The most common alteration after neoadjuvant chemo-radiotherapy is eosinophilic cytoplasm due to nuclear atypia. Irradiated adenocarcinomas can exhibit changes such as neuroendocrine differentiation. These changes can be readily recognized by routine microscopic examination. Neuroendocrine cells can be demonstrated by using antibodies to chromogranin.

The treatment results and insignificant toxicity prove this method as a standard in the combined treatment for locally advanced rectal carcinoma. A longer follow up period and a larger study with significant number of patients is necessary to find the correlation between treatment results and observed grades of tumor regression, which assess tumor response more precisely, compared to the tumor response evaluated by the change of tumor infiltration depth and the changes in lymph nodes or downsatging.

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