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Markers of Cellular Adhesion in Diagnosis and Therapy Control of Colorectal Carcinoma

L. HOLUBEC JR.[1], O. TOPOLCAN[1], J. FINEK[1], S. HOLDENRIEDER[3], P. STIEBER[3], M. PESTA[1], R. PIKNER[1], L. HOLUBEC SEN.[1], A. SUTNAR[1], V. LISKA[1], S. SVOBODOVA[2], V. VISOKAI[2] and S. KORMUNDA[1]

[1]*Charles University Prague, Medical Faculty Pilsen;*
[2]*Charles University Prague, First Medical Faculty Prague, Czech Republic;*
[3]*Institut fuer Klinische Chemie, LMU-Klinikum Grosshadern, University of Munich, Germany*

Abstract. *Aim: Early diagnosis of the progressive tumor disease and control of the effect of therapy in colorectal carcinoma are most frequently performed by monitoring CEA or CA 19-9 tumor markers. Their clinical application is, however, limited. The aim of our study was to demonstrate the contribution of adhesive molecule assessment to the early diagnosis of progression. We also wanted to find out if changes in the levels of cellular adhesion parameters correlate with the effect of antitumor therapy. Materials and Methods: Intercellular cell adhesive molecule-1 (ICAM-1) and Vascular cell adhesive molecule-1 (VCAM-1) were assessed using the ELISA method, and the results were correlated with CEA and CA 19-9 tumor markers. Three hundred and sixty-four patients with colorectal carcinoma in Dukes' stages B-D were monitored. The results were processed with the SAS 6.2. statistical program and Statistica. Results: In 92 patients with first clinical progression (occurrence of distant metastases irrespective of localization), significantly increased ICAM-1 and VCAM-1 values were demonstrated. In ROC evaluation of curves, we also demonstrated high sensitivity of adhesive molecules against both the control healthy group (n=89) and the no evidence of disease group (NED) (n=183). Adhesive molecule levels were closely connected with the type and course of therapy and are presented in the form of case reports. Conclusion: Soluble adhesive molecules are a prospective parameter both for the early diagnosis of progression and for control of the effect of therapy. There is a need for a large-scale study, preferably multicentric, which would verify the suitability of introducing cellular adhesion parameter assessment into routine practice.*

Correspondence to: Lubos Holubec, MD.,Ph.D., Dept.of Oncology and Radiotherapy, E.Benese 13, 305 99 Pilsen, Czech Republic, e-mail: HOLUBEC@fnplzen.cz

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With the development of modern surgical techniques in curative therapy of secondary metastases from colorectal carcinoma, there are increasing demands for their early diagnosis (1, 2). Apart from the use of screening methods, the assessment of the markers of tumor biological activity (which characterize individual steps in the metastatic process) seems to offer good prospects. Cellular adhesion markers, which find their application in the nidation of tumor cells and their reverse penetration into tissue, as well as in the formation and growth of metastases in new tissue, may be considered to represent such a parameter (4, 10, 14). Our previous pilot studies demonstrated significant changes of adhesive molecule values in patients with colorectal carcinoma (13). The aim of this study was to demonstrate the contribution of adhesive molecule assessment for an early diagnosis of progression, and also to determine whether changes in the levels of cellular adhesion parameters correlate with the effect of antitumor therapy.

Materials and Methods

Serum values of soluble cell adhesive markers ICAM-1 (Intercellular cell adhesive molecule-1) and VCAM-1 (Vascular cell adhesive molecule-1) were assessed using the ELISA method (Bender Medsystems). The results were correlated with serum values of the recommended CEA (carcinoembryonic antigen) tumor markers and CA 19-9 (carbohydrate antigen) tumor markers using the IRMA method (Immunotech in Prague, Cis Bio International, France).

For the tumor marker assessment, venous blood from the cubital vein was sampled in standard conditions between 7 and 9 a.m., in order to exclude the effects of daily rhythm. The serum obtained by centrifugation was stored at a temperature of –70˚C until the laboratory analysis.

Statistical analysis of the data was performed by using the S.A.S program, version 6.12 and the Statistica program. Descriptive statistics (average, median, standard deviation, maximum, minimum) were calculated for the whole group of patients, as well as for individual subgroups.

Table I. *Medians (MED) and the statistical significance of differences for the individual compared groups (Healthy, NED, RD).*

	Serum levels (median)			
Groups	ICAM-1	VCAM-1	CEA	CA 19-9
Healthy controls	206.0	358.0	1.2	10.5
No evidence of disease (NED)	277.0	640.0	1.4	7.8
Recurrence of disease (RD)	433.0	1111.0	16.5	41.2
	p-values (Wilcoxon test)			
Compared groups	ICAM-1	VCAM-1	CEA	CA 19-9
Healthy x RD	0.0001	0.0001	0.0001	0.0001
NED x RD	0.0001	0.0001	0.0001	0.0001

Profiles of specificities and corresponding sensitivities for individual referential groups are illustrated with received operating characteristics (ROC) curves in accordance with EGTM recommendations (5). Comparison of the groups according to different criteria was made with the Wilcoxon non-pair test.

Patients were monitored during the follow-up period at regular 3-month intervals for the first 2 years and at 6-moth periods afterwards. Patients were examined by the clinical oncologists using physical examination and imaging methods (X-ray, ultrasound, CT and colonoscopy), according to the recommendation of European Society for Medical Oncology (ESMO). A blood test for tumor markers and basic biochemistry screening was performed for every patient at every regular visit. Inclusion criteria for this clinical trial were normal liver and renal function tests (serum urea, serum creatinine, SGPT, SGOT).

We monitored patients with colorectal carcinoma in Dukes' stages B-D. This included a total of 183 patients with no evidence of disease (NED) and 92 patients with recurrence of disease (RD). The definition of the patients' clinical condition was as follows:

- The first clinical progression of the disease irrespective of localization (without distinguishing locoregional relapse and distant metastases) was considered as recurrence of disease (RD).
- Patients with no evidence of the disease symptoms within at least one year during follow-up were considered as remission patients (NED).

As a control group, we used a group of 89 healthy individuals, whose median age (58 years) corresponded roughly to the median age of the patients with colorectal carcinoma (64 years). The dynamics of tumor marker changes in patients after adjuvant chemotherapy, following primary surgery of colon carcinoma, are presented as case reports. In both cases these were patients in the Dukes' stage C, and both patients were treated with 5-fluorouracil potentiated with leucovorin, according to the MAYO regimen.

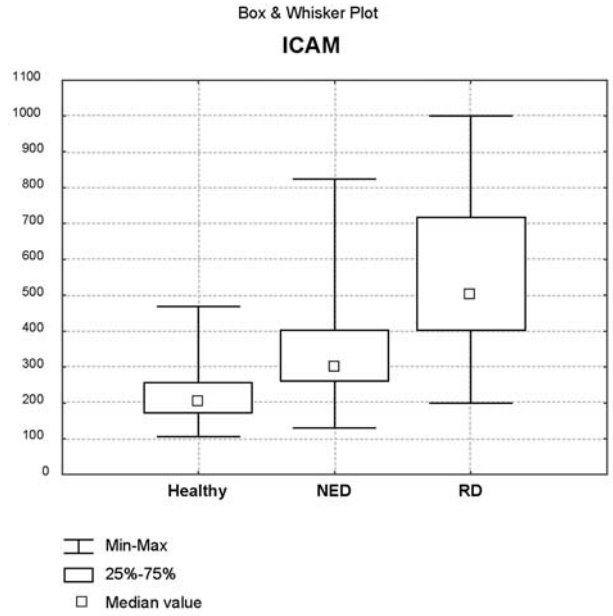

Figure 1. *The basic descriptive statistics for ICAM-1.*

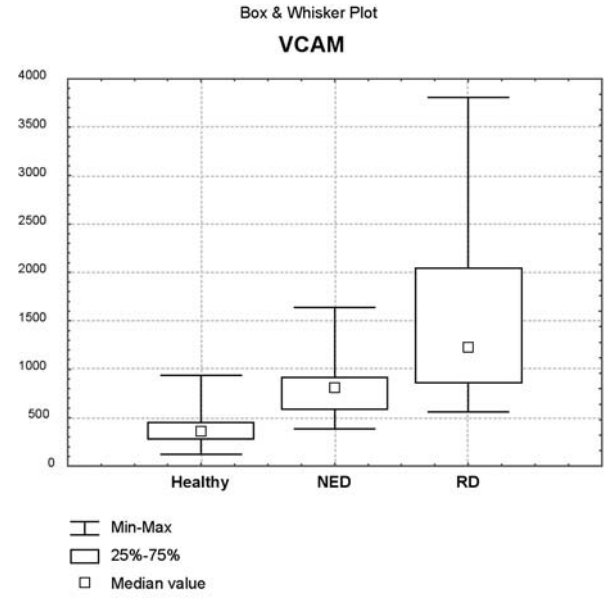

Figure 2. *The basic descriptive statistics for VCAM-1.*

Results

Table I presents medians (MED) for the individual compared groups (healthy, NED, RD), and the statistical significance of differences between the groups. In patients with clinical progression (occurrence of distant metastases irrespective of localization), we demonstrated significantly increased values of ICAM-1 and VCAM-1 compared to both healthy and remission groups. Best correlation was achieved between the levels of adhesive molecules ICAM-1 and VCAM-1 in patients with clinical progression (Spearman rank correlation coefficient = 0.72; $p<0.001$).

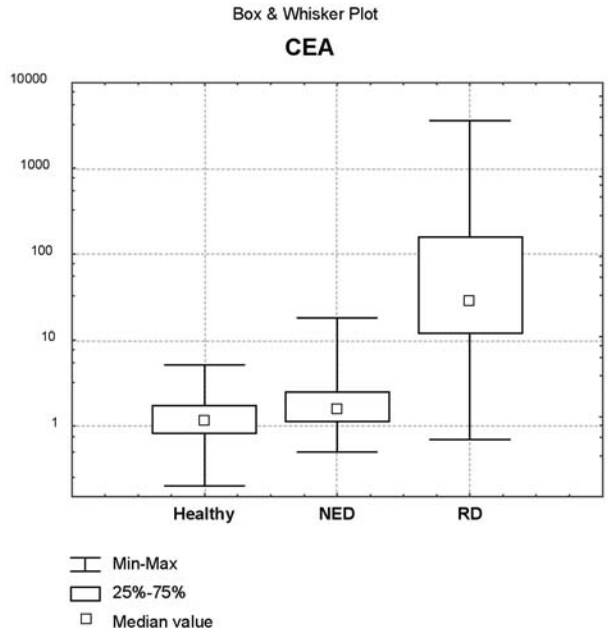

Figure 3. *The basic descriptive statistics for CEA.*

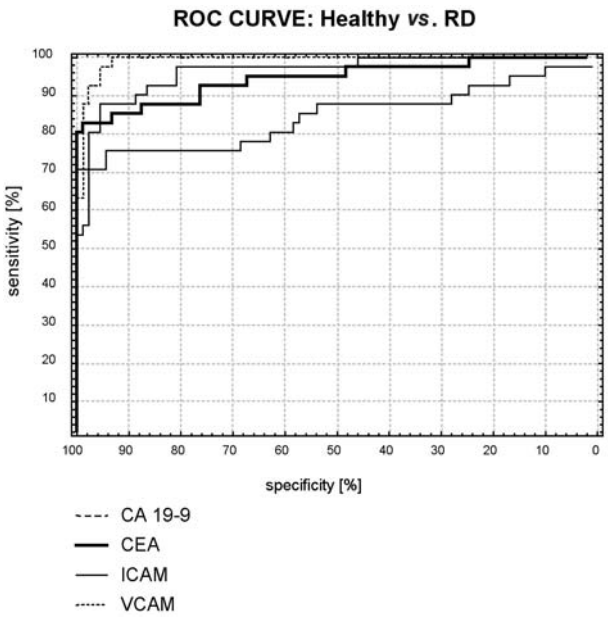

Figure 5. *ROC curves assessment: recurrence of disease versus healthy control group.*

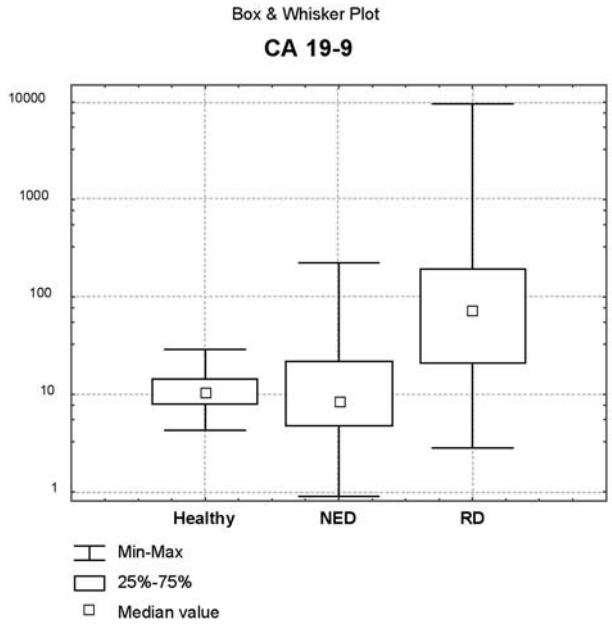

Figure 4. *The basic descriptive statistics for CA 19-9.*

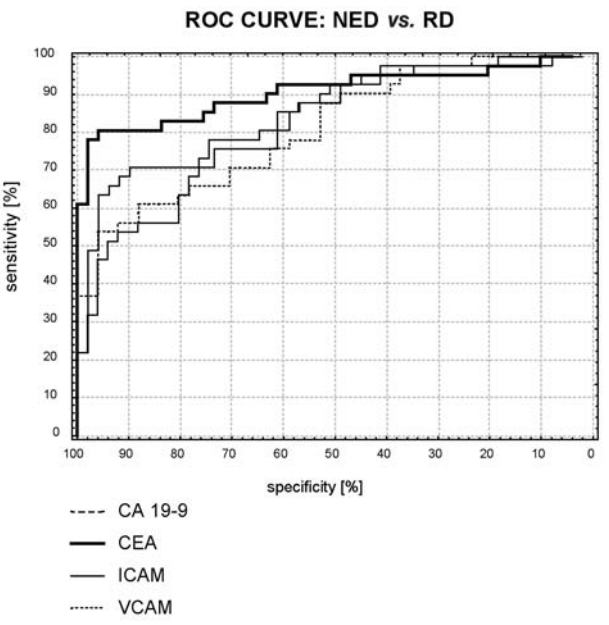

Figure 6. *ROC curves assessment: recurrence of disease versus no evidence of disease.*

The basic descriptive statistics for individual compared markers (ICAM-1, VCAM-1, CEA and CA 19-9) and individual compared groups (healthy, NED, RD) are given in the form of box plots in Figures 1-4.

We also demonstrated high sensitivity of adhesive molecules during ROC curves assessment, both against the control healthy group and NED group (see Figures 5, 6). Adhesive molecule levels were closely connected with the type and course of the therapy, and are presented in the form of case reports. The first case was a 62-year-old patient with Dukes' stage C colon tumor. The patient underwent adjuvant chemotherapy and, since its completion, he has

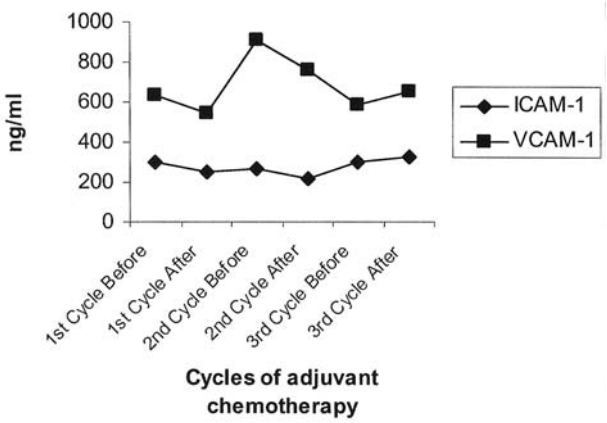

Figure 7. *Case report: changes in the level dynamics of ICAM-1 and VCAM-1 during the adjuvant chemotherapy, favorable prognosis.*

been in a long-term remission. During the chemotherapy, we observed normal values for ICAM-1 and slightly increased values for VCAM-1. A typical example of the course of changes in the level dynamics is shown in Figure 7. Other tumor marker levels did not demonstrate any substantial changes during the therapy and were mostly within the range of normal values (see Table II).

Figure 8 shows the dynamics of changes in adhesive molecule values in a 57-year-old patient with Dukes' stage C colon tumor. In this patient, disease progression occurred within 6 months after the end of adjuvant chemotherapy. Already during the chemotherapy pathological values of ICAM-1 and VCAM-1 had been registered. The progression did not influence other tumor marker values: only the values for TPS cytokeratin were slightly increased (Table III).

Discussion

Thanks to the development of new therapeutic possibilities for colorectal carcinoma, such as new surgical techniques for liver metastases or new types of chemotherapy drugs and their combinations with immunotherapy, more attention is being paid to the early diagnosis of tumor diseases (9, 11). The situation in colorectal carcinoma, however, is highly unsatisfactory, since more than 50% of tumors are diagnosed in late stages (Dukes' C or D) when locoregional glands are already affected or distant metastases are present. The evidential value of classical tumor markers is limited and, apart from CEA, other markers do not have sufficient sensitivity (7, 8, 12, 15). There is, therefore, an effort to find new markers of tumor biological activity which will replace present markers or, in combination with them, improve their diagnostic possibilities (6, 10). Adhesive molecules represent a group of markers which find their application in the cascade

Table II. *Case report: changes in the level dynamics of tumor markers during the adjuvant chemotherapy, favorable prognosis.*

Chemotherapy	ICAM-1 ng/ml	VCAM-1 ng/ml	CEA ng/ml	CA 19-9 U/ml	TPA U/l	TPS U/l
1st Cycle Before	297	634	0.7	7.3	31	35
1st Cycle After	254	539	0.8	4.1	31	31
2nd Cycle Before	266	906	0.6	8.4	14	51
2nd Cycle After	220	760	0.8	5.0	10	48
3rd Cycle Before	303	580	0.7	11.5	10	47
3rd Cycle After	324	654	0.7	8.2	14	24

Table III. *Case report: changes in the level dynamics of tumor markers during the adjuvant chemotherapy, unfavorable prognosis.*

Chemotherapy	ICAM-1 ng/ml	VCAM-1 ng/ml	CEA ng/ml	CA 19-9 U/ml	TPA U/l	TPS U/l
1st Cycle Before	430	1388	0.7	7.3	40	130
1st Cycle After	474	1661	0.8	4.1	45	133
2nd Cycle Before	593	1961	0.6	8.4	43	280
2nd Cycle After	624	2933	0.8	5.0	42	245
3rd Cycle Before	712	2244	0.7	11.5	73	340
3rd Cycle After	673	2308	0.7	8.2	49	354

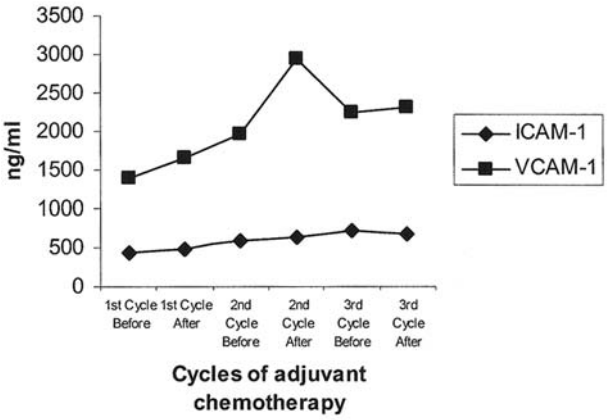

Figure 8. *Case report: changes in the level dynamics of ICAM-1 and VCAM-1 during the adjuvant chemotherapy, unfavorable prognosis.*

of metastatic processes during the formation and growth of metastases in new tissue (13). We directed our attention to soluble receptors of ICAM-1 and VCAM-1 immunoglobulins which, in interaction with cytokines, participate in endothelium activation and subsequent adhesion of thrombocyte and metastatic tumor cell aggregations to endothelium. In this way, they assist the formation of distant metastases (4, 14). Median values of both these parameters were statistically significantly higher in patients with

progression than in patients with no evidence of disease (NED) and in healthy control groups. While evaluating the specificity-sensitivity profile of ROC curves, we demonstrated high sensitivity of adhesive molecules particularly in comparison with the healthy control group. CEA remained the best marker in comparison with the NED group, and ICAM-1 also demonstrated a good profile. One possible reason for the difference in adhesive molecule profiles between the control healthy group and patients with NED is the anticipated long lead time in these markers, so that some "non-specific" cytoadhesion elevations are in fact already connected with future progression of the tumor. We confirmed this assumption in some cases of patients who underwent adjuvant chemotherapy. When the marker profile did not change during chemotherapy, the prognosis for these patients was good and they are still in a long-term remission of the disease. When gradual elevation of adhesive molecules occurred during the therapy, these patients were diagnosed with progression of the tumor disease.

Adhesive molecules have good prospects as parameters, both for early diagnosis of progression and control of the effect of therapy. There is a need for a large-scale study, preferably multicentric, which would verify the suitability of introducing the assessment of cellular adhesion parameters into routine practice.

Acknowledgements

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