GENDER-RELATED DIFFERENCES IN PATHOLOGICAL AND CLINICAL TUMOUR RESPONSE BASED ON IMMUNOHISTOCHEMICAL PROTEINS EXPRESSION IN RECTAL CANCER PATIENTS TREATED WITH SHORT COURSE OF PREOPERATIVE RADIOTHERAPY

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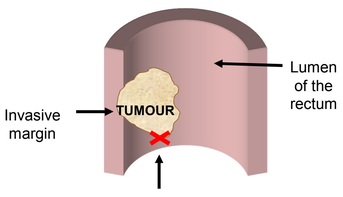
PURPOSE

Assessment of prognostic value of pretreatment expression of six proteins in rectal cancer for early pathological tumour response (pTR), clinical tumour response (CTR) to preoperative radiotherapy (RT), and the potential difference between these parameters depending on patient gender.

MATERIAL AND METHODS

Between November 2003 and June 2006 we recruited 111 patients with resectable rectal carcinoma for whom abdominal surgery was planned. Patients were treated with short preoperative course of RT (SCRT) with 5 Gy dose per fraction during 5 days, followed by surgery 3 to 53 days (mean 21 days) later. Expression of CD34, Ki-67 (MIB-1), GLUT-1, Ku70, BCL-2, and P53 proteins was assessed immunohistochemically.
Association between proteins expression and pTNM, pTR and CTR was analysed separately for short (≤ 15 days) and long (> 15 days) break between RT and surgery and males and female patients.

MATERIAL



Tumour samples were taken twice: before RT and during surgery from the same region (the lowest part of the tumour mass

SUMMARY

Methods and material: 111 patients were treated with short preoperative course of RT (SCRT) with 5 Gy dose per fraction during 5 days, followed by surgery 3 to 53 days (mean 21 days) later. Expression of GLUT-1, Ku70, BCL-2, P53 proteins was assessed immunohistochemically. Tumour regression after RT was assessed by surgeons at the time of operation (CTR), and by pathologist on the excised tumour mass (pTR). Results:There were 76 men and 35 women. There were 27 cTNM stage I, 69 stage II and 15 stage III tumours. We found 26 well-differentiated, 80 moderately-differentiated and 3 poorly-differentiated tumours. Significant differences in Ki-67, GLUT-1, Ku70 and BCL-2 expression between male and female tumours were observed for pathological stage (pTNM) and grade. Association between proteins expression and pTNM, pTR and CTR was analysed separately for short (≤ 15 days) and long (> 15 days) break between RT and surgery and males and female patients. For SCRT with short break none protein was significantly related to pTNM, and for pTR higher Ki-67 and lower BCL-2 expression were correlated with pTR. In the male subgroup, BCL-2 overexpression was predictive. For SCRT with long break none of the proteins was predictive for pTR but Ki-67, Ku70 (in female subgroup) and BCL-2 expression were positively correlated with pTNM, what was shown for the first time. BCL-2 overexpression was associated with CTR in female subgroup, only.
Conclusions: In SCRT, long break in the treatment should be avoided because correlation between Ki-67, Ku70 and BCL-2 expression and pTNM after RT might indicate tumour progression reflecting tumour cell repopulation.

CLINICAL TUMOUR RESPONSE

Tumour regression after RT was assessed at time of operation by surgeons according to the following RECIST criteria:
CTR1. Progressive disease (PD), increase of tumour volume ≥ 20 %
CTR2. Stable disease (SD), tumour regression < 20 - 30 %
CTR3. Partial response (PR), tumour regression <100 ≥ 30 %
CTR4. Complete response (CR), 100 % tumour regression

PATHOLOGICAL ASSESSMENT

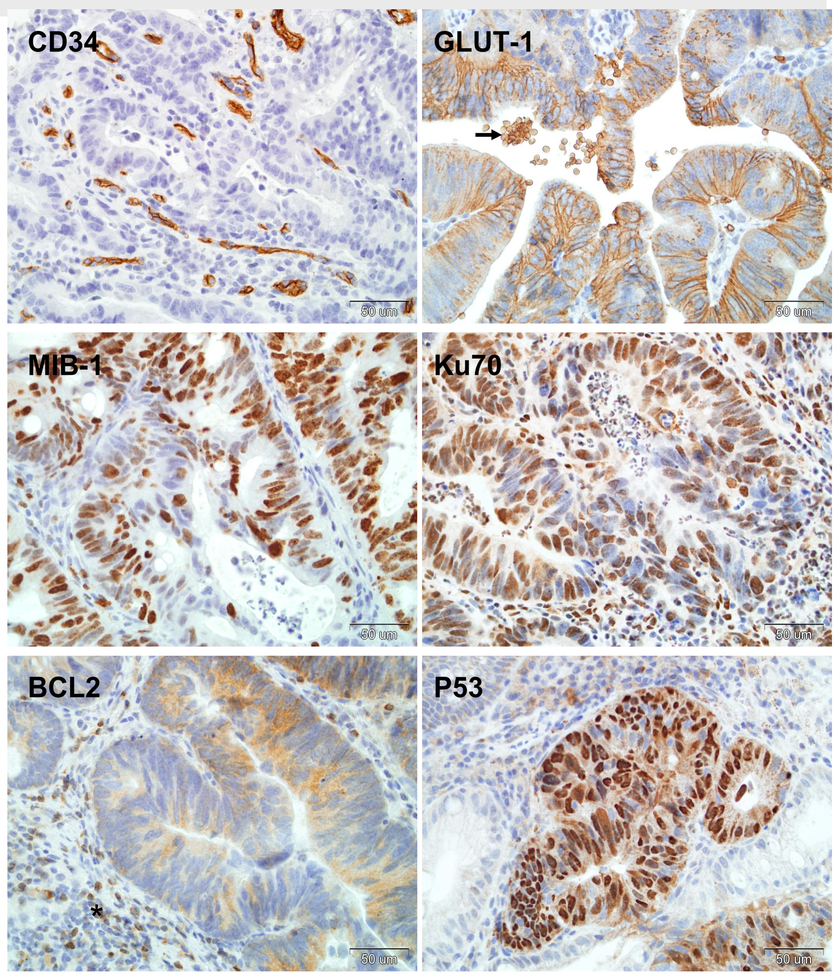
Surgical specimen was examined by a pathologist and the following criteria of tumour regression after RT according to Dworak's classification were applied:

D0 – no regression
D1: dominant tumour mass with obvious fibrosis and/or vasculopathy
D2: dominantly fibrotic changes with few tumour cells or groups
D3: very few (difficult to find microscopically) tumour cells in fibrotic tissue
D4: no tumour cells, only fibrotic mass (total regression or response)

Procedures for immunohistochemical staining

Antigen	Clone	Manufacturer	Antigen retrival	Dilution
CD34	QBend10	DAKO[1]	10 mM sodium citrate buffer (pH=6.0), microwave, 3x5 min (800W, 650W, 650W)	1:50
P53	PAb 1801	Leica Biosystems[2]		1:40
BCL2	124	DAKO[1]		1:40
Ku70	polyclonal	Santa Cruz Biotechnology[3]	10 mM sodium citrate buffer (pH=6.0), microwave, 2x5 min (500W)	1:75
Ki-67	MIB-1	DAKO[1]	10 mM sodium citrate buffer (pH=6.0), microwave, 4x5 min (800W)	1:100
GLUT-1	polyclonal	Merck Millipore[4]	TRS pH=6.1 (DAKO), water bath, 96^{0}C, 20 min.	1:300

[1] DakoCytomation Denmark A/S, Glostrup, Denmark
[2] Leica Biosystems Newcastle Ltd, Newcastle, United Kingdom
[3] Santa Cruz Biotechnology, Inc. Dallas, Texas U.S.A.
[4] Merck Millipore, Billerica, Massachusetts U.S.A.



Microphotographs (x 400) of colorectal cancer from pretreatment biopsy. Immunohistochemical nuclear staining for MIB-1, P53, Ku70 membranous staining pattern for CD34 and GLUT-1 with red blood cells acting as positive (arrow) internal control, and cytoplasmatic staining for BCL-2 was shown.

Statistical analysis

Statistical analysis was performed with STATISTICA vs.9. For determination of mean values for biomarkers 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.

The correlation between proteins expression and patient clinicopathological factors and pathological and clinical tumour response in rectal cancer

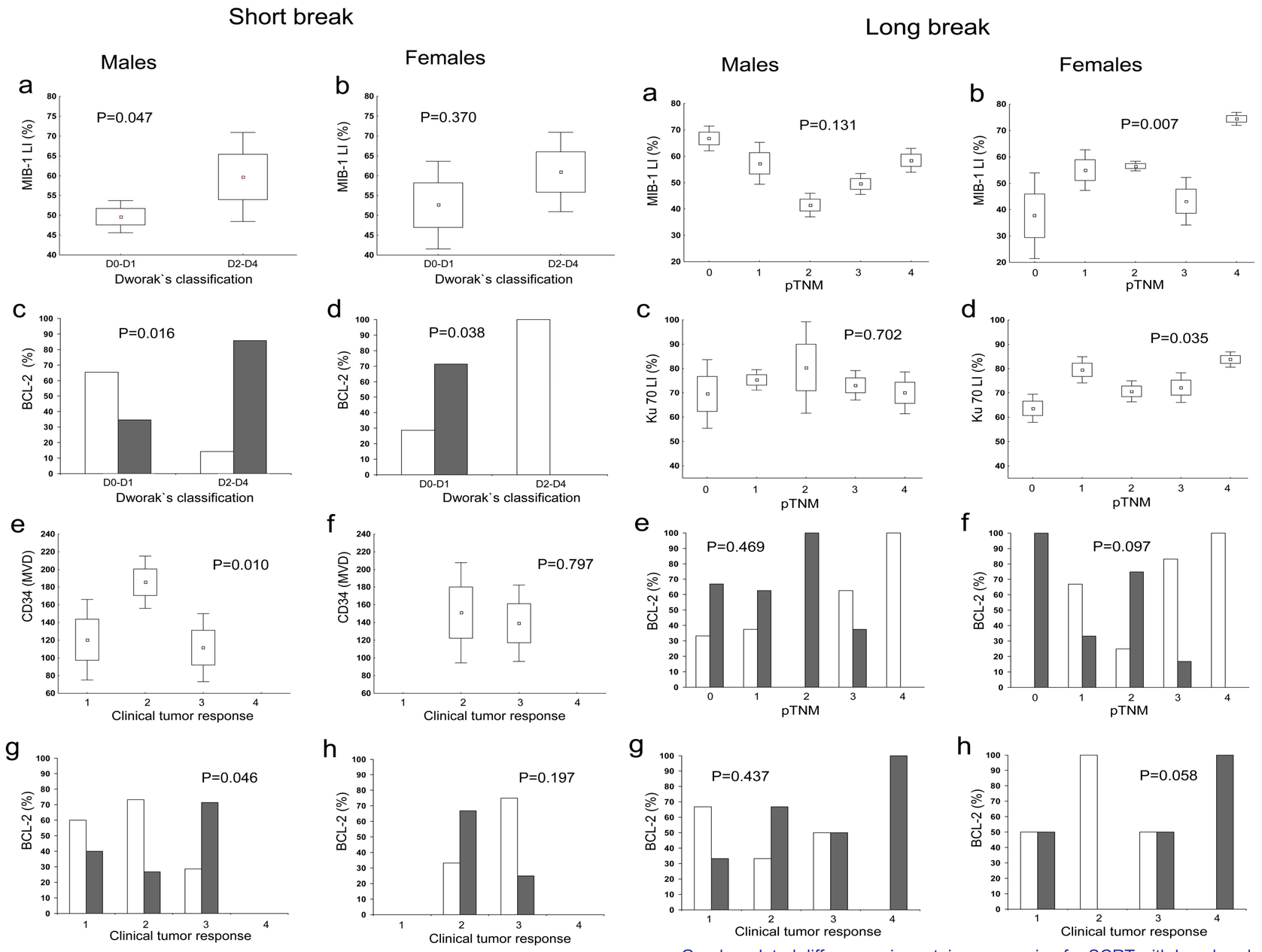
Characteristics	N	Sex		P-value
		Men	Women	
Age mean (range) (years)	111	76* 61.1 (30-77)	35 60.7 (43-82)	P=0.829
Tumour stage TNM				
I	27	20 (26.3 %)**	7 (20.0 %)	
II	69	50 (65.8 %)	19 (54.3 %)	P=0.038
III	15	6 (7.9 %)	9 (25.7 %)	
pTNM				
0	5	3 (4.0 %)	2 (5.7 %)	
1	46	35 (46.0 %)	11(31.4 %)	
2	16	9 (11.8 %)	7 (20.0 %)	P=0.603
3	39	26 (34.2 %)	13 (37.1 %)	
4	5	3 (4.0 %)	2 (5.7 %)	
Histological grade				
G1	26	15 (19.8 %)	11(33.3 %)	
G2	80	59 (77.6 %)	21(63.6 %)	P=0.300
G3	3	2 (2.6 %)	1 (3.0 %)	
Interval between RT and surgery (days) mean (range):	111	76 20.6 (3-45)	35 23.5 (4-53)	P=0.241
Short break (≤ 15 days)	52	40 9.6 (3-15)	12 8.7 (4-12)	P=0.328
Long break (>15 days)	59	36 32.7 (17-45)	23 31.3 (20-53)	P=0.413
pTR				
D0	21	14 (18.7 %)	7 (20.6 %)	
D1	60	42 (56.0 %)	18 (52.9 %)	
D2	18	13 (17.3 %)	5 (14.7 %)	P=0.871
D3	4	3 (4.0 %)	1 (2.9 %)	
D4	6	3 (4.0 %)	3 (8.8 %)	
CTR				
1	14	12 (15.8 %)	2 (5.7 %)	
2	37	24 (31.6 %)	13 (37.2 %)	P=0.425
3	56	38 (50.0 %)	18 (51.4 %)	
4	4	2 (2.6 %)	2 (5.7 %)	

*number of cases, ** percentage of cases within the subgroups

Gender-related clinico-pathological characteristics of rectal cancer patients

Parameter	CD34 (MVD*) Mean ± SE	MIB-1LI (%) Mean ± SE	GLUT-1LI (%) Mean ± SE	KU 70 LI(%) Mean ± SE	P53 LI(%) Mean ± SE	BCL-2 negative N (%)	BCL-2 positive N (%)
TNM							
I	(23)** 148.6 ± 12.7	(27) 52.8 ± 2.4	(26) 16.7 ± 4.6	(26) 76.3 ± 1.7	(27) 40.4 ± 6.8	14 (60.9)***	9 (39.1)
II	(55) 133.9 ± 7.9	(69) 53.7 ± 1.6	(68) 11.6 ± 1.9	(63) 73.0 ± 1.2	(65) 42.5 ± 4.5	27 (47.4)	30 (52.6)
III	(12) 157.2 ± 18.7	(15) 51.6 ± 4.5	(15) 23.6 ± 6.6	(15) 78.1 ± 2.3	(14) 49.2 ± 9.7	7 (53.8)	6 (46.2)
pTNM							
0	(4) 143.9 ± 7.3	**(5) 55.1 ± 7.7**[2]	(5) 4.2 ± 3.4	(5) 67.2 ± 4.3	(5) 37.9 ± 17.5	1 (20.0)	**4 (80.0)** [4]
1	(42) 130.0 ± 9.0	**(46) 56.1± 2.0**	(45) 15.4 ± 2.6	(44) 76.6 ± 1.3	(45) 37.2 ± 5.6	20 (46.5)	**23 (53.5)**
2	(10) 170.8 ± 21.1	**(16) 54.9 ± 3.0**	(16) 22.5 ± 6.8	(13) 73.6 ± 2.8	(14) 49.7 ± 8.7	4 (30.8)	**9 (69.2)**
3	(31) 142.8 ± 10.9	**(39) 48.0 ± 1.9**	(38) 12.6 ± 3.2	(38) 73.1 ± 1.5	(37) 49.7 ± 5.6	20 (69.0)	**9 (31.0)**
4	(3) 165.9 ± 41.5	**(5) 59.4 ± 7.9**	(5) 4.4 ± 2.8	(4) 76.9 ± 4.4	(5) 28.8 ± 17.7	3 (100.0)	**0 (0.0)**
Grade							
1	(22) 135.5 ±16.2	(26) 54.1 ± 2.4	(26) 20.2 ± 4.5	**(25) 76.2 ± 2.1**[3]	(26) 39.8 ± 6.9	6 (25.0)	**18 (75.0)** [5]
2	(63) 141.2 ± 7.0	(80) 53.1 ± 1.5	(78) 12.8 ± 2.1	**(74) 74.6 ± 1.0**	(75) 46.1 ± 4.2	40 (61.5)	**25 (38.5)**
3	(3) 177.4 ± 10.2	(3) 47.7 ± 13.1	(3) 4.0 ± 3.0	**(3) 60.9 ± 5.3**	(3) 12.6 ± 7.3	2 (100.0)	**0 (0.0)**
pTR							
D0	(18) 125.1 ± 11.0	(21) 51.3 ± 2.7	(21) 15.9 ± 4.6	(21) 73.3 ± 1.8	(20) 56.2 ± 7.6	10 (62.5)	6 (37.5)
D1	(49) 148.3 ± 9.5	(60) 52.5 ± 1.7	(59) 14.7 ± 2.7	(55) 75.5 ± 1.3	(58) 41.8 ± 4.8	29 (55.8)	23 (44.2)
D2	(14) 135.6 ± 16.0	(18) 54.2 ± 3.5	(17) 15.9 ± 4.9	(17) 72.7 ± 2.1	(16) 34.3 ± 8.7	5 (35.7)	9 (64.3)
D3	(4) 122.7 ± 28.0	(4) 62.6 ± 5.8	(4) 11.5 ± 5.0	(4) 78.9 ±6.0	(4) 9.8 ± 5.3	2 (50.0)	2 (50.0)
D4	(4) 152.6 ± 10.3	(6) 58.5 ± 6.4	(6) 4.7 ± 2.8	(5) 70.6 ±5.8	(6) 54.0 ± 15.6	1 (20.0)	4 (80.0)
CTR							
1	**(12) 126.4 ± 16.9**[1]	(14) 51.3 ± 4.0	(14) 11.8 ± 7.2	(13) 73.4 ± 2.8	(14) 44.5 ± 9.4	6 (60.0)	**4 (40.0)** [6]
2	**(33) 164.1 ± 11.3**	(37) 54.5 ± 2.5	(35) 15.8 ± 3.3	(34) 74.9 ± 1.4	(35) 47.4 ± 6.1	21 (63.6)	**12 (36.4)**
3	**(42) 126.8 ± 8.2**	(56) 52.9 ± 1.6	(56) 14.9 ± 2.5	(53) 75.1 ± 1.3	(53) 39.6 ± 5.0	21 (45.7)	**25 (54.3)**
4	**(3) 137.7 ± 5.3**	(4) 51.7 ± 8.9	(4) 4.9 ± 4.3	(4) 66.9 ± 5.6	(4) 41.2 ± 22.2	0 (0.0)	**4 (100.0)**
All tumors	(90) 140.8 ± 6.3	(111) 53.2 ± 1.3	(109) 14.4 ± 1.9	(104) 74.5 ± 0.9	(106) 42.9 ± 3.5	48 (51.6)	45 (48.4)

RESULTS



Gender related differences in proteins expression significant for pTR (a-d) and clinical tumor response (e – h) for patients treated with SCRT and short break (≤ 15 days) before surgery. Association between pTR (Dworak's classification: D0-D1 vs D2 – D4) and MIB-1 (a, b), BCL-2 (c, d) CD34 (e, f), and BCL-2 (g,h). P value for (a, b, e, f) one-way Anova test and for (c, d, g, h) Pearson Ch2 test. BCL-2 negativity – open columns, BCL-2 positivity – solid columns. (a, b, e, f - mean values ± S.E. are shown.

Gender related differences in proteins expression for SCRT with long break (> 15 days). Association between pTNM and MIB-1 (a, b), Ku70 (c, d), BCL-2 (e - f). The relationship between BCL-2 expression and clinical tumor response (g, h). P value from one-way Anova test (a - d), from Pearson's Chi2 test (e – h). BCL-2 negativity – open columns, BCL-2 positivity – solid columns. (a-d) - mean values ± S.E. are shown.

CONCLUSIONS

In SCRT, long break in the treatment should be avoided because correlation between Ki-67, Ku70 and BCL-2 expression and pTNM after RT might indicate tumour progression reflecting tumour cell repopulation.