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

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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,

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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.

3.4. Statistical analysis

Statistical analysis was performed with STATISTICA vs.9. For determination of mean values for variables and standard errors of means (SE), the descriptive statistics were used. Intergroup differences in the mean values were tested with one-way ANOVA test or Student's t-test. Associations between investigated categorical parameters and clinicopathological variables were evaluated by Pearson's Chi^2 test. Differences were considered significant at P value of <0.05. Survival was estimated using the Kaplan–Meier method.[13] The difference in survival rates between groups was assessed by the log-rank test. In univariate analysis, for the break between RT and surgery, that is the time interval between the last day of RT and surgery, and overall treatment time (OTT), that is the time from the beginning of RT to surgery, median values were used as cut-off points. For biological variables, these were mean or median values. Survival time was calculated from the date of surgery to the date of death, local relapse, metastasis or last follow-up. Multivariate analysis to determine prognostic factors for OS, local RFS and MFS was carried out by means of Cox's regression model in which all significant prognostic factors from the univariate analysis were entered. The Cox analysis was performed for all patients and separately for the male and female subgroups.

4. Results

4.1. Patients

A total of 126 consecutive patients were included in the study. Clinical and pathomorphological characteristics of the analyzed patients are presented in Table 1.

The time interval between the end of irradiation and surgery averaged 22.0 days (range 3–53) and did not differ significantly for males and females (Table 1). In the whole group, OTT ranged from 7 to 58 days (Table 1).

At the time of recruitment no significant differences between the male and female subgroups were found for all clinical parameters apart from the pT category (Table 1). In addition, the mean time to recurrence and metastasis did not differ between male and female patients (23.5 vs. 15.2 months, $P = 0.234$; 14.0 vs. 23.2 months, respectively, $P = 0.092$) (Table 1).

There was also no significant difference in the assessed prognostic parameters for patients treated with short or long break in the treatment apart from pTNM, and tumor grade (Table 1). In addition, no significant difference in prognostic parameters (listed in Table 1) was indicated between a short/long break within the male and female cohorts.

4.2. Biological markers

BrdUrdLI, SPF and DNA ploidy was assessed in all tumors. Immunostaining for CD34, Ki-67, GLUT-1, Ku70, P53 and BCL-2 protein was performed on 88, 108, 106, 102, 106 and 90 tumors, respectively, as in some cases there was insufficient material left in the paraffin block. Expression of CD34, Ki-67 and Ku70 was observed in all cases. Immunonegativity was noted in 21 (19.8%) cases of GLUT-1, 44 (41.5%) of P53 (LI $\leq$ 25%), and 63 (70.0%) of BCL-2. The mean MVD was 140.8 vessels/mm^2, mean MIB-1LI, GLUT-1LI, Ku70LI and P53LI was 52.8%, 14.7%, 74.5% and 43.6%, respectively. These values did not differ between the male and female subgroups. In the whole group, median BrdUrdLI was 7.9% $\pm$ 0.5 and mean SPF was equal to 21.0 $\pm$ 0.9. Sixty-six (50.8%) aneuploid tumors were identified. There was no significant correlation between analyzed markers and cTNM and pTNM stage or grade.

4.3. Overall survival analysis

Thirty (23.8%) not cancer-specific deaths (20 males and 10 females) were indicated. The median follow-up time was 53.5 months (Table 1). Overall, 10-year survival was 69% in females and 59% in males ($P = 0.832$). Younger patients ($\leq$62.0 years) with well differentiated tumors ($P = 0.013$) and those with less advanced pTNM ($P = 0.040$) survived significantly better (Table 2). However, in the male subgroup, only patients' age and tumor grade were significant, and in the female subgroup none of these parameters were important (data not shown). In the whole patient cohort a break ($\leq$17 days) in the treatment, OTT ($\leq$22 days), and P53 negativity were significant prognostic factors for overall survival (Table 2). Patients treated with SCRT with a break longer than 17 days survived significantly worse than those treated with a shorter break ($P = 0.030$). This was particularly important for male patients (Fig. 1a) and not significant in the female subgroup (Fig. 1b). Only when the population was separated by gender, was favorable association of low Ku70 expression with OS observed in the female patients (Fig. 1c, d), and it was the only parameter significant for OS in the female subgroup. P53 negativity was a predictive factor for long OS but significant only in the male subgroup (Fig. 1e).

4.4. Recurrences

In 18 (14.3%) patients, local recurrences were indicated: 10 (11.9%) in male and 8 (18.2%) in female patients. In a break >17 days, recurrences appeared in 6 (60%) males and in 6 (75%) females. The univariate analysis revealed that higher pTNM, a break $\geq$ 21.5 days (significant cut-off point), MVD > 140.8/mm^2, BrdUrdLI > 7.9% and tumor aneuploidy (border significance) were unfavorable prognostic factors for RFS (Table 2). The different influence of tumor biological factors on RFS was noticed for males and females (Fig. 2a–d). Male patients with low tumor proliferation (Fig. 2a) and low MVD (Fig. 2c) had higher probability of RFS. In females, these differences were not significant (Fig. 2b, d).

4.5. Metastases

Metastases appeared in 23 (18.2%) patients: 14 (16.7%) males and 9 (21.4%) female patients. In treatment with a break $\leq$17 days, metastases were made in 7 males (50%) and in 5 females (55%). While in treatment with a long break, cancer spread was observed in 7 (50%) males and 4 (44.4%) female patients. There was no statistical difference in MFS between male and female patients ($P = 0.561$) (Table 2). In univariate analysis,

Table 1 – Gender-related clinicopathological and treatment characteristics of rectal cancer patients.

Characteristics	All	Sex			Break in treatment		
		Men	Women	P-value	≤17 days	>17 days	P-value
Age (years)							
Median	126[a], 62.0	84, 62.0	42, 61.5	0.844	64, 61.5	62, 63.5	0.370
(mean ± SD)	(61.3 ± 9.8)	(61.5 ± 9.2)	(61.1 ± 11.1)		(60.6 ± 10.3)	(62.1 ± 9.4)	
Clinical stage (AJCC)							
I	33[a] (26.2%)[b]	24 (28.6%)	9 (21.4%)	0.369	13 (20.3%)	20 (32.3%)	0.308
II	76 (60.3%)	51 (60.7%)	25 (59.5%)		42 (65.6%)	34 (54.8%)	
III	17 (13.5%)	9 (10.7%)	8 (19.1%)		9 (14.1%)	8 (12.9%)	
Clinical tumor category							
T2	34[a] (27.0%)[b]	25 (29.7%)	9 (21.4%)	0.282	14 (21.9%)	20 (32.3%)	0.417
T3	85 (67.5%)	56 (66.7%)	29 (69.1%)		46 (71.9%)	39 (62.9%)	
T4	7 (5.5%)	3 (3.6%)	4 (9.5%)		4 (6.2%)	3 (4.8%)	
Clinical node category							
N0	109[a] (86.5%)[b]	75 (89.3%)	34 (80.9%)	0.197	55 (85.9%)	54 (87.1%)	0.849
N1	17 (13.5%)	9 (10.7%)	8 (19.1%)		9 (14.1%)	8 (12.9%)	
Pathological stage (AJCC)							
0	6[a] (4.8%)[b]	4 (4.8%)	2 (4.8%)	0.567	0 (0.0%)	6 (9.7%)	0.031
1	56 (44.4%)	40 (47.6%)	16 (38.1%)		28 (43.8%)	28 (45.1%)	
2	22 (17.5%)	12 (14.3%)	10 (23.8%)		15 (23.4%)	7 (11.3%)	
3	42 (33.3%)	28 (33.3%)	14 (33.3%)		21 (32.8%)	21 (33.9%)	
Pathological tumor category							
pT0	6[a] (4.8%)[b]	4 (4.4%)	2 (4.8%)	0.045	0 (0.0%)	6 (9.7%)	0.086
pT1	10 (7.9%)	6 (7.2%)	4 (9.5%)		4 (6.3%)	6 (9.7%)	
pT2	51 (40.5%)	39 (46.4%)	12 (28.6%)		27 (42.2%)	24 (38.7%)	
pT3	52 (41.3%)	28 (33.3%)	24 (57.1%)		28 (43.7%)	24 (38.7%)	
pT4	7 (5.5%)	7 (8.3%)	0 (0.0%)		5 (7.8%)	2 (3.2%)	
Pathological node category							
pN0	84[a] (66.7%)[b]	56 (66.7%)	28 (66.7%)	0.551	43 (67.2%)	41 (66.1%)	0.462
pN1	20 (15.9%)	15 (17.8%)	5 (11.9%)		8 (12.5%)	12 (19.4%)	
pN2	22 (17.4%)	13 (15.5%)	9 (21.4%)		13 (20.3%)	9 (14.5%)	
Histological grade							
G1	32[a] (25.8%)[b]	19 (22.6%)	13 (32.5%)	0.495	11 (17.2%)	21 (35.0%)	0.025
G2	89 (71.8%)	63 (75.0%)	26 (65.0%)		50 (78.1%)	39 (65.0%)	
G3	3 (2.4%)	2 (2.4%)	1 (2.5%)		3 (4.7%)	0 (0.0%)	
OTT (days)							
Median	126[a], 22.0	84, 19.0	42, 29.5	0.261	64, 15.0	62, 37.5	–
(mean ± SD)	(26.3 ± 12.7)	(25.4 ± 12.3)	(28.1 ± 13.3)		(14.8 ± 3.4)	(38.1 ± 6.0)	
Interval between RT and surgery (days)							
Median	126[a], 17.0	84, 14.0	42, 29.5	0.256	64, 10.0	62, 32.5	–
(mean ± SD)	(21.3 ± 12.7)	(20.4 ± 12.4)	(28.1 ± 13.3)		(9.8 ± 3.4)	(33.1 ± 6.0)	
Type of surgery							
Abdominoperineal excision	53[a] (42.1%)[b]	40 (47.6%)	13 (30.9%)	0.074	35 (54.7%)	38 (61.3%)	0.453
Anterior resection	73 (57.9%)	44 (52.4%)	29 (69.1%)		29 (45.3%)	24 (38.7%)	
Overall survival (months)							
Median	126[a], 53.5	84, 53.5	42, 53.3	0.403	64, 54.3	62, 50.8	0.182
(mean ± SD)	(53.3 ± 24.0)	(54.6 ± 24.2)	(50.8 ± 23.6)		(56.2 ± 25.1)	(50.4 ± 22.7)	
Time to recurrence (months)							
Median	18[a], 17.7	10, 23.5	8, 15.2	0.234	6, 13.0	12, 20.5	0.947
(mean ± SD)	(19.7 ± 10.8)	(22.4 ± 12.6)	(16.2 ± 7.5)		(19.4 ± 15.8)	(19.8 ± 8.2)	
Time to metastasis (months)							
Median	23[a], 17.5	14, 14.0	9, 23.2	0.093	12, 22.8	11, 16.0	0.583
(mean ± SD)	(19.3 ± 11.7)	(16.0 ± 11.0)	(24.4 ± 11.5)		(20.6 ± 13.2)	(17.8 ± 10.4)	

[a] Number of cases.
[b] Percentage of cases within the subgroups.

Table 2 – Univariate analysis for overall survival (OS), local recurrence-free survival (RFS), and metastasis-free survival (MFS) for 126 rectal cancer patients.

Variable	OS			RFS			MFS		
	RR	95% CI	P-value*	RR	95% CI	P-value*	RR	95% CI	P-value*
Age (years)									
≤62.0	1.00	Reference		1.00	Reference		1.00	Reference	
>62.0	4.61	1.98–10.75	<0.001	1.33	0.53–3.35	0.551	0.67	0.28–1.58	0.355
Gender									
Male	1.00	Reference		1.00	Reference		1.00	Reference	
Female	1.09	0.51–2.33	0.832	1.76	0.69–4.45	0.236	1.28	0.55–2.96	0.561
Grade									
1	1.00	Reference		1.00	Reference		1.00	Reference	
2–3	5.08	1.21–21.39	0.013	1.74	0.50–6.02	0.372	2.33	0.69–7.86	0.157
Clinical stage (AJCC)									
I	1.00	Reference		1.00	Reference		1.00	Reference	
II–III	2.04	0.78–5.34	0.125	1.03	0.37–2.90	0.950	1.78	0.61–5.24	0.279
Pathological stage (AJCC)									
0–1	1.00	Reference		1.00	Reference		1.00	Reference	
2–3	2.17	1.02–4.65	0.040	2.88	1.03–8.08	0.036	11.92	2.79–50.86	<0.001
Break in the treatment									
≤17 days	1.00	Reference		1.00	Reference		1.00	Reference	
>17 days	2.26	1.06–4.84	0.030	2.58	0.97–6.89	0.050	0.99	0.44–2.26	0.991
Cut off point 21.5 days									
OTT									
≤22 days	1.00	Reference		1.00	Reference		1.00	Reference	
>22 days	2.32	1.09–4.97	0.030	2.32	0.87–6.18	0.100	1.03	0.46–2.34	0.991
Ki-67LI									
≤ 52.8%	1.00	Reference		1.00	Reference		1.00	Reference	
>52.8%	0.74	0.36–1.54	0.422	0.57	0.22–1.48	0.246	0.41	0.16–1.09	0.065
Ku70									
≤74.9%	1.00	Reference		1.00	Reference		1.00	Reference	
>74.9%	1.53	0.72–3.24	0.264	1.21	0.48–3.06	0.690	0.98	0.39–2.48	0.964
GLUT-1									
<14.7%	1.00	Reference		1.00	Reference		1.00	Reference	
≥14.7%	0.84	0.38–1.87	0.675	0.49	0.16–1.49	0.193	0.46	0.15–1.39	0.155
CD34 (MVD)									
<140.8 vessels/mm^2	1.00	Reference		1.00	Reference		1.00	Reference	
≥140.8 vessels/mm^2	1.23	0.52–2.84	0.634	3.17	0.97–10.30	0.043	1.85	0.69–4.96	0.216
P53									
≤25.0%	1.00	Reference		1.00	Reference		1.00	Reference	
>25.0%	3.29	1.24–8.79	0.010	1.12	0.43–2.94	0.820	2.94	0.97–8.95	0.043
BrdUrdLI									
≤7.9%	1.00	Reference		1.00	Reference		1.00	Reference	
>7.9%	2.03	0.96–4.29	0.058	2.99	1.06–8.40	0.029	3.95	1.47–10.64	0.003
BCL-2									
Positivity	0.62	0.27–1.42	0.073	0.38	0.12–1.23	0.404	0.39	0.12–1.23	0.145
Negativity	1.00	Reference		1.00	Reference		1.00	Reference	
SPF									
≤21.0%	1.00	Reference		1.00	Reference		1.00	Reference	
>21.0%	1.57	0.76–3.25	0.215	1.47	0.58–3.72	0.414	2.23	0.95–5.27	0.059
DNA ploidy									
Diploid	1.00	Reference		1.00	Reference		1.00	Reference	
Aneuploid	0.97	0.47–1.99	0.936	2.60	0.93–7.29	0.059	2.14	0.88–5.20	0.085

* P values from log-rank test.

higher pTNM, P53 immunopositivity and BrdUrdLI >7.9% were unfavorable prognostic factors for MFS (Table 2). In addition, for MFS the different biological factors were significant for male and female patients (Fig. 3a–f). Female patients with slowly proliferating (Fig. 3b) and diploid tumors (Fig. 3d) had significantly higher probability of MFS. In male patients, P53 negative tumors less frequently created metastasis (Fig. 3e) than in females with the same status (Fig. 3f).

In our series, no female patient with slowly proliferating and only one with a diploid tumor developed metastases, whereas, for aneuploid and fast proliferating tumors, MFS in females was lower than in males (Fig. 3a–d).

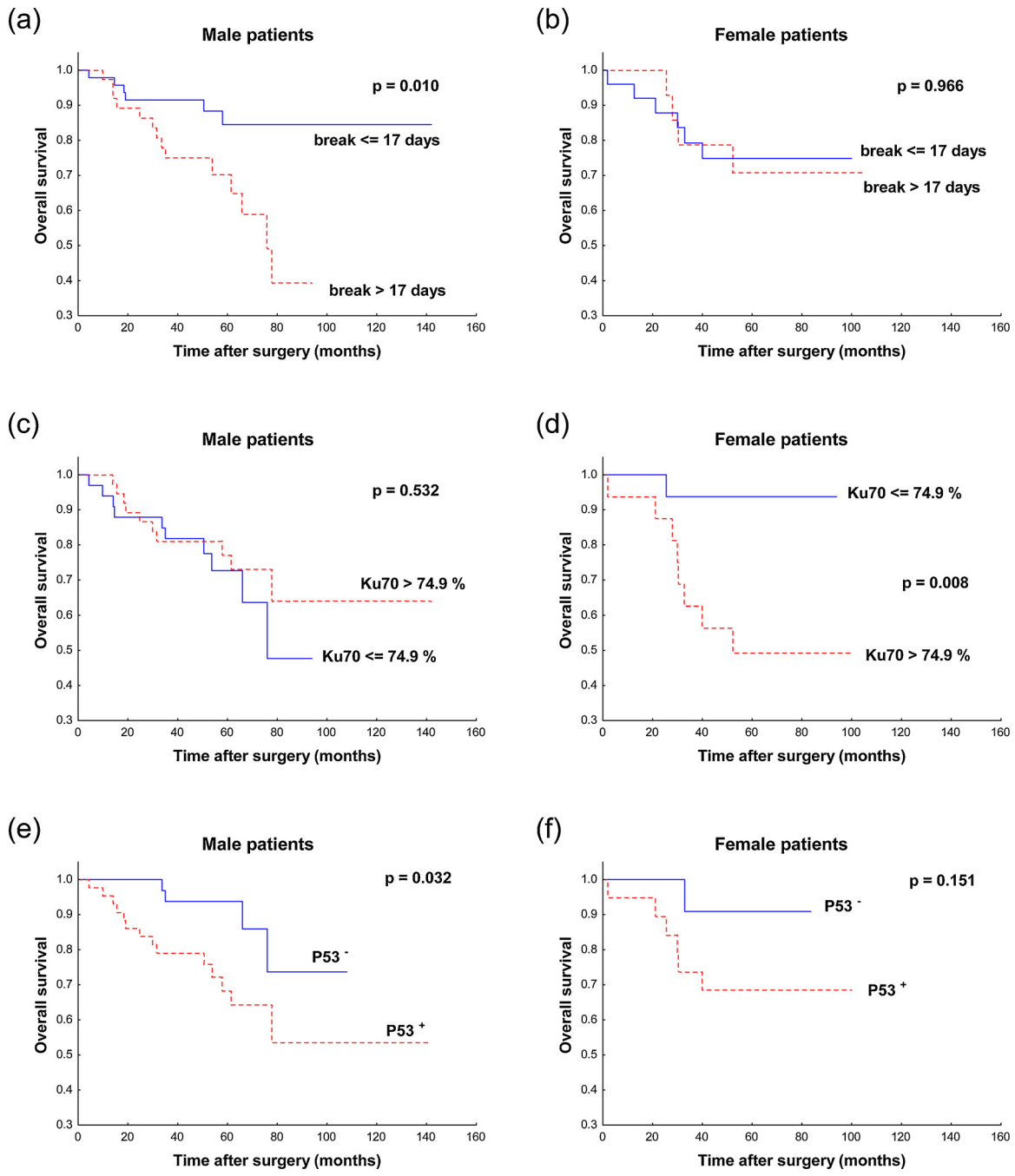

Fig. 1 – Gender-related differences in the influence of break in the treatment, Ku70, and P53 expression on overall survival of male (a, c, e) and female (b, d, f) rectal cancer patients.

4.6. *Multivariate Cox analysis*

Univariate analysis revealed a different influence of biological parameters between the patient genders. Multivariate Cox analysis demonstrated that for the whole patients' cohort higher tumor grade, pTNM stage, a break > 17 days and P53 positivity + BrdUrdLI > 7.9% appeared to be independent negative factors for OS. In the male patients subgroup, however, only age > 62 years and a long break were significant (Table 3). In the female subgroup multivariable analysis was not performed because only one variable (Ku70) was significant in univariate analysis.

In the whole group high MVD and tumor aneuploidy were negative prognosticators for RFS. Higher tumor proliferation (BrdUrdLI > 7.9%) was a negative prognostic factor for RFS in the male subgroup. In the female subgroup the multivariate analysis could not be performed because only one variable (pTNM stage) was significant. Higher pTNM stage, and P53 positivity combined with higher proliferation (BrdUrdLI > 7.9%) were important for MFS in the whole cohort (Table 3). In female

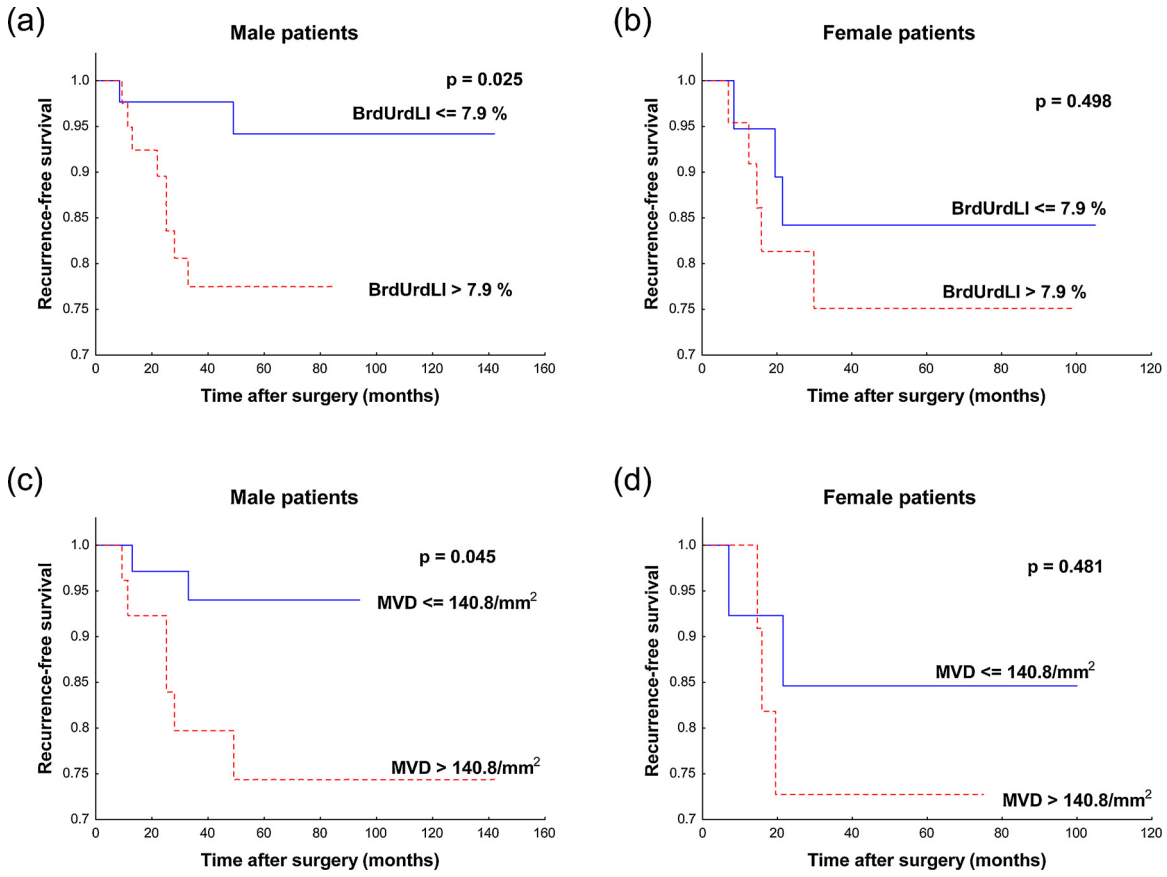

Fig. 2 – The difference in recurrence-free survival between male and female rectal cancer patients based on BrdUrdLI (a, b) and microvessel density (MVD) (c, d).

patients the analysis for MFS could not be performed because there are a low number of uncensored cases in some variables (100% MFS for females with lower pTNM stage and slowly proliferating tumors).

5. Discussion

We analyzed patients with locally advanced rectal cancer after short-course preoperative RT. The difference in overall survival between male and female patients was not found, although, gender-related difference in the influence of clinical and tumor biological factors for OS, RFS and MFS was shown.

Multivariate Cox analysis revealed, for the first time, that the interval between RT and surgery ≤17 days, lower tumor grade and pTNM stage, P53 negativity, or low tumor proliferation (BrdUrdLI ≤ 7.9%) are related to higher OS in patients with locally advanced rectal cancer. Tumor diploidy and lower MVD were identified as independent prognostic factors for higher RFS. Higher pTNM stage and P53 overexpression together with faster tumor proliferation (BrdUrdLI > 7.9%) led to lower MFS.

A gender-related difference in the influence of biological parameters on OS, RFS and MFS was observed. We believe that these differences were not probably influenced by clinical factors as differences in clinicopathological factors (apart from a higher pT in the male subgroup), and treatment characteristics between the male and female subgroups were not statistically important. These parameters were also not different in patients' cohorts treated with a short and long break, apart from pTNM stage and grade. Stage pTNM = 0 observed in 6 patients treated with a long break was expected, as about two weeks after RT (short break) downstaging is difficult to indicate. Significantly higher grade in male tumors treated with SCRT with a short break if important should give adverse clinical effect than observed.

Sex-specific differences in biological factors and relevant underlying mechanisms may be caused, for example, by hormonal signaling (estrogen, progesterone) which can act concurrently, either stimulating or suppressing immune response,[14] cell proliferation and radiosensitivity. It was previously shown that gender differences in tumor response may be caused by: epigenetic changes (higher frequency of DNA hypermethylation in males rather than females), which could be down-regulated by estrogen,[15,16] or estrogen receptor α expression which can protect p53 from deactivation.[17] This could confirm our results showing slightly higher OS in female rather than male patients with negative P53 (Fig. 1e, f).

In addition, as shown in our study, higher tumor cell radiosensitivity (less effective DNA damage repair in Ku70 ≤74.9% subgroup) in female rather than male tumors was probably the cause of higher female survival. This finding is in agreement with recent results of Alsbeih et al.,[18]

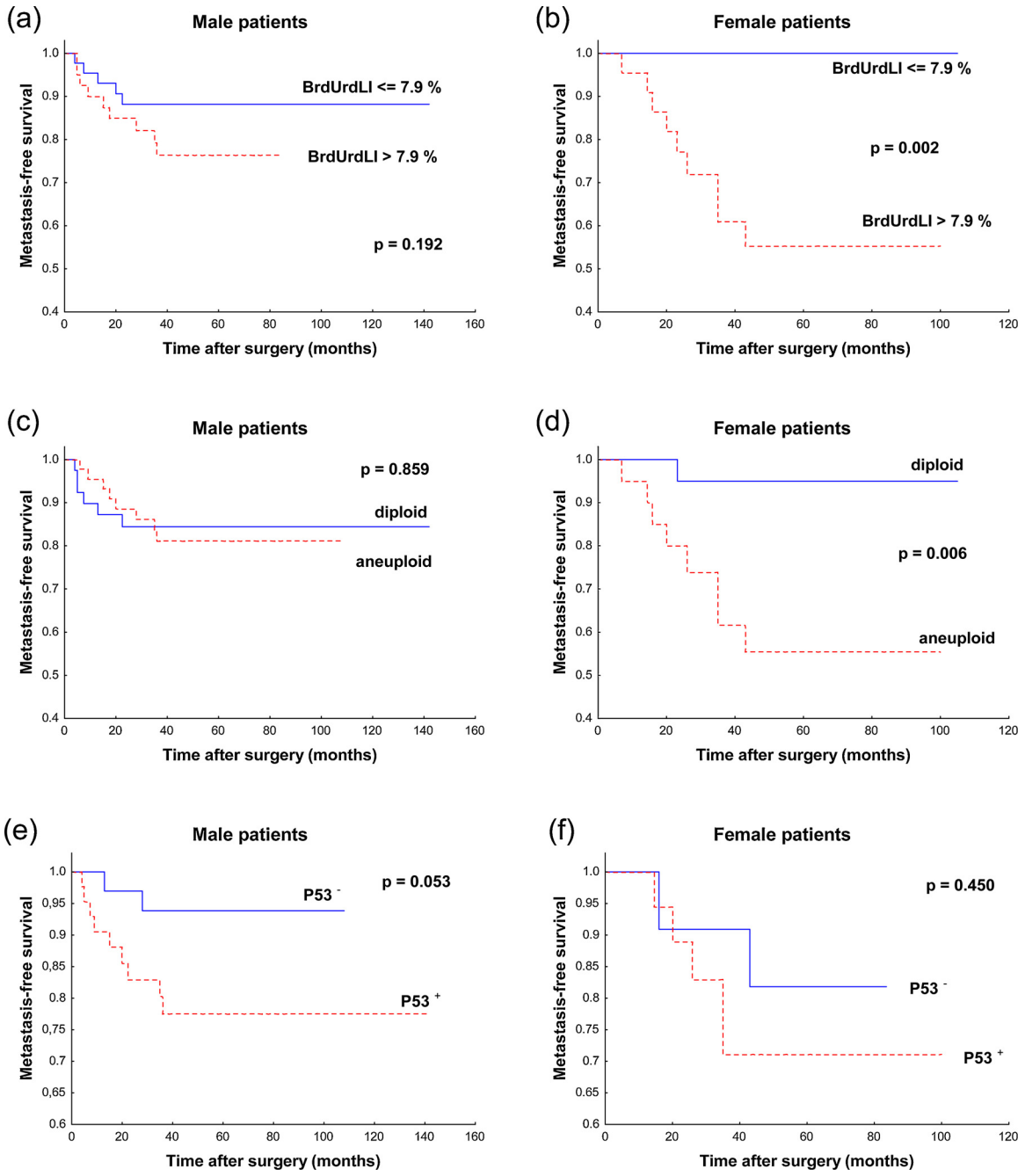

Fig. 3 – Influence of patient's gender and BrdUrdLI (a, b), tumor DNA ploidy (c, d) and P53 expression (e, f) on metastasis-free survival of rectal cancer patients.

where based on non-transformed fibroblast cell strains, difference in radiosensitivity between males and females was shown.

Tumor cell proliferation, efficiency of DNA repair damage and P53 activity are known important factors in RT response. These parameters may be responsible for tumor repopulation within the interval between RT and surgery. In our study time from RT to surgery >17 days appeared to be an unfavorable prognostic factor for OS, especially in males. Other authors who used SCRT or different adjuvant treatments did not show the significant influence of break length on OS (but they did not analyze tumor biological factors).[2–4] We believe that the lack of break influence on OS may be true only for patients treated more intensively: CHT/RT with dose of 50.4 Gy,[19] SCRT with immediate surgery, or two-week course of preoperative chemoradiotherapy[20] but not for those treated with SCRT with a long break and BED_{10} of 37.5 Gy (NTD = 31.2 Gy), like in the case of our series. Therefore, our data are in strong contradiction with the results of Rega et al.[6] who after SCRT recommend a break before the surgery longer than 8 weeks. Our opinion may support the results of Lefevre and colleagues[21] who reported that surgical morbidity was higher when surgery was delayed for 11 weeks rather than for 7 weeks after radiochemotherapy.

Table 3 – Multivariate Cox analysis performed to determine prognostic factors for overall survival (OS), recurrence-free survival (RFS) and metastasis-free survival (MFS) for rectal cancer patients.

All patients				Male patients			
Variable	RR	95% CI	P-value	Variable	RR	95% CI	P-value
OS							
Grade				Age			
1	1.00	Reference		≤62 years	1.00	Reference	
2–3	6.48	1.43–29.35	0.015	>62 years	4.68	1.55–14.08	0.006
Break in the treatment				Break in the treatment			
≤17 days	1.00	Reference		≤17 days	1.00	Reference	
>17 days	4.69	1.89–11.60	0.001	>17 days	2.78	1.06–7.28	0.037
Pathological stage (AJCC)							
0–1	1.00	Reference					
2–3	2.84	1.19–6.78	0.019				
P53 and BrdUrdLI							
P53 ≤ 25.0% or BrdUrdLI ≤7.9%	1.00	Reference					
P53 > 25.0% and BrdUrdLI > 7.9%	2.82	1.26–6.30	0.011				
RFS							
CD34 (MVD)				BrdUrdLI			
<140.8 vessels/mm^2	1.00	Reference		≤7.9%	1.00	Reference	
≥140.8 vessels/mm^2	3.82	1.16–12.52	0.027	>7.9%	4.97	1.05–23.45	0.043
DNA ploidy							
Diploid	1.00	Reference					
Aneuploid	4.18	1.14–15.31	0.031				
MFS							
Pathological stage (AJCC)				Pathological stage (AJCC)			
0–1	1.00	Reference		0–1	1.00	Reference	
2–3	8.31	1.90–36.37	0.005	2–3	7.94	1.77–35.50	0.007
P53 and BrdUrdLI							
P53 ≤ 25.0% or BrdUrdLI ≤ 7.9%	1.00	Reference					
P53 > 25.0% and BrdUrdLI > 7.9%	2.99	1.17–7.61	0.022				

In the entire group Ku70 expression and MVD were not important prognostic factors but appeared significant in at least one subgroup when analyzed separately for males and females. This shows a potential importance of analyzing rectal cancer data both with and without gender as a stratifying factor.

In our study, P53 expression was a strong predictor in multivariate analysis of distal metastases and OS, which is in agreement with other studies analyzing patients after surgery[22] or neoadjuvant CHT and/or RT.[23] It has been shown that in rectal cancer, the loss of P53 function induces mitochondrial genomic instability, and mitochondrial mutations promote aggressive tumor behavior and apoptotic resistance.[24] In our study patients with P53 positivity and higher tumor proliferation (BrdUrdLI > 7.9%) indicated higher tumor cell survival after RT, which led to lower patients survival, and which may support an earlier study.[25] In female patients cohort, low (≤74.9%) Ku70 expression was the strongest parameter for high OS, which was not true for the male subgroup (Fig. 1c, d). This may suggest high effectiveness of the DNA repair process in male tumors and greater cell radioresistance. After RT, male tumor cells with Ku70 ≤ 74.9% expression might have more efficient DNA damage repair than female cells and in P53 positive patients they may cause lower tumor cell apoptosis which, in consequence, causes lower OS, particularly when the break between RT and surgery is longer than 17 days.

Multivariate analysis pointed out that aneuploid and better oxygenated tumors had higher potential to create recurrences, which caused lower probability of patients' RFS, and has been confirmed by other studies.[26,27] Moreover, only in male patients was lower proliferation a positive prognostic factor and indicated lower incidence of local recurrences. For MFS, in the whole patient cohort, negative biological factors were – BrdUrdLI > 7.9% and P53 immunopositivity, which led to higher incidence of metastasis and lower MFS, particularly in male patients with a higher pTNM stage. In the female cohort, tumor diploidy and low proliferation indicated highest MFS. Therefore, in clinical practice negative biomarkers, especially in male patients, should be used for more aggressive treatment.

Our data also showed that tumor biological factors, like DNA repair, hypoxia, and tumor proliferation, are important players in tumor response after RT. These findings emphasize the clinical utility of tumor biological markers assessment in rectal cancer for personalized therapy. We also think that the impact of gender difference on the effect of cancer treatment should be considered in clinical trials and the results should be reported sex-specifically. This strategy can facilitate individualization of treatment.

6. Conclusions

Our study revealed that in male rectal patients treated with SCRT, intervals between RT and surgery >17 days should be avoided because they negatively influence patients' survival.

Negative biological factors may be helpful in personalized therapy.

Conflict of interest

None declared.

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