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Preoperative serum levels of c-erbB-2 do not seem to be useful in management of patients with rectal cancer

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Abstract

Background and aims: Soluble c-erbB-2 oncoprotein has been proven as a useful marker in the management of breast cancer patients but its value in diagnostics and follow-up of colorectal cancer patients remains controversial. The aim of this study was to evaluate the usefulness of serum c-erbB-2 monitoring in diagnostics and prediction of disease outcome in rectal cancer patients. *Patients and methods:* Serum samples from 88 patients with rectal adenocarcinoma before surgery and 41 healthy controls were tested for the presence of c-erbB-2 oncoprotein by ELISA and the patients were followed-up for at least 5 years after surgery. *Results:* Preoperative serum c-erbB-2 levels were significantly higher in Stage IV patients than in healthy controls ($P < 0.001$) and did not show correlation with preoperative CEA levels. Elevated preoperative serum c-erbB-2 levels showed relatively high specificity (88%) and low sensitivity (44%) in diagnosis of rectal cancer. Elevated preoperative oncoprotein levels were predictive neither for overall survival nor for development of local recurrence/distant metastases. *Conclusion:* Although preoperative serum c-erbB-2 levels were significantly higher in rectal cancer patients than in healthy controls, the soluble c-erbB-2 does not seem to be useful in diagnosis of rectal cancer due to its low sensitivity. Preoperative serum levels of this oncoprotein were predictive neither for overall survival nor for local recurrence/distant metastases in rectal cancer patients.

Key words: Rectal cancer, soluble c-erbB-2/HER-2/neu, tumor markers, follow-up.

Introduction

The c-erbB-2 (HER-2/neu) oncoprotein is a 185-kD epidermal growth factor receptor-like transmembrane glycoprotein with tyrosine kinase activity. The extracellular domain of c-erbB-2 can be shed from cancer cells in cultures overexpressing c-erbB-2 [1]. The antigen was also found in the sera of patients with breast [2, 3], hepatocellular [4], gastric [5], and ovarian cancers [6]. Possible clinical significance of soluble c-erbB-2 has been so far more extensively studied only in breast cancer patients, where its levels were hypothesized to correlate with the disease stage [7], with response to hormone therapy [8] and chemotherapy [9], and predicts for shortened survival [7]. Data related to serum c-erbB-2 levels in patients with rectal cancer is, to our knowledge, limited to 6 reports [10, 11, 12, 13, 14, 15]. Two of these reports are based on a small number of patients [10, 11] and no one has been dealing particularly with rectal cancer patients. The aim of this study was to assess the association of preoperative serum c-erbB-2 levels with tumor stage, local recurrence, distant metastases, and overall survival of rectal cancer patients.

Subjects and methods

Subjects

Healthy controls. Forty-one healthy volunteers, aged 25 -75 years (median 53) among them 20 females (49%) and 21 males (51%) served as blood donors for determination of soluble c-erbB-2.

Patients. A cohort of 88 patients with primary rectal adenocarcinoma, aged 28 - 79 years (median 60), among them 35 females (40%) and 53 males (60%) were included in this prospective study. Preoperative diagnosis was established by standard diagnostic procedures, i.e. endoscopy and radiography, and when required by ultrasonography and computerized tomography. Regarding tumor localization, all patients had rectal cancer; colon, cecal and anal cancers were not included in the study. All primary tumors were resected and pathohistologically confirmed to be adenocarcinomas. Tumor staging was carried out by AJCC Cancer Staging system [16]. There were 9 (10%) stage 0 patients, 24 (27%) stage I patients, 17 (19%) stage II patients, 20 (23%) stage III patients, and 18 (21%) stage IV patients (13 with liver, 1 with pulmonary, 1 with brain, 2 with bone, and 1 with peritoneal metastases). In the first year following surgery, the patients were followed-up according to a standardized protocol that included laboratory tests (routine blood cell count, CEA), ultrasonography of abdomen, computerized tomography of the pelvis, chest radiography and rectoscopy at 3-month intervals in the first year, at 6-months intervals in the second year after surgery and at yearly intervals afterwards. The study was carried out in compliance with the Helsinki Declaration II [17] and informed consent was obtained from all patients and healthy controls prior to their enrollment in the study.

Methods

Serum c-erbB-2 determination. Blood samples were drawn by venepuncture 1-3 days before surgery between 7:00 and 8:00 a.m. and centrifuged. Sera were separated and stored in aliquots at -20^{o} C until assayed. Levels of soluble c-erbB-2 oncoprotein were determined by enzyme-linked immunosorbent assay (Quantitative ELISA Assay neu/c-erbB-2, Oncogene Research Products, Calbiochem, USA) using monoclonal antibody specific for human p185 $^{neu/c\text{-}erbB\text{-}2}$ as well as for its extracellular fragment p105 (Ab-3). Results were expressed in human *neu* units (HNU)/mL.

Serum CEA determination. Serum CEA levels were determined in serum samples obtained and stored as described above. An immunoradiometric assay for the quantitative determination of carcinoembryonic antigen in human serum or plasma was used (ELSA2-CEA, Cat. # 38C17, CIS Bio International, France), with the level of 5 ng/mL as a cut-off value.

Statistical analysis

The results are presented as medians with corresponding interquartile ranges and were analyzed by Kruskal-Wallis and Mann-Whitney U post-hoc tests with correction for multiple testing. P values < 0.05 were considered statistically significant. Differences between categories were analyzed by χ^2 test. Correlation between c-erbB-2 and CEA levels was analyzed by the Fisher's exact test and by calculation of Pearson correlation coefficient. Survival rates were calculated by the Kaplan-Meier method and compared using the log-rank test, with $p<0.05$ being considered statistically significant. Predictive value of preoperative c-erbB-2 levels was tested by ROC analysis.

Results

On the basis of measurements in healthy volunteers, the concentration of 51.5 HNU/ml c-erbB-2 was used as a cut-off value by the ROC analysis. Specificity, sensitivity, accuracy, false positive/negative ratios, and positive/negative predictive values of soluble c-erbB-2 in diagnosis of rectal cancer are shown in Table 1. With 88% specificity at the selected cut-point, the sensitivity was 44% (39 out of 88 patients with elevated c-erbB-2 values) and was lower than the sensitivity of CEA (48%, 42 out of 88 patients with elevated preoperative CEA levels, Table 2) in diagnosis of rectal cancer. There was no significant correlation between preoperative serum c-erbB-2 and CEA levels ($r = 0.33$, $P=0.086$). Preoperative serum levels of c-erbB-2 were significantly higher in stage IV patients than in healthy controls while there were no significant differences between patients with other disease stages and healthy individuals (Table 2). Taking the value of 51.5 HNU/mL c-erbB-2 as a cut-point, significantly more positive findings were noted among stage IV than in stage 0 patients; there were no significant differences between stage I - III and stage 0 patients (Table 3). During 5-year follow-up (mean follow-up time 63.4 months) 45 out of 70 patients who were disease-free after primary tumor resection remained disease-free, 10 developed local recurrence and 15 developed distant metastases. There was no difference in preoperative serum c-erbB-2 levels between disease-free patients and those who developed local recurrence but in those who developed distant metastases preoperative serum c-erbB-2 levels were significantly higher than in those who were disease-free (Figure 1). The proportion of patients with preoperative elevated c-erbB-2 values (> 51.5 HNU/mL) was also significantly higher among patients who developed distant metastases in comparison with those who remained disease-free (Figure 1). Cut-off value of 51.5 HNU c-erbB-2/mL was also used for the

survival analysis. Patients with elevated preoperative oncoprotein levels showed slightly shorter but nonsignificant overall survival compared to those with normal oncoprotein levels (42.9 months, 95% CI = 34.5-51.3 vs. 45.0 months, 95% CI = 38.9-51.1, respectively; log rank test = 0.63; p = 0.426; Figure 2). ROC analysis of elevated preoperative oncoprotein predictive value gave AUC of 0.540 (95% CI = 0,386-0.694) for local recurrence and AUC of 0.583 (95 % CI = 0.444-0.723) for distant metastases.

Discussion

Preoperative serum c-erbB-2

In this study, we detected significantly higher preoperative serum c-erbB-2 levels in stage IV rectal cancer patients before surgery than in healthy individuals indicating a possible role for c-erbB-2 in colorectal cancer progression, as already suggested in two recently published reports [14, 15]. Regarding the diagnostic value of c-erbB-2, its sensitivity was the same (44%) as reported by Polychronidis and coworkers (44%) [15] and higher than the one reported by Tsigris and coworkers (41%) [14]. The specificity found in our study was also almost the same as in Polychronidis' study (88% vs. 89% respectively) [15]. However, it was lower than reported by Tsigris et al. (97%) [14], probably because the sensitivity in their study was lower than in ours (41% vs. 44%, respectively). Significantly more patients with elevated c-erbB-2 were detected among those with distant metastases than among those with localized disease. Since the sensitivity of serum c-erbB-2 in diagnosis of rectal cancer was lower than the sensitivity of CEA, and since there was no correlation between serum levels of these two markers, serum c-erbB-2 does not seem to be of any value in increasing the sensitivity of rectal cancer diagnosis.

Preoperative serum c-erbB-2 and survival

Although a tendency for shorter overall survival of patients with elevated preoperative serum c-erbB-2 levels when compared to those with normal levels was noted this difference was not significant (log rank test, p=0.426). Polychronidis et al [15] reported similar results in their series of 52 colorectal cancer patients and ascribed the lack of significance to too small number of patients. However, we also failed to demonstrate statistical significance although our cohort comprised 88 patients. On the other hand, Tsigris et al [14] demonstrated the correlation of elevated serum c-erbB-2

levels with overall survival in their series of 63 colorectal cancer patients. This difference could be explained by more restrictive cut-off value for c-erbB-2 levels and correspondingly higher sensitivity obtained in our and Polychronidis' than in Tsigris' study. In addition, our cohort was more homogeneous with respect to tumor localization, comprising only rectal cancer patients. We also did not find elevated preoperative serum c-erbB-2 levels to be predictive for neither local recurrence nor distant metastases. Chamberlain et al [13] also reported that elevated serum c-erbB-2 concentrations were not predictive of disease outcome in their cohort of 88 colorectal cancer patients. However, they did not specify whether this applied to overall survival or to a disease recurrence. In conclusion, elevated serum c-erbB-2 levels showed high specificity and positive predictive value and low sensitivity and negative predictive value so that these levels do not seem to be useful as a tumor marker in rectal cancer diagnosis. They also do not seem to be useful predictors of neither overall survival nor local recurrence/distant metastases in rectal cancer patients.

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Table 1 Diagnostic value of elevated serum c-erbB-2 and CEA levels in patients with rectal cancer

	Serum c-erbB-2 > 51.5 HNU/mL	Serum CEA > 5 ng/mL
Specificity	88%	88%
Sensitivity	44%	48%
Accuracy	58%	59%
False positive ratio (FP)	12%	12%
False negative ratio (FN)	56%	52%
Positive predictive value (PV+)	89%	92%
Negative predictive value (PV-)	42%	38%

Table 2. Soluble c-erbB-2 levels in healthy subjects and rectal cancer patients before surgery regarding the stage of disease

Subjects	Stage of disease	Number of subjects	c-erbB-2, HNU/mL
Healthy controls		41	42 (32-47)*
Rectal cancer patients	0	9	38 (31-53)
	I	24	44 (35-74)
	II	17	47 (37-63)
	III	20	52 (37-72)
	IV	18	55 (39-72)‡

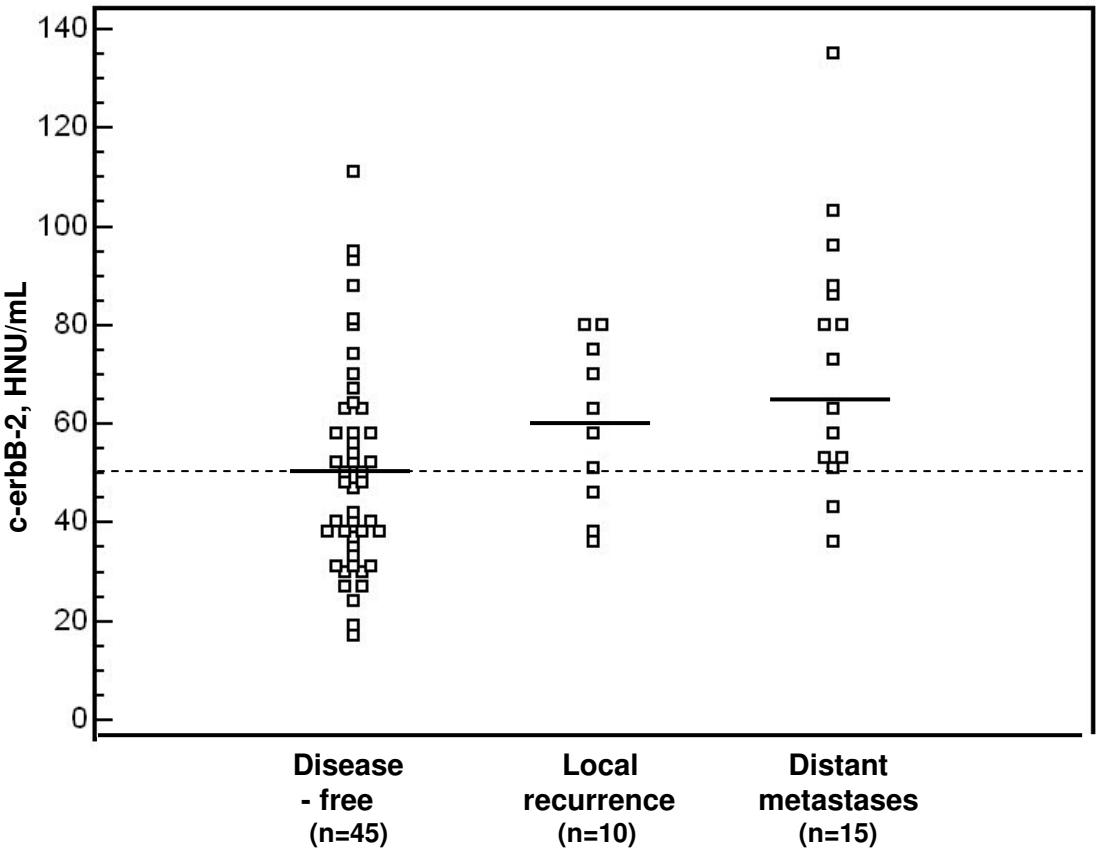
* Median (interquartile range); ‡p <0.001 vs. Healthy controls (Kruskal-Wallis test with Mann-Whitney post-hoc corrected for multiple tests).

Table 3. Number and proportions of rectal cancer patients with elevated preoperative serum c-erbB-2 levels regarding stage of disease

Stage of disease	c-erbB-2 >51.5 HNU/mL		χ^2	*P* vs. Stage 0
	Number of patients	Proportion of patients		
0	1/9	0		
I	6/20	30	0.40	NS
II	8/18	44	1.67	NS
III	9/17	53	2.76	NS
IV	15/24	63	5.02	0.025
Total	39/88	44		

NS = not significant (χ^2 test).

Figure 1. Preoperative serum c-erbB-2 levels in rectal cancer patients who were disease-free after primary tumor resection in relation to disease status after 5-year follow-up



C-erbB-2 values: significantly higher in Distant metastases vs. Disease free (P<0.016, Mann Whitney, uncorrected); number of patients with elevated (>51.5 HNU/mL) c-erB-2: significantly higher in Distant metastases vs. Disease free (P=0.011, χ^2=6.49); dashed line: cut-off value; straight lines: medians.

Figure 2. Kaplan-Meier survival curve showing no significant difference in survival rates between rectal cancer patients with elevated and those with normal preoperative serum c-erbB-2 levels