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Gender-related prognostic significance of clinical and biological tumor features in rectal cancer patients receiving short-course preoperative radiotherapy

Anna Gasinska[a,*], *Zbigniew Darasz*[b], *Agnieszka Adamczyk*[a], *Beata Biesaga*[a], *Joanna Niemiec*[a], *Marian Reinfuss*[c]

[a] *Department of Applied Radiobiology, Maria Sklodowska – Curie Memorial Cancer Centre and Institute of Oncology, Cracow Branch, Poland*

[b] *Department of Surgery, Maria Sklodowska – Curie Memorial Cancer Centre and Institute of Oncology, Cracow Branch, Poland*

[c] *Department of Radiation Oncology, Maria Sklodowska – Curie Memorial Cancer Centre and Institute of Oncology, Cracow Branch, Poland*

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ABSTRACT

Aim: To study the prognostic value of clinical and biological features of rectal cancer and potential gender differences in patients' overall survival (OS), local recurrence-free survival (RFS) and metastasis-free survival (MFS) after short-course preoperative radiotherapy (SCRT) with short or long interval between RT and surgery (break).

Background: The length of the interval between RT and surgery in SCRT is debatable and gender-related differences in patients survival are not established yet.

Materials and methods: 126 patients received SCRT with 5 Gy dose per fraction during 5 days, followed by radical surgery after short break ≤17 days, and a long break >17 days. Pre-treatment tumor proliferation (bromodeoxyuridine labeling index, BrdUrdLI and S-phase fraction) was evaluated by flow cytometry and proteins: CD34, Ki-67, GLUT-1, Ku70, BCL-2, P53 expression was studied immunohistochemically.

Results: The studied group included 84 men and 42 women. There were 33, 76, and 17 cTNM (AJCC) tumor stages I, II, III, respectively. The median follow-up time was 53.3 months (range 2–142 months). For the whole group Cox multivariate analysis revealed that tumor grade (G > 1), interval between RT and surgery >17 days, pTNM stage >1 and P53 positivity + BrdUrdLI > 7.9% were negative prognostic factors for OS. Tumor aneuploidy and MVD > 140.8 vessels/mm^2 were important for RFS. pTNM stage > 1 and P53 positivity combined with BrdUrdLI > 7.9% were risk predictors for MFS. Based on tumor biological features,

* *Corresponding author at*: Department of Applied Radiobiology, Maria Sklodowska – Curie Memorial Cancer Centre and Institute of Oncology, Cracow Branch, Garncarska 11, 31-115 Cracow, Poland.
E-mail address: z5gasins@cyf-kr.edu.pl (A. Gasinska).

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gender-related difference in OS, RFS, and MFS were observed. In multivariate analysis, male patients age > 62 years and break >17 days only appeared to be significant for OS.
Conclusions: In male rectal patients treated with SCRT, breaks between RT and surgery >17 days should be avoided because they negatively influence patients' survival.

1. Background

Treatment of locally advanced but resectable rectal carcinoma mainly utilizes two modalities of preoperative radiotherapy (RT): either(1) long course radio-chemotherapy with 50.4 Gy in 25–28 fractions and surgery after 4 to 8 weeks break, or (2) short course RT (SCRT) with 5×5 Gy followed by immediate surgery.[1–3] In Europe, SCRT has become the standard treatment for patients with resectable cancer, and seems to offer the same reduction of local recurrence rate.[2,4] However, in some centers, apart from treatment with 5×5 Gy with a short (5–7 days) break, also 5×5 Gy and a long (4–5 weeks) break schedule before surgery is applied, and both were used in our study. Some authors[1,5–7] suggest that SCRT with delayed surgery is suitable for early cancer.

Personalized treatment based on predictions for patients' outcome requires early characterization of patients' sensitivity to treatment. It is important to verify if some tumor biological factors may be predictive for early and late tumor response (recurrence and metastasis rate) and patients survival.

Therefore, prognostic value of selected proteins was assessed: the P53 – tumor suppressor protein, BCL2 – anti-apoptotic protein, Ki-67 (MIB1), a marker of cell proliferation, GLUT-1 – a hypoxia-regulated membrane protein glucose transporter-1, allowing the energy-independent transport of glucose across the cell membrane, CD-34 – a protein that indicates endothelial cells of blood vessels and, therefore, determines tumor vascularization and Ku70 – a protein involved in the DNA repair process. Earlier, we studied the pretreatment tumor proliferation rate (bromodeoxyuridine labeling index; BrdUrdLI, S-phase fraction; SPF)[8,9] and expression of six proteins to assess prognostic significance for early tumor response.[10] We showed that higher Ki-67 and lower BCL2 expression were correlated with pathological tumor responses.

2. Aim

Currently, we would like to assess (1) prognostic significance of tumor proliferation (BrdUrdLI, S-phase fraction (SPF), Ki-67 LI) and expression of six proteins for patients' overall survival (OS), local recurrence-free survival (RFS) and metastasis-free survival (MFS) after SCRT with a short (≤17 days) or long (>17 days) interval between RT and surgery (break), (2) to check if potential gender differences exist in patients' long term characteristics.

3. Materials and methods

3.1. *Patients*

A total of 126 patients with rectal carcinoma were included in the study in which a tumor biopsy was taken before preoperative RT performed between November 2003 and January 2006. There were 84 males and 42 females with a mean age for the entire group of 62.0 (range 30–82) years.

3.2. *Treatment*

This is a retrospective analysis of two patient cohorts treated with SCRT (5 Gy/5 days) and surgery: one with an interval ≤17 days (median) and the other >17 days before surgery. In the first cohort there were 64 and in the second 62 patients. Inclusion and exclusion criteria and detailed information on irradiation and surgery were presented earlier.[8] Tumors were classified according to the WHO classification of intestinal carcinoma[11] and clinical (cTNM) and pathological (pTNM) stages according to the AJCC TNM 2010 classification.[12]

3.3. *Flow cytometric analysis and immunohistochemistry*

Tumor samples from each of the 126 patients were taken before RT. One fragment was used for flow cytometric analysis (BrdUrdLI, SPF) and the other for immunohistochemical evaluation of proteins expression. The details of flow cytometric analysis were described earlier.[9] BrdUrdLI was calculated as a percentage of cells which incorporated BrdUrd. DNA ploidy and SPF were calculated from the DNA profile with ModFit software.[8] The presence of aneuploidy was estimated by evaluating the DNA index, i.e. the ratio of the modal DNA fluorescence of abnormal to normal G1/G0 cells.

Expression of examined proteins was visualized by immunohistochemical staining. Detailed information on the staining procedure was presented earlier.[10] Immunoreactivity of Ki-67, GLUT-1 and Ku 70 was scored as the number of positive tumor cells to the total number of counted tumor cells – labeling index (LI). P53 protein expression was considered positive if >25% of tumor cells showed immunopositivity and BCL-2 if expression was noted in any cancer cell. For CD34 expression, 7–10 high power (400×) tumor fields were counted for each patient. The mean vessel count per 1 mm^2 of tumor area (microvessel density; MVD) was used in the analysis. Immunohistochemical assessments were performed by researchers blinded to clinical outcome.