Tumor markers in staging and prognosis of colorectal carcinoma

M. LEVY[1*], V. VISOKAI[1], L. LIPSKA[1], O. TOPOLCAN[2]

[1] Thomayer Teaching Hospital, 1.st Medical Faculty of Charles University Prague Surgical Department, Videnska 800, 14059 Prague 4, Czech Republic, e-mail: miroslav.levy@ftn.cz; [2] Faculty Hospital Pilsen, Medical Faculty Pilsen, Charles University Prague, Imunoanalytical laboratory, E. Benese 13, 305 99 Pilsen, Czech Republic

Received July 16, 2007

The precise preoperative staging of colorectal cancer is fundamental for surgical strategy, incomplete staging means incomplete treatment and poor outcome. Large-scale clinical evaluations of predictive markers are currently in progress, including determination of their ability to predict response of patients to therapy for advanced disease and for adjuvant treatment. Lack of specificity and sensitivity preclude the use of all existing serum markers for the early detection of colorectal carcinoma. The aim of the study was to investigate the clinical significance of serum tumor markers and biological activity markers – oncofetal tumormarker CEA, mucin tumormarkers CA19-9, CA242, proliferative tumor markers Thymidine kinase, soluble cytoceratines fragments TPS, TPA, adhesive molecules ICAM – 1, VCAM -1, IGF-1, and adipocytokinins Adiponectin, Leptin in patients with colorectal cancer before primary operation. The study included 142 patients between the ages of 35 – 89 years. Operated between November 2003 to March 2006. We have confirmed that CA19-9 is besides CEA an important marker in colorectal cancer. Comparing CA19-9 and CA242 in preoperative staging, CA242 is more specific. Statistical significant difference between early and metastatic stage of colorectal cancer was not confirmed in markers: ICAM-1, VCAM, adiponectin, leptin. Statistical significant difference between early and metastatic stage of colorectal cancer was confirmed in markers: CEA, CA19-9, CA242, TPS, TPA, TK, IGF-1. None of the used markers was able to distinguish stage II and III, in other words to identify patients with infiltration of lymph nodes. This fact is very important in our aspirations to find which marker from periferal blood could help to poit out patients in risk of lymphatic infiltration and to indicate these patients for adjuvant therapy. Combination of CEA and either CA19-9 or CA242 can be recommended for preoperative investigation. CA 242 in this study seems to have slightly better results in preoperative staging.

Key words: tumor markers, colorectal carcinoma, staging, prognosis

Colorectal cancer (CRC) remains a major public health problem throughout the world. Currently, CRC is the second most common cancer in Europe both in terms of incidence and mortality.

The goal of all cancer research and treatment is to prevent people dying from the disease. Knowledge has been accruing rapidly about actions and interventions that could lead to a reduction in death from colorectal cancer by reducing the risk of developing the disease, identifying the disease at a stage when it is more curable, or improving the outcome of treatment. The precise preoperative staging is fundamental for surgical strategy, incomplete staging means incomplete treatment and poor outcome [1,2].

Large-scale clinical evaluations of predictive markers are currently in progress, including determination of their ability to predict response of patients to therapy for advanced disease and for adjuvant treatment[3,4,5]. Lack of specificity and sensitivity preclude the use of all existing serum markers for the early detection of CRC [6].

Our Surgical Department has a long time experience using CEA and CA19-9 in staging and follow up of patients with colorectal cancer. In this thesis, results of study examining other markers are presented.

Aim of the study. To investigate the clinical significance of serum tumor markers and biological activity markers – oncofetal tumormarker CEA, mucin tumormarkers CA19-9, CA242, proliferative tumor markers Thymidine kinase, soluble cytoceratines fragments TPS, TPA, adhesive molecules ICAM – 1, VCAM -1, IGF-1, and adipocytokinins Adiponectin,

* Corresponding author

Leptin in patients with colorectal cancer before primary operation.

To determine relations of above stated markers with the earliest and the most advanced stages of colorectal cancer.

To select from the above mentioned markers those, with statistically significant differences between levels in early and advanced stages of colorectal cancer .

To correlate these selected markers with TNM stages of colorectal cancer.

To identify the optimal combination of serum tumor markers in colorectal cancer staging.

To suggest markers for preoperative staging of patients with colorectal cancer.

Methods

This study is presenting prospectivelly collected data concerning patients with colorectal adenocarcinoma verified by biopsy – before primary operation. Positive biopsy was the inclusion criterion. Exclusion criterion: absence or lack of blood sample.

Standard preoperative staging according to local (Dept. of Surgery Thomayer Teaching Hospital) protocol was performed[7]. Before urgent operations patients had limited staging according to the individual conditions.

Periferal blood samples were collected in the period of 3 weeks prior to the operation and the day of surgery. The analysis was done in Dept. of Oncology, Immunoanalytical laboratory, University Hospital Pilsen – the methodology vide infra.

All the patients were operated at the Surgical Department of Thomayer Teaching Hospital and the1st Faculty of Medicine Charles University Prague according our standard.

In emergency operations of colorectal cancer with curative intent, the same principles of radicality as in elective procedures are followed.

Histological specimens were evaluated at the Department of Pathology Thomayer Teaching Hospital. Lymph nodes are retrieved by surgeons from the native specimen.

Stage of the cancer used in statistical analysis in the study was according to the UICC TNM classification 1997 and was based on clinical data, imaging results, operation records and surgical specimens.

For N status description at least 12 lymphonodes must be histologically examined, otherwise stated as NX stage.

Patients were devided according to stage of the disease in three groups.

In the first group (early) patients with T category Tis and T1 were included, so that there was the highest probability of non metastatic tumor. In the second group (metastatic) there were patients with evident disseminated disease- N and M positivity, in the third group (non- early, non- metastatic) there were non-early non-metastatic patients.

Table 1. Group characteristics

	No
Males	90 patients
Females	52 patients
Colon cancer	72 patients
Rectal or rectosigmoid cancer	70 patients
TNM I	26 patients
TNM II	42 patients
TNM III	31 patients
TNM IV	43 patients

Material. The study included 142 patients between the ages of 35 – 89 years. Operated between November 2003 to March 2006 (Tab. 1.).

Analysis of samples

For the tumor marker assessment venous blood from the cubital vein was sampled in standard conditions between 7 and 9 a.m. The serum acquired through centrifugation was stored until laboratory analysis at a temperature of -70°C. Tumor markers were assessed with commercial laboratory kits, in accordance with the manufacturers' recommendations. The following tumor markers were assessed: CEA and CA 19-9 (MEIA, AxSYM Abbott), TPA (IRMA, Byk Sangtec), TPS (IRMA, Beki), TK (REA, Immunotech), CA 242 (ELISA, CanAg Diagnostics AB). ICAM-1 and VCAM-1 were assessed by multiplex analysis (LUMINEX, Linco Research), Adiponectin, Leptin and IGF-1 were assessed by isotopic methods. All the patients were also clinically examined by an surgeon during every blood sampling for tumor markers.

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. Comparison of the groups according to different criteria was made with the Wilcoxon non-pair test. The values equal to or less than 0.05 were considered as significant. The data were also analyzed using the Spearman correlating coefficient; the values equal to or less than 0.05 were considered as significant.

Results

Significant differences in serum levels of markers were confirmed between early stage colorectal cancer and colorectal cancer of metastatic stage in CEA, CA242, TK, TPA, TPS and IGF. Between early stage colorectal cancer and non-early non-metastatic stage were significant differences not confirmed in any of the markers. Between non-early non-metastatic stage and metastatic stage were differences confirmed in CA 19-9 and CA 242 only. CEA,

Table 2. Descriptions of markers, early stage

Marker	N	Median	Lower Quartil	Upper Quartil	Minimum	Maximun
CEA	19	1,50	1,00	3,60	0,30	12,90
CA 19-9	20	10,75	7,45	19,70	4,00	30,00
CA – 242	16	6,75	4,50	9,40	1,80	22,80
TK	17	3,60	2,70	5,60	1,00	13,90
TPA	17	31,00	0,00	44,00	0,00	159,00
TPS	20	30,00	3,50	52,50	0,00	179,00
ICAM	15	131,00	112,00	161,00	78,00	248,00
VCAM	15	610,00	439,00	1038,00	274,00	1266,00
Adiponectin	11	24,70	20,60	34,60	13,40	62,90
Leptin	11	4,80	3,20	15,40	1,90	32,50
IGF 1	19	274,10	213,40	389,90	150,50	695,30

Table 3. Descriptions of markers, metastatic stage

Marker	N	Median	Lower Quartil	Upper Quartil	Minimum	Maximun
CEA	91	3,20	2,00	13,70	0,30	585,00
CA 19-9	90	15,45	8,90	61,00	0,00	2842,00
CA – 242	84	10,15	5,40	36,10	0,70	150,00
TK	76	5,65	3,80	8,55	0,40	295,00
TPA	76	51,50	28,50	97,00	0,00	1266,00
TPS	80	53,00	21,50	131,00	0,00	1544,00
ICAM	72	142,00	117,00	206,00	70,00	655,00
VCAM	72	801,50	498,00	1121,00	222,00	1752,00
Adiponectin	58	23,05	16,40	32,35	7,30	72,10
Leptin	58	5,30	2,95	10,20	1,10	73,10
IGF 1	87	223,60	157,20	277,90	93,50	478,20

Table 4. Descriptions of markers, non-early non-metastatic stage

Marker	N	Median	Lower Quartil	Upper Quartil	Minimum	Maximun
CEA	21,00	2,20	1,30	5,00	0,00	85,00
CA 19-9	21,00	8,10	5,90	13,40	0,70	104,80
CA – 242	17,00	6,40	3,70	10,00	0,10	98,70
TK	14,00	5,15	3,70	6,80	1,00	80,00
TPA	14,00	41,50	27,00	52,00	0,00	94,00
TPS	16,00	36,50	17,00	56,00	3,00	687,00
ICAM	16,00	130,50	107,50	170,50	94,00	463,00
VCAM	16,00	845,50	543,00	1148,50	277,00	1608,00
Adiponectin	13,00	26,10	18,30	35,80	6,00	66,70
Leptin	13,00	7,00	5,70	40,60	2,30	51,80
IGF 1	17,00	237,90	170,80	393,50	125,10	679,10

CA19-9, CA242, TK, TPA, TPS and IGF were selected for further investigation. (Tab. 2-5)

Using Spearman Correlation Coefficients between TMN stage and markers, statistically significant correlation between TNM stage and markers were confirmed in CEA, CA 19-9, CA 242, TK, TPS, TPA, IGF.

Comparing levels of selected markers in TNM stages, significant differences in serum levels of markers were between

Table 5. Comparison of early stage (1) versus metastatic stage (2) versus non-early non-metastatic stage (3)

Marker	Statistically significant differences p <		
	1 versus 2	1 versus 3	2 versus 3
CEA	**0,0005**	0,1834	0,6290
CA 19-9	0,08	0,1952	**0,0520**
CA – 242	**0,04**	0,8722	**0,0340**
TK	**0,0058**	0,1933	0,4060
TPA	**0,0084**	0,4409	0,6780
TPS	**0,0157**	0,3148	0,1990
ICAM	0,2900	1,0	0,3770
VCAM	0,4320	0,4021	0,7916
Adiponectin	0,3502	0,9315	0,4420
Leptin	0,5395	0,2947	0,0810
IGF 1	**0,0112**	0,3810	0,2700

TNM stage I colorectal cancer and stage IV in all selected markers, between TNM stage II and stage IV in CEA, CA19-9, CA242, TPS and TPA, between TNM stage III and stage IV in CEA, CA19-9, CA242, TPS and TPA, between TNM stage I and stage II in CEA and TK and between TNM stage I and stage III in CEA and TK. None of the selected markers showed statistically significant difference comparing levels in TNM II and III stage.

The differences among behaviors of particular markers throughout examined groups were noticed and so we were interested if some fix context can be detected or if each marker is independent. That is why correlations in all groups were performed.

Spearman Correlation Coefficients in group 1 (early): there are correlations between CEA and CA19-9, CEA and CA242. Suprisingly TK, TPS, TPA do not correlate with any other marker.

Spearman Correlation Coefficients in group 2 (metastatic): TK correlates with TPA, TPS and CA19-9. TK does not correlate with CEA and CA242. TPA correlates with TPS, CEA and CA242. TPA does not correlate with CA242. TPS correlates with TPA, CEA and CA19-9; does not correlate with CA242. CA 242 correlates with CEA, CA19-9 but does not correlate with TPS, TPA and TK.

Spearman Correlation Coefficients in group 3 (non-early non-metastatic): CEA correlates with TPA in a negative way, CEA does not correlate with CA19-9, CEA does not correlate with CA19-9, TPS. CEA correlates with CA242. CA19-9 correlates with CA242 and with nothing else.

Discussion

The aim of this work was to evaluate the role and help of markers of biologic activity of colorectal cancer in preoperative staging. For this intention three groups of biological factors were chosen. Different types of tumor markers- CEA, mucin markers, markers of cytoskeleton etc., adipocytokines, which are known risk factors for colorectal carcinogenesis and adhesive molecules, known for relations with metastatic process.

During the last 10 years there were about 240 papers concerning adiponectin, leptin and especially IGF. These articles dealing mostly with cancerogenesis of colorectal cancer with links to metabolic syndome X, hypertension, diabetes mellitus, where IGF is studied as one of potential risk factors. This presented study is one of few which is monitoring levels IGF, adiponectin and leptin according to the stage of colorectal cancer. It seems that IGF, leptin and adiponectin play an important role in genesis of colorectal cancer, but as they are locally active substances, it is questionable if they can in periferal blood reflect the stage of tumor. In our study there was no statistical significance in differnces of leptin levels between patients in early stage and metastatic stage.

Adiponectin and IGF are mentioned in a series of papers published by Wei, Giovannucci et al. as risk factors in etiopathogenesis ef colorectal cancer[8], but in literature we did not find a study concerning adiponectin levels related to colorectal cancer stage in the same way as it is in this study.

In total we have found seven papers on adhesion molecules and colorectal cancer, in these, in agreement with our experience, is significant difference between patients with colorectal cancer and a control group. There are still controversies in literature regarding correlations of levels of adhesion molecules and stage of colorectal cancer. Velikova[9] in 1998 investigated the concentrations of the soluble adhesion molecules in 48 patients with colorectal cancer before treatment, levels of circulating ICAM-1 and VCAM-1 were increased both in patients with local and those with metastatic disease. In our results neither ICAM nor VCAM levels correlated with dissemination of colorectal cancer when compared early stage and metastatic stage of the disese.

In pub-med search have been found only three articles concerning thymidine kinase and colorectal cancer. In 1994 Tanigawa studied in 127 patients with colorectal cancer, the relations between thymidine uptake by cancer cells in semisolid media, their clinico-pathologic features and survival times [10]. In 1995 Thomas presented a study on TK concerning patients with asymptomatic colorectal carcinoma and patients known to have hepatic metastases from colorectal tumours [11]. The TK activity in patients with asymptomatic cancer was lower comparing to TK activity in patients with metastatic disease and the difference was statistically significant. In our study the same result was confirmed – the difference between levels of TK in patients with colorectal carcinoma stage TNM I versus TNM IV was statistically significant. Moreover there was statistically significant difference between stage TNM I versus TNM II and the same in TNM I versus TNM III. Only the stage TNM II versus TNM III has no statistically significant difference in levels of TK, similarity with other markers will be shown below. In a study published by Topolcan[12] in 2005 thymidine kinase seems to be a suitable parameter for monitoring the effect of adjuvant and palliative chemotherapy in colorectal cancer.

The changes of cytokeratine markers in colorectal cancer were in previous literature often discussed and most of the authors came to the cunclussion, that TPA and TPS are of none or diminished prognostic significance. We have found statistically significant differences between early and metastatic colorectal carcinoma, but there were no significant differences between “non-metastatic non-early “ and metastatic stages or between “non-metastaic non-early“ and early stages. It seems that TPS and TPA is able to confirm advanced disease, but comparing to routinelly used CEA and CA19-9, cytokeratines do not provide any additional information.

Papers concerning CA242 are published predominantly in Scandinavia and China. In the years 1991-1994 most of the articles presented results of investigating CA242 in relations to diagnosis and monitoring of colorectal cancer[13,14,15]. A longitudinal evaluation of serum CA242 levels demonstrated that this marker was indicative of the status of colorectal cancer disease in a paper of authors from Rome[16]. After surgery of colorectal cancer, CA 242 emerged as a significant predictor of survival, in multivariate analysis, entering the tumour markers as continuous variables, Dukes’ stage was the strongest prognostic factor, followed by CA 242, whereas age, gender, CEA and TPA were not[17].

The issue of CEA and CA19-9 is well known from literature and our current results endorse these findings. We have confirmed that CA19-9 is besides CEA an important marker in colorectal cancer. Comparing CA19-9 and CA242 in preoperative staging, CA242 is more specific. Correlation coeffitients between CA19-9 and CA242 are about 0,7 thus these markers are not identical, but in colorectal cancer is the advantage of CA242 over CA19-9 not so evident as in cancer of pancreas. Regarding results of our study, CA242 seems to be more sensitive in preoperative staging than CA19-9, especially comparing early and metastatic stages.

From the clinician point of view is the comparison of marker levels in TNM stage II and III very interesing. None of the used markers was able to distinguish stage II and III, in other words to identify patients with infiltration of lymph nodes. This fact is very important in our aspirations to find which marker from periferal blood could help to poit out patients in risk of lymphatic infiltration and to indicate these patients for adjuvant therapy. As well interesting is, that the same comparison of the same markers in stage TNM I and III is suggesting two markers – TK and CEA. In this consequence it is necessary to mention issue of N understaging despite of meticulous effort to identify and examine the lymphonodes from the specimen as described above in methodology. It is possible that some patient with infiltrated lymph nodes are hidden in the TNM stage II. That is the reason why at our department we indicate patient pT3 and pT4, N0, M0 for chemotherapy.

Conclussions

Statistical significant difference between early and metastatic stage of colorectal cancer was not confirmed in markers: ICAM-1, VCAM, adiponectin, leptin.

Statistical significant difference between early and metastatic stage of colorectal cancer was confirmed in markers: CEA, CA19-9, CA242, TPS, TPA, TK, IGF-1.

Correlations between levels of markers and TNM stage were confirmed.

Combination of CEA and either CA19-9 or CA242 can be recommended for preoperative investigation. CA 242 in this study seems to have slightly better results in preoperative staging.

References

[1] DANIELS IR, FISHER SE, HEALD RJ, MORAN BJ., Accurate staging, selective preoperative therapy and optimal surgery improves outcome in rectal cancer: a review of the recent evidence, Colorectal Dis. 2007; 9(4): 290–301.

[2] STEIN U, SCHLAG PM. Clinical, biological, and molecular aspects of metastasis in colorectal cancer. Recent Results Cancer Res. 2007; 176: 61–80.

[3] KANEKO I, TANAKA S, OKA S, et al. Immunohistochemical molecular markers as predictors of curability of endoscopically resected submucosal colorectal cancer. World J Gastroenterol. 2007; 13(28): 3829–35.

[4] OGINO S, KAWASAKI T, KIRKNER GJET al. Evaluation of Markers for CpG Island Methylator Phenotype (CIMP) in Colorectal Cancer by a Large Population-Based Sample.J Mol Diagn. 2007; 9(3): 305–314.

[5] FAMULSKI W, SULKOWSKA M, WINCEWICZ A et al. P53 correlates positively with VEGF in preoperative sera of colorectal cancor patients. Neoplasma. 2006; 53(1): 43–8.

[6] DUFFY MJ, van DALEN A, HAGLUND C et al. Tumour markers in colorectal cancer: European Group on Tumour Markers (EGTM) guidelines for clinical use. Eur J Cancer. 2007; 43(9): 1348–60.

[7] VISOKAI V, LIPSKA L, BERGMANN P et al. Multiorgan resections for advanced colorectal cancer.Anticancer Res. 2006; 26(4B): 3183–6.

[8] Wei EK, Giovannucci E, Fuchs CSet al. Low plasma adiponectin levels and risk of colorectal cancer in men: a prospective study.J Natl Cancer Inst. 2005; 97(22): 1688–94

[9] VELIKOVA G, BANKS RE, GEARING A et al. .Serum concentrations of soluble adhesion molecules in patients with colorectal cancer. Br J Cancer. 1998; 77: 1857–63.

[10] TANIGAWA N, MASUDA Y, MURAOKA R et al. Prognostic significance of in vitro thymidine uptake in patients with colorectal carcinoma.J Surg Oncol. 1994; 55(4): 209–14

[11] THOMAS WM, ROBERTSON JF, McKENNA PG et al. Serum thymidine kinase in colorectal neoplasia.Eur J Surg Oncol. 1995; 21(6): 632–4

[12] TOPOLCAN O, HOLUBEC L Jr, FINEK J et al. Changes of thymidine kinase (TK) during adjuvant and palliative chemotherapy. Anticancer Res. 2005; 25(3A): 1831–3

[13] ESKELINEN M, PASANEN P, KULJU A et al.Clinical evaluation of serum tumour markers CEA, CA 50 and CA 242 in colorectal cancer. Anticancer Res. 1994; 14(3B): 1427–32.

[14] KUUSELA P, HAGLUND C, ROBERTS PJ. Comparison of a new tumour marker CA 242 with CA 19-9, CA 50 and carcinoembryonic antigen (CEA) in digestive tract diseases. Br J Cancer. 1991; 63(4): 636–40

[15] KAWA S, TOKOO M, HASEBE O et al. Comparative study of CA242 and CA19-9 for the diagnosis of pancreatic cancer. Br J Cancer. 1994; 70(3): 481–6.

[16] SPILA A, FERRONI P, COSIMELLI M et al.. Evaluation of the CA 242 tumor antigen as a potential serum marker for colorectal cancer. Anticancer Res. 1999; 19(2B): 1363–8.

[17] CARPELAN-HOLMSTROM M, HAGLUND C, LUNDIN J et al. Independent prognostic value of preoperative serum markers CA 242, specific tissue polypeptide antigen and human chorionic gonadotrophin beta, but not of carcinoembryonic antigen or tissue polypeptide antigen in colorectal cancer. Br J Cancer. 1996; 74(6): 925–9.