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Adjuvant Radiochemotherapy of Stage II and III Rectal Adenocarcinoma: Role of CEA and CA 19-9

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Abstract. *Background: This analysis was undertaken to evaluate the impact of pre-radiotherapy CEA and CA 19-9 values on clinical outcome of locally advanced rectal cancer. Patients and Methods: Retrospective data were collected from patients (n=203) with UICC stage II and III rectal adenocarcinomas, who underwent low anterior or abdominoperineal resection and received post-operative or pre-operative radiochemotherapy from January 1989 until July 2002. The rates of survival and distant and local recurrences were evaluated using Kaplan-Meier survival analysis, Log-rank test and Cox's proportional hazards (median follow-up 8 years). Multivariate analysis was used to assess the prognostic value of CEA and CA 19-9. Results: The 5-year actuarial rates for patients with normal (n=118) and elevated (n=88) CEA values were as follows: overall survival 62.4% and 32.0% (p<0.001), local control 73.5% and 55.0% (p=0.007), and absence of distant metastasis 83.3% and 88.0% (n.s.), respectively. Similar results were obtained for patients with normal (n=82) and elevated (n=10) CA 19-9 values: overall survival 60.7% and 14.0% (p=0.007), local control 83.7% and 80.0% (n.s.), and absence of distant metastasis 64.9% and 75.0% (n.s.), respectively. After adjustment for TNM stage, sex, age, LDH, tumor site and grading, the elevation of CEA proved to be an independent prognostic factor for overall survival (relative risk of 1.01 per ng/ml, CI 1.002 – 1.01; p=0.005). Conclusion: This study confirmed the prognostic value of pre-radiotherapy CEA and CA 19-9 in patients with stage II or III rectal carcinoma.*

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An improved therapeutic strategy for stage II and III rectal adenocarcinomas is urgently needed because up to 30% of patients still develop recurrent disease after curative surgical resection (1). Several studies are ongoing, aiming at the evaluation of new multimodality treatment strategies (2). Recent results have raised the question of whether the current "monolithic approaches" (3) or new risk-adapted strategies should prevail in future. Thus, in future, decision-making will need more accurate data about individual risk of tumor relapse, preferably obtained by non-invasive methods or routine laboratory diagnostics.

Several functions have been attributed to CEA (oncofetal glycoprotein antigen): involvement in cell adhesion, inhibition of cell death induced by loss of anchorage to the extracellular matrix, and cooperation in cellular transformation with proto-oncogenes like Bcl2 and C-Myc (4). CEA is present in embryonic tissues and certain epithelial malignancies. Progressive elevation of CEA may indicate tumor recurrence 1 – 3 years before clinical evidence of metastases. Local recurrences are accompanied by a small rise of CEA, hepatic metastasis by a large rise. CEA has been shown to be the most cost-effective approach to detect potentially resectable metastases from colon cancer (5).

With the long-term outcome in mind, our study aimed to evaluate the impact of increased levels of CEA and CA 19-9 in the combined-modality treatment for stage II and III rectal carcinoma, performed during the past 14 years at the Department of Radiotherapy, University Hospital of Freiburg, Germany.

Patients and Methods

Inclusion and exclusion criteria. Male or female patients between 20 and 90 years, with histologically confirmed rectal adenocarcinoma scheduled for conventional radiotherapy of the pelvic region (6 / 18 MeV linear accelerator) in the adjuvant or neoadjuvant setting, were included in this study. Exclusion criteria were: heavy smoking

Table I. *Patient characteristics.*

	No. of patients (N=203)	%
Sex		
Female	132	65.1%
Male	71	34.9%
Age		
Median	60	
Range	34 – 83	
Stage		
II	66	32.5%
III	137	67.5%
Treatment setting		
Adjuvant	166	81.8%
Neoadjuvant	37	18.2%
CEA		
<3 ng/ml	118	58.1%
≥3 ng/ml	85	41.9%
CA 19-9		
<60 U/ml	82	89.1%
≥60 U/ml	10	10.9%

Table II. *Overall survival, cancer-specific survival, disease-free survival, rates of local relapse and distant metastases at 5 years. P values of the Log-rank test for univariate analysis are given.*

	Overall survival	Local control	Distant metastasis
All stage II & III	49.4%	66.0%	14.9%
CEA	$p<0.001$	$p=0.007$	$p=0.546$
<3 ng/ml	62.4%	73.5%	16.7%
≥3 ng/ml	32.0%	55.0%	12.0%
CEA during RT	$p<0.001$	$p<0.001$	$p=0.524$
decrease	68.2%	84.9%	92.7%
increase	43.1%	42.8%	85.3%
CA 19-9	$p=0.007$	$p=0.541$	$p=0.408$
<60 U/ml	60.7%	83.7%	35.1%
≥60 U/ml	14.0%	80.0%	25.0%

(>40 cigarettes per day), pancreatitis, hypothyroidism, current inflammation including infections, inflammatory bowel disease, or cirrhosis, and any non-colorectal second malignancies. Further, only patients with reliable data about pre-radiotherapy CEA levels were encompassed. Patients with metastatic or recurrent tumors were not included. Pre-treatment evaluation included complete blood test, chemistry profile, chest radiography, liver ultrasonography and computer tomography (CT) of the abdomen and pelvis.

Blood samples for estimation of tumor markers were collected prior to radiotherapy, at three-monthly intervals for two years afterwards, and at six-monthly intervals for five years afterwards. CEA and CA 19-9 were defined as "elevated" if they exceeded 3 ng/ml and 60 U/ml, respectively (6). For smokers the same cut-off value for CEA was taken.

Regarding surgical intervention, patients treated by low anterior resection (LAR) or by abdominoperineal resection (APR) were eligible. Patients who were defined as "radically resected" (proximal and distal surgical margins were microscopically free of tumor, R0) were scheduled for 6 cycles of chemotherapy according to the NIH protocol: bolus application of 500 mg/m^2 5-FU for 3 days during cycles 1-3 and for 5 days during cycles 4-6. In contrast, patients with microscopic or macroscopic residual tumors received continuous infusion over 24 hours for 14 days: 350 mg/m^2 5-FU *i.v.* Additionally, bolus applications of 200 mg/m^2 leucovorin and 4 mg/m^2 mitomycin C were given daily.

Statistical methods. Kaplan-Meier curves (7) were used to estimate the distribution of overall survival (OAS). For analysis adjusting the rates of treatment failures, local-relapse-free survival (LRS) and distant-relapse-free survival (DRS) were determined as life-table analysis referring to freedom of locoregional relapse and freedom of distant metastases. Log-rank tests (Cox-Mantel) were used to compare the survival distributions between different patient subgroups (8). The influence of CEA and CA 19-9 was studied by Cox's proportional-hazard regression analysis (9).

Results

Patient data. Complete data about CEA were available for 132 men and 71 women with stage II or III tumors treated with pre- or post-operative adjuvant radiochemotherapy (between January 1989 and July 2002). These 203 patients were enrolled in the study. Ages ranged from 34 to 83 years old (median 60 years). The distribution of stages is shown in Table I. One hundred and sixty-six (81.8%) and 37 (18.2%) patients were treated by post-operative radiochemotherapy and pre-operative radiochemotherapy, respectively. The radiotherapy courses included a total dose of 45 - 56 Gy in 25 - 31 sessions using 6 and 18 MeV linear accelerators. Survival data and CEA values were available for all 203 patients but only 92 patients had documented CA 19-9 values.

Kaplan-Meier survival analysis of 203 patients revealed a calculated 5-year overall survival rate of 49.4% (Figure 1). The rate of 5-year local control was 68.3%, and the rate of distant metastases was 14.9%. Median follow-up was 8 years. There was no difference in survival between the post-operative or pre-operative radiochemotherapy groups (5-year overall survival: 48.8% and 50.1%, respectively), but there was a difference between stage II and III tumors (59.1% and 44.4%, respectively).

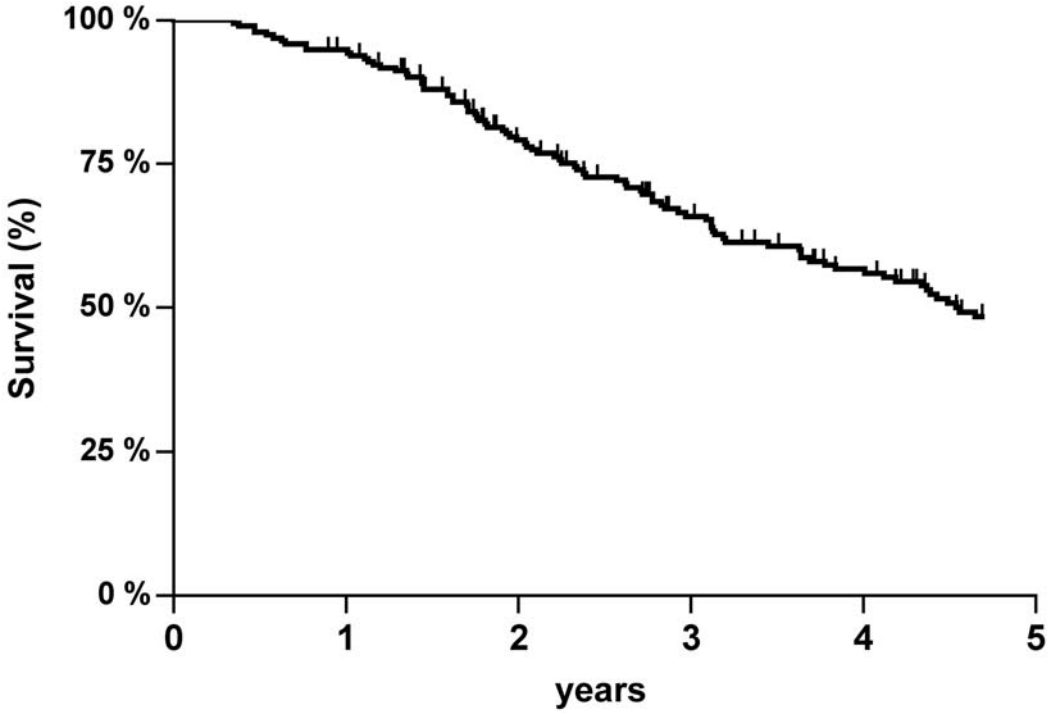

Figure 1. *Kaplan-Meier analysis of overall survival.*

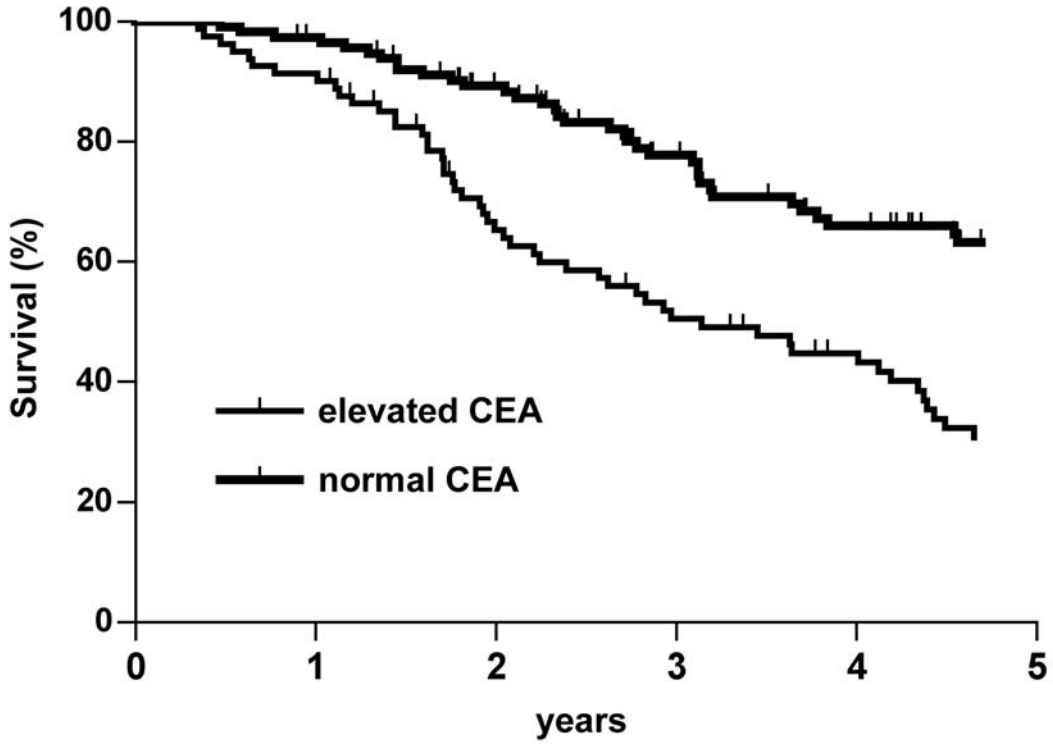

Figure 2. *Survival curves for normal and elevated levels of CEA.*

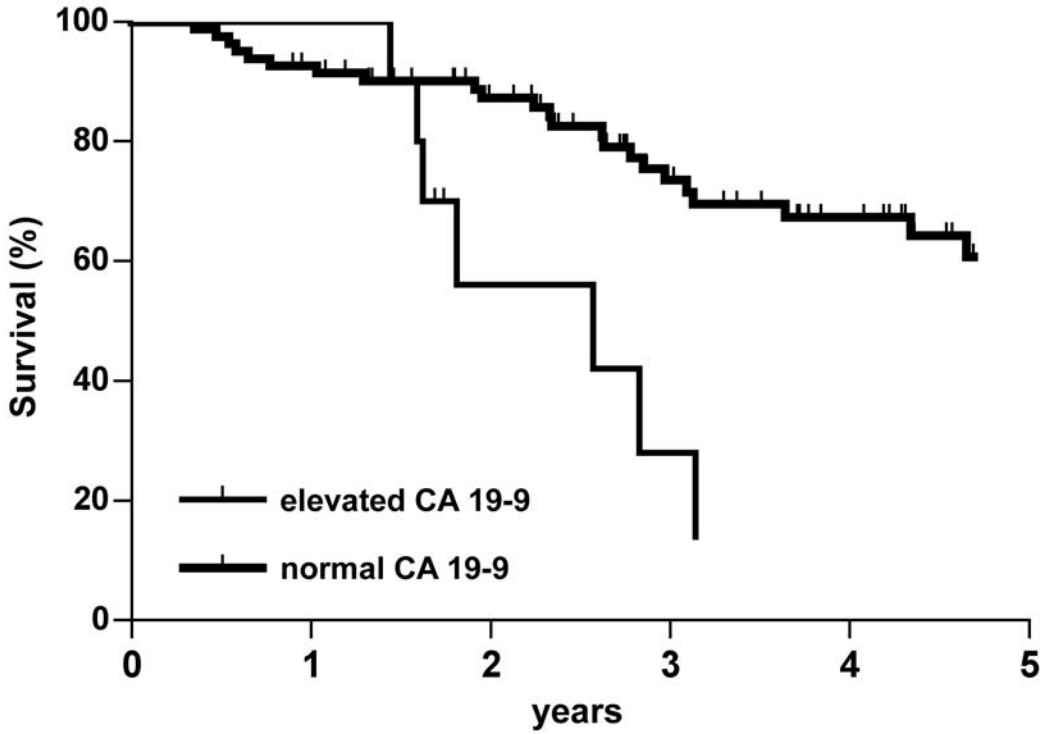

Figure 3. *Survival curves for normal and elevated levels of CA 19-9.*

Table III. *Patient characteristics (Chi-square test).*

	Increase of CEA (85/203 tumors)		Increase of CA 19-9 (10/92 tumors)	
	No.	%	No.	%
Age	$p=0.56$		$p=0.46$	
≤Median age of 60	39	45.9%	5	50.0%
>Median age of 60	46	54.1%	5	50.0%
Stage	$p=0.02$		$p=0.28$	
II	20	23.5%	2	20.0%
III	65	76.5%	8	80.0%
Treatment	$p=0.04$		$p=0.03$	
Post-operative RCT	64	75.3%	6	60.0%
Pre-operative RCT	21	24.7%	4	40.0%
Grading	$p=0.26$		$p=0.76$	
G I	4	5.8%	0	0.0%
G II	49	71.0%	8	80.0%
G III	16	23.2%	2	20.0%
Tumor localization	$p=0.95$		$p=0.93$	
≤Median of 7 cm ab ano	39	54.2%	3	50.0%
>Median of 7 cm ab ano	33	45.8%	3	50.0%

Tumor markers CEA and CA 19-9. According to the inclusion criteria, pre-radiotherapy CEA values were available for all patients (Table III). 118 (58.1%) patients showed normal levels of CEA (<3 ng/ml) whereas 85 (41.9%) patients showed elevated levels of CEA (≥3 ng/ml). The 5-year overall survival rate (Figure 2) was significantly lower if CEA was elevated (32.0% *versus* 62.4%). Median survival decreased from 9.8 to 3.1 years, if CEA was elevated (Hazard ratio 2.4, CI 1.7 – 3.8; $p<0.001$). Similarly, the local control decreased from 73.5% to 55.0%, if CEA was elevated, whereas the rate of distant metastases was not significantly different.

Eighty-two (89.1%) patients showed normal levels of pre-radiotherapy CA 19-9 (<60 U/ml), whereas 10 (10.9%) patients showed elevated levels of CA 19-9 (≥60 U/ml). CA 19-9 data were missing for 111 patients. Similarly to CEA, elevated levels of CA 19-9 (Figure 3) were associated with a significantly lower 5-year overall survival rate (14.0% *versus* 60.7%) and median survival (2.6 *vs.* 6.1 years, Hazard ratio 3.0, CI 1.6 – 19.1; $p<0.001$) compared to normal CA 19-9 levels, but not with the rates of local control or distant metastases.

As potential prognostic factors, age, sex, stage, CEA, LDH, localization of tumor and grading were tested in a multivariate model. CEA (relative risk [RR] 1.01 per ng/ml,

CI 1.00 – 1.02; $p=0.04$), age (relative risk [RR] 1.03 per year, CI 1.00 – 1.05; $p=0.02$) and, as a trend, stage (II *vs.* III, relative risk [RR] 0.78, CI 0.58 – 1.02; $p=0.06$) turned out to be independent prognostic factors for overall survival.

Using the Chi-square test, we searched for potential associations between increase of CEA and CA 19-9 and age, stage, treatment (pre- or post-operative radio-chemotherapy), grading and tumor localization (Table III). Significant associations with CEA increase were found for stage (II *vs.* III: 23.5% *vs.* 76.5%, respectively) and treatment (post-operative *vs.* pre-operative radio-chemotherapy: 75.3% *vs.* 24.7%). Using a patient cohort of smokers of less than 40 cigarettes per day, no correlation between CEA levels and smoking was seen. Further, treatment (post-operative *vs.* pre-operative radio-chemotherapy) was significantly correlated with an increase of CA 19-9. However, the sample size was low as only 10 patients showed elevated levels of CA 19-9.

Discussion

Prognostic factors CEA and CA 19-9. Patients who showed elevated CEA values in our study had significantly lower 5-year overall survival rates and local control (32.0% and 55.0%) compared to patients with normal CEA values (62.4% and 73.5%, respectively). Similarly, patients with elevated CA 19-9 values had significantly lower overall survival rates than other patients (14.0% *vs.* 60.7%, $p=0.007$). This is consistent with several previous studies (10-16) which demonstrated that CEA and CA 19-9 levels are reliable tumor markers for rectal cancer. In a study of Behbehani *et al.* (17), patients with pre-operatively elevated CEA levels had a 2-year disease-free survival of 23%, whereas patients without CEA elevation had a 2-year disease-free survival of 71%. In a multivariate analysis, Lee *et al.* identified a non-significant trend of CEA as an independent prognostic factor for survival (18). Similar results were described by Myerson *et al.* (19).

However, the majority of studies referred to pre-operative CEA and CA 19-9 values and no data about long-term outcome were given. Our study revealed the importance of pre-radiotherapy values of CEA and CA 19-9 in long-term prognosis. Furthermore, after adjustment TNM stage, sex, age, LDH, tumor site and grading, CEA values continued to provide independent predictive information on survival in multivariate analysis. Additionally, an increase of CEA values during radiotherapy was associated with a decreased overall survival rate (Table II). Using Chi-square tests, a significant association between stage and elevation of CEA was observed. If lymph nodes were involved, the patient had higher levels of CEA, indicating that CEA was associated with dissemination of tumor cells and progressive disease.

Using a nude mouse model with rectal carcinoma xenografts (20), we had previously shown that CEA was significantly associated with increased levels of multidrug resistance, which is known to predict poor outcome even if a broad panel of chemotherapeutics is used (21). A possible interaction between MDR and sensitivity to radiotherapy was reported by Wenz *et al.* (22), while other studies negated such interactions (23, 24). Nevertheless, data about sensitivity to radio- or chemotherapy is extremely helpful (*e.g.* in the selection of the sequence of pre- or post-operative adjuvant therapy), and the CEA value before treatment may provide some additional information to estimate the prognosis of rectal cancer.

Our patient collective was not homogeneous since patients with pre-operative and post-operative radio-chemotherapy were included. However, analysis of survival data showed no differences concerning overall survival, local control, or distant metastases. Smokers of more than 40 cigarettes per day were excluded; other smokers showed not correlation between number of cigarettes and CEA levels.

Physicians seem not to be convinced of the potential benefits from CEA or CA 19-9 measurements, or they may not know of the recommendations for the use of tumor markers in colorectal cancer given by the ASCO guidelines (25). Nevertheless, routine determination of CEA and CA 19-9 is easy, established in clinical practice, and the results are reliable and comparable between different laboratories (26).

In most reports about the prognostic value of CEA and CA 19-9, adjuvant chemotherapy and/or radiotherapy was not routinely applied or the information about it was incomplete. Furthermore, different cut-off levels for CEA (3 μg/l to 5 μg/l) and CA 19-9 (14 kU/l to 60kU/l) have been used. For our patient cohort, complete data about radio-chemotherapy courses were available, and routinely CEA and CA 19-9 were defined as "elevated" if they exceeded 3 ng/ml and 60 U/ml, respectively. Smokers of more than 40 cigarettes per day were excluded. Smokers of less than 40 cigarettes per day were included without adjustment of the cut-off value. Further, radiation therapy, as well as the hepatotoxicity of anti-neoplastic drugs, is known to induce a transient rise in CEA. Since all patients received complete courses of the same radiochemotherapy scheme, any potential effects applied to all patients.

Perspectives of rectal cancer. Recently, much effort has been put into identifying novel prognostic factors. Alterations in DNA ploidy has been investigated, assuming that a aneuploid DNA content may preclude a higher radiosensitivity than that of diploid tumors. The pre-irradiation biopsy specimens of 72 patients with colorectal canter treated by pre-operative radiotherapy were investigated by Qiu *et al.* (27). Among the examined parameters of microsatellite instability, microvessel count, immunohistochemistry for proliferating cell nuclear

antigen, p53, p21, bcl-2 and VEGF, only the presence of positive nodes and p21 expression were significantly associated with tumor response to pre-operative radiation. Considering that patients with low thymidylate synthase levels, an enzyme involved in DNA biosynthesis, showed improved outcome, measurement of this enzyme was regarded as useful in predicting response to 5-FU-based therapy (28). Rödel *et al.* reported promising results analyzing survivin, a novel member of the inhibitor of apoptosis family (29). Low survivin expression was related to an increased rate of disease-free survival, a reduced risk of distant metastases and local failure.

An important milestone for cancer treatment would be to establish new screening methods with the following properties: easy-to-handle in day-to-day clinical practice, reliable and showing significant impact on outcome (*i.e.* correlation between elevation of values and survival, local relapse and distant recurrences). Though CEA results should only be used in conjunction with further clinical evidence, our study confirmed, for a defined collective of patients treated with standard adjuvant or neoadjuvant radiochemotherapy, that CEA and CA 19-9 are valuable prognostic tumor markers.

Conclusion

Our study demonstrated that patients with locally advanced colorectal cancer and elevated CEA values had significantly lower 5-year overall survival rates and local control. Elevation of the CA 19-9 values also indicated significantly lower rates of overall survival.

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References

1 Saltz LB and Minsky B: Adjuvant therapy of cancers of the colon and rectum. Surg Clin North Am *82*: 1035-1058, 2002.

2 Kachnic LA and Willett CG: Radiation therapy in the management of rectal cancer. Curr Opin Oncol *13*: 300-306, 2001.

3 Sauer R: Adjuvant and neoadjuvant radiotherapy and concurrent radiochemotherapy for rectal cancer. Pathol Oncol Res *8*: 7-17, 2002.

4 Gold P and Goldenberg N: The carcinoembryonic antigen (CEA): past, present, and future. McGill J Med *3*: 66, 1997.

5 Castells A, Bessa X, Daniels M, Ascaso C, Lacy AM, Garcia-Valdecasas JC, Gargallo L, Novell F, Astudillo E, Filella X and Pique JM: Value of postoperative surveillance after radical surgery for colorectal cancer: results of a cohort study. Dis Colon Rectum *41*: 714-723, 1998.

6 Welch CE and Malt RA: Abdominal surgery (second of three parts). N Engl J Med *308*: 685-695, 1983.

7 Kaplan EL and Meier P: Nonparametric estimation from incomplete observations. J Am Stat Assoc *53*: 457-481, 1958.

8 Peto R and Peto J: Asymptocally efficient rank invariant test procedures. J R Stat Soc A *135*: 185-206, 1972.

9 Cox DR: Regression models and life tables. J R Stat Soc B *34*: 187-220, 1972.

10 Nakayama T, Watanabe M, Teramoto T and Kitajima M: Slope analysis of CA19-9 and CEA for predicting recurrence in colorectal cancer patients. Anticancer Res *17*: 1379-1382, 1997.

11 Reiter W, Stieber P, Reuter C, Nagel D, Lau-Werner U, Pahl H and Fateh-Moghadam A: Preoperative serum levels of CEA and CA 19-9 and their prognostic significance in colorectal carcinoma. Anticancer Res *17*: 2935-2938, 1997.

12 Filella X, Molina R, Pique JM, Grau JJ, Garcia-Valdecasas JC, Biete A, Novell F, Astudillo E, Bordas JM and Campo E: CEA as a prognostic factor in colorectal cancer. Anticancer Res *14*: 705-708, 1994.

13 Nelson RL: The usefulness of carcinoembryonic antigen in postoperative colorectal cancer patients. Dis Colon Rectum *40*: 866-867, 1997.

14 Dawson LA, Franssen E and Davey P: Postoperative borderline elevated CEA predicts for earlier relapse in patients with rectal cancer receiving adjuvant postoperative therapy. Cancer J Sci Am *5*: 374-379, 1999.

15 Wang WS, Lin JK, Chiou TJ, Liu JH, Fan FS, Yen CC, Lin TC, Jiang JK, Yang SH, Wang HS and Chen PM: Preoperative carcinoembryonic antigen level as an independent prognostic factor in colorectal cancer: Taiwan experience. Jpn J Clin Oncol *30*: 12-16, 2000.

16 Fucini C, Tommasi MS, Cardona G, Malatantis G, Panichi S and Bettini U: Limitations of CEA monitoring as a guide to second-look surgery in colorectal cancer follow-up. Tumori *69*: 359-364, 1983.

17 Behbehani AI, Al Sayer H, Farghaly M, Kanawati N, Mathew A, al Bader A and van Dalen A: Prognostic significance of CEA and CA 19-9 in colorectal cancer in Kuwait. Int J Biol Markers *15*: 51-55, 2000.

18 Lee YT: Local and regional recurrence of carcinoma of the colon and rectum: II. Factors relating to operative technique. Surg Oncol *5*: 1-13, 1996.

19 Myerson RJ, Michalski JM, King ML, Birnbaum E, Fleshman J, Fry R, Kodner I, Lacey D and Lockett MA: Adjuvant radiation therapy for rectal carcinoma: predictors of outcome. Int J Radiat Oncol Biol Phys *32*: 41-50, 1995.

20 Weissenberger C, Fiebig HH, Lutterbach J, Barke A, Momm F, Muller M, Witucki G, Guttenberger R and Berger DP: Is there any correlation between MDR1, GST-pi-expression and CEA? Anticancer Res *20*: 5139-5144, 2000.

21 Bradley G and Ling V: P-glycoprotein, multidrug resistance and tumor progression. Cancer Metastasis Rev *13*: 223-233, 1994.

22 Wenz F, Engling A, Schäfer J, Fruehauf S and Weber KJ: [Genetherapeutic and pharmacological modulation of radiation-induced apoptosis] Gentherapeutische und pharmokologische Modulation der bestrahlungsinduzierten Apoptose. *In:* [Proceedings of the 9th Symposium: Experimental and Clinical Radiation Biology] Experimentelle Strahlenbiologie und Klinische Strahlenbiologie (Beck-Bornholdt HP, Baumann M, Raabe A and Peterson C, eds). Hamburg, pp. 115, 2000.

23 Ruth AC and Roninson IB: Effects of the multidrug transporter P-glycoprotein on cellular responses to ionizing radiation. Cancer Res *60*: 2576-2578, 2000.
24 Weissenberger C, Fiebig HH, Guttenberger R, Hinkelbein W and Frommhold H: Correlation between expression of drug resistance, tumour markers and radioresistance. *In:* 3rd Balkan Congress of Oncology (Antypas G, ed). pp. 141-146, 2000.
25 Bast RC Jr, Ravdin P, Hayes DF, Bates S, Fritsche H Jr, Jessup JM, Kemeny N, Locker GY, Mennel RG and Somerfield MR: 2000 update of recommendations for the use of tumor markers in breast and colorectal cancer: clinical practice guidelines of the American Society of Clinical Oncology. J Clin Oncol *19*: 1865-1878, 2001.
26 Persijn JP and Korsten CB: The development of a radioimmuno-assay for carcino-embryonic antigen with some applications. Clinical evaluation of carcino-embryonic antigen, I. J Clin Chem Clin Biochem *14*: 377-387, 1976.
27 Qiu H, Sirivongs P, Rothenberger M, Rothenberger DA and Garcia-Aguilar J: Molecular prognostic factors in rectal cancer treated by radiation and surgery. Dis Colon Rectum *43*: 451-459, 2000.
28 Leichman CG, Lenz HJ, Leichman L, Danenberg K, Baranda J, Groshen S, Boswell W, Metzger R, Tan M and Danenberg PV: Quantitation of intratumoral thymidylate synthase expression predicts for disseminated colorectal cancer response and resistance to protracted-infusion fluorouracil and weekly leucovorin. J Clin Oncol *15*: 3223-3229, 1997.
29 Rodel F, Hoffmann J, Grabenbauer GG, Papadopoulos T, Weiss C, Gunther K, Schick C, Sauer R and Rodel C: High survivin expression is associated with reduced apoptosis in rectal cancer and may predict disease-free survival after preoperative radiochemotherapy and surgical resection. Strahlenther Onkol *178*: 426-435, 2002.

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