Targ Oncol (2015) 10:549–555
DOI 10.1007/s11523-015-0365-x

ORIGINAL RESEARCH

The Association of Serum Carcinoembryonic Antigen, Carbohydrate Antigen 19-9, Thymidine Kinase, and Tissue Polypeptide Specific Antigen with Outcomes of Patients with Metastatic Colorectal Cancer Treated with Bevacizumab: a Retrospective Study

Ondrej Fiala[1,2] · Jindrich Finek[1] · Tomas Buchler[3] · Vit Martin Matejka[1] · Lubos Holubec[1,2] · Jana Kulhankova[1] · Zbynek Bortlicek[4] · Vaclav Liska[2,5] · Ondrej Topolcan[6]

Published online: 15 April 2015

Abstract The aim of our retrospective study was to analyze the association of selected tumor markers (TMs) including serum carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA 19-9), thymidine kinase, and tissue polypeptide specific antigen with outcomes in patients with metastatic colorectal cancer (mCRC) treated with bevacizumab. There is an increasing body of evidence from retrospective/observational studies that some serum TMs may be predictive of effect of targeted therapies in mCRC. In our study, the cohort included 152 patients treated with bevacizumab-based therapy between years 2005 and 2014 at Department of Oncology and Radiotherapy, Medical School and Teaching Hospital Pilsen. Serum samples for measurement of TMs were collected within 1 month before the initiation of bevacizumab-based treatment. In multivariate Cox analysis that included serum tumor markers and clinical baseline parameters, the number of metastatic sites (hazard ratio [HR]=2.00, p=0.001) and CEA levels (HR=2.80, p<0.001) were significantly associated with progression-free survival, whereas CA 19-9 levels (HR=2.25, p=0.008) were the only studied parameter associated with overall survival. Quantification of serum CEA and CA 19-9 is simple and readily available, and their candidate prognostic importance in the setting of antiangiogenesis therapy deserves to be studied in prospective trials.

✉ Ondrej Fiala
fiala.o@centrum.cz

[1] Department of Oncology and Radiotherapy, Medical School and Teaching Hospital in Pilsen, Charles University in Prague, alej Svobody 80, 304 60 Pilsen, Czech Republic

[2] Biomedical Center, Faculty of Medicine in Pilsen, Charles University in Prague, Pilsen, Czech Republic

[3] Department of Oncology and First Faculty of Medicine, Charles University and Thomayer Hospital, Prague, Czech Republic

[4] Institute of Biostatistics and Analyses, Faculty of Medicine, Masaryk University, Brno, Czech Republic

[5] Department of Surgery, Medical School and Teaching Hospital in Pilsen, Charles University in Prague, Pilsen, Czech Republic

[6] Department of Nuclear Medicine, Medical School and Teaching Hospital in Pilsen, Charles University in Prague, Pilsen, Czech Republic

1 Introduction

Colorectal cancer (CRC) is one of the most common cancer-related causes of morbidity and mortality in developed countries [1, 2]. Targeted therapies based on monoclonal antibodies against vascular endothelial growth factor (VEGF) or epidermal growth factor receptor (EGFR) are widely used in the treatment of metastatic CRC (mCRC). Bevacizumab is a humanized monoclonal antibody that blocks angiogenesis by inhibition of the most important angiogenic growth factor, VEGF. So far, no reliable biochemical or molecular predictors of response to bevacizumab have been validated.

The measurement of serum tumor markers is a simple and non-invasive method for assessing the response to systemic therapies in mCRC and, in some cases, the prognosis [3, 4]. Molecules used as serum tumor markers play a role in several cancer-related processes including cell adhesion, proliferation, and tumor angiogenesis and hypothetically could be useful as

a readily available surrogate marker for molecular characteristics of a tumor [5–11].

The aim of our retrospective study was to evaluate the association of baseline serum levels of carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA 19-9), thymidine kinase (TK), and tissue polypeptide specific antigen (TPS) with outcomes of patients with mCRC treated with bevacizumab.

2 Patients and Methods

Patients and Treatment We retrospectively analyzed clinical data of 152 adult patients with histologically confirmed mCRC treated with bevacizumab-based therapy between years 2005 and 2014 at Department of Oncology and Radiotherapy, Medical School and Teaching Hospital Pilsen, Czech Republic. Bevacizumab (Avastin, F. Hoffman-La Roche Ltd., Basel, Switzerland) was administered in combination with chemotherapy or as a single agent in a standard approved doses (5.0 mg/kg every 14 days or 7.5 mg every 21 days). The chemotherapy consisted of the following schedules: fluorouracil and leucovorin in combination with oxaliplatin (FOLFOX) or irinotecan (FOLFIRI), or alone (FUFA); capecitabine alone; oxaliplatin alone; and irinotecan alone. None of the patients had previously received antiangiogenic therapies.

Data Source Data were obtained from the clinical registry CORECT. The clinical registry CORECT (http://corect.registry.cz/) is a non-interventional post-registration population-based database of epidemiological and clinical data of patients with mCRC treated with targeted therapies in the Czech Republic. The registry contains anonymized individual patient data including demographic parameters, initial staging and disease characteristics, baseline patient information at the start of targeted therapy, as well as data on survival and adverse events. The entries are updated at least twice a year.

The protocol of our study was approved by the independent ethics committee of the University Hospital Pilsen and complied with the International Ethical Guidelines for Biomedical Research Involving Human Subjects, Good Clinical Practice guidelines, the Declaration of Helsinki, and local laws. Data from patients treated at the Medical School and Teaching Hospital Pilsen were extracted from the CORECT registry for the purposes of our study. Clinical data from the registry were validated against hospital medical records. Data on serum tumor markers were extracted from the hospital information system and merged to the registry data. The data have been analyzed retrospectively.

Clinical Monitoring of Data in the CORECT Registry and Statistics The treatment was prospectively monitored, and the clinical course of patients was continuously assessed at pre-specified time points. Physical examination and routine laboratory tests were performed every 2 weeks; computed tomography (CT) or positron emission tomography—(PET)-CT was performed every 3 months of the treatment. The objective tumor response was assessed by the attending physician using Response Evaluation Criteria in Solid Tumors (RECIST) [12].

Tumor Marker Measurement Serum samples were collected, and the measurement was performed within 1 month before the initiation of bevacizumab treatment. Serum levels of CEA and CA 19-9 were measured using chemiluminescent method on a DxI 800 analyzer (BeckmanCoulter, Brea, CA, USA). Serum levels of TK were measured using radioenzymatic assay (REA) on an Stratec 300 analyzer (Immunotech, Czech Republic). Serum levels of TPS were measured using immunoradiometric assay (IRMA) on a Stratec 300 analyzer (IDL Biotech, Sweden). The measurements were performed in the Central Immunoanalytic Laboratory at the Department of Nuclear Medicine, Pilsen University Hospital, using the following cutoff values: CEA 3 μg/l; CA 19-9 28 μg/l; TK 8 U/l, and TPS 90 μg/l. These are the upper normal values for the tumor markers measured by the used tests.

Statistical Analysis Standard frequency tables and descriptive statistics were used to characterize sample data set. Progression-free survival (PFS) and overall survival (OS) were estimated using the Kaplan-Meier method, and all point estimates were accompanied by 95 % confidence intervals. PFS was determined from the date of bevacizumab initiation until the date of first documented progression or death. OS was determined from the date of bevacizumab initiation until the date of death. Statistical significance of the differences in Kaplan-Meier estimates according to tumor marker levels was assessed using the log-rank test. Based on the results from univariate Cox proportional hazards models, multivariable Cox regression model was used to adjust results in terms of number of metastatic sites and line of therapy as the most clinical relevant potential confounders. As a level of statistical significance, alpha=0.05 was used.

3 Results

Patient Characteristics The study included 152 patients. The median age was 61.1 years (range 32.6–83.1 years). One hundred and four (68.4 %) patients were male, 86 (56.6 %) had a primary tumor localized in the colon, 101 (66.4 %) had metastatic disease at diagnosis, and 131 (86.2 %) received the bevacizumab-containing regimen in the first line. Bevacizumab was combined with FOLFOX in

118 (77.6 %) patients, FOLFIRI in 16 (10.5 %) patients, FUFA in 6 (3.9 %), capecitabine in 5 (3.3 %) patients, irinotecan in 3 (2.0 %) patients, oxaliplatin in 2 (1.3 %) patients, and 2 (1.3 %) patients received bevacizumab in monotherapy. The baseline patient characteristics are summarized in Table 1. The median follow-up of the cohort was 18.9 months, the median OS was 35.5 months (95 % CI 24.1–46.9), and the median PFS was 11.6 months (95 % CI 9.2–14.1).

Relation Between Baseline Levels of Serum Tumor Markers and Survival Baseline serum levels of CEA, CA 19-9, TK, and TPS are shown in Table 2. The median PFS and OS for patients with high CEA was 9.7 and 35.1 compared to 21.0 and 56.0 months for patients with low CEA ($p< 0.001$ and p=0.107) (Fig. 1a, b). The median PFS and OS for patients with high CA 19-9 was 9.7 and 29.0 compared to 13.5 and 56.0 months for patients with low CA 19-9 ($p= 0.034$ and p=0.003) (Fig. 1a, b). The median PFS and OS for patients with high TK was 9.9 and 33.8 compared to 12.9 and 45.5 months for patients with low TK (p=0.735 and p= 0.179). The median PFS and OS for patients with high TPS was 10.1 and 35.1 compared to 13.5 and 42.5 months for patients with low TPS (p=0.797 and p=0.563). The PFS and OS data are summarized in Table 2, and survival curves for CEA and CA 19-9 are shown in Fig. 1.

Baseline clinical parameters were assessed together with serum tumor marker levels in univariate and multivariate models. The univariate Cox proportional hazards model revealed that the number of metastatic sites (HR=1.75, p= 0.003), lines of therapy (HR=1.80, p=0.020), CEA (HR= 2.70, p<0.001), and CA 19-9 (HR=1.49, p=0.035) were significantly associated with PFS, whereas CA 19-9 (HR=2.33, p=0.004) was significantly associated with OS (Table 3). The multivariate Cox proportional hazards model revealed that the number of metastatic sites (HR=2.00, p=0.001), and CEA (HR=2.80, p<0.001) were significantly associated with PFS, whereas CA 19-9 (HR=2.25, p=0.008) was the only studied parameter associated with OS (Table 4).

Table 1 Baseline patient characteristics

	Total n=152
Males, *n* (%)	104 (68.4)
Age at treatment initiation (years)	
Median (min–max)	61.1 (32.6–83.1)
Localization, *n* (%)	
Colon	86 (56.6)
Rectum	66 (43.4)
Thromboembolism in anamnesis, *n* (%)	11 (7.2)
Hypertension in anamnesis, *n* (%)	64 (42.1)
Primary metastatic, *n* (%)	
M0	51 (33.6)
M1	101 (66.4)
Adenocarcinoma, *n* (%)	151 (99.3)
Prior surgery, *n* (%)	146 (96.1)
Prior radiotherapy, *n* (%)	24 (15.8)
Adjuvant chemotherapy, *n* (%)	40 (26.3)
Line of therapy, *n* (%)	
1st line	131 (86.2)
2nd and higher line	21 (13.8)
Site of metastatic disease, *n* (%)	
Liver	111 (73.0)
Lung	49 (32.2)
Lymph nodes	40 (26.3)
Peritoneum	26 (17.1)
Other	20 (13.2)
Number of metastatic sites, *n* (%)	
1	85 (55.9)
2	46 (30.3)
3 and more	21 (13.8)
KRAS gene status, *n* (%)	
KRAS mutated	49 (32.2)
Wild-type *KRAS*	48 (31.6)
Unknown	55 (36.2)
Chemotherapy regimens, *n* (%)	
FOLFOX	118 (77.6)
FOLFIRI	16 (10.5)
Modified FUFA	6 (3.9)
Capecitabine	5 (3.3)
Irinotecan	3 (2.0)
Oxaliplatin	2 (1.3)
No chemotherapy	2 (1.3)
Subsequent treatment with anti EGFR monoclonal antibodies, *n* (%)	
Yes	25 (16.4)
No	127 (83.6)

4 Discussion

Randomized phase III clinical trials as well as observational studies have provided evidence for the efficacy and safety of bevacizumab in the treatment of patients with mCRC [13–17]. Despite the rapid developments in the field of predictive oncology in recent years, there is still no available biomarker predicting treatment efficacy of bevacizumab-based therapy. Several candidate predictive biomarkers including angiopoietin-2, circulating levels of short VEGF-A isoforms, soluble VEGFR-1, and intramural expression of VEGFR-2 or neuropilins have been studied but not sufficiently validated for routine clinical use [18–20]. CEA, CA 19-9, TK, and TPS are serum tumor markers used as a part of the diagnostic work-up and monitoring of patients with CRC. In the present study, we focused on their association with the efficacy of bevacizumab-based therapy in patients with mCRC.

Table 2 Progression-free survival and overall survival according to baseline levels of serum tumor markers

	n (%)	Median PFS (months) (months, 95 % CI)	1 year PFS (%) (%; 95 % CI)	Log rank test *p* value	Median OS (months, 95 % CI)	2 years OS (%; 95 % CI)	Log rank test *p* value
CEA				*<0.001*			0.107
≤3 μg/l	32 (21.1)	21.0 (10.3–31.6)	71.0 (55.0–86.9)		56.0 (33.8–78.2)	76.3 (60.7–91.8)	
>3 μg/l	120 (78.9)	9.7 (7.5–11.8)	41.6 (32.4–50.9)		35.1 (27.9–42.4)	65.6 (55.2–76.1)	
CA 19-9				*0.034*			*0.003*
≤28 μg/l	66 (44.3)	13.5 (11.7–15.2)	58.2 (46.0–70.5)		56.0 (30.7–81.3)	77.1 (65.4–88.7)	
>28 μg/l	83 (55.7)	9.7 (7.9–11.5)	40.4 (29.3–51.4)		29.0 (19.9–38.0)	59.1 (46.2–72.1)	
TK				0.735			0.179
≤8 U/I	67 (46.2)	12.9 (10.1–15.7)	53.1 (40.6–65.6)		45.5 (32.3–58.7)	75.4 (63.1–87.7)	
>8 U/I	78 (53.8)	9.9 (6.4–13.4)	42.9 (31.6–54.1)		33.8 (22.7–44.8)	62.0 (49.0–75.0)	
TPS				0.797			0.563
≤90 μg/l	42 (29.4)	13.5 (10.4–16.5)	55.1 (39.7–70.5)		42.5 (20.7–64.2)	68.2 (52.5–84)	
>90 μg/l	101 (70.6)	10.1 (7.0–13.1)	43.2 (33.2–53.3)		35.1 (21.9–48.3)	66.0 (54.9–77.2)	

CEA carcinoembryonic antigen, *CA 19-9* carbohydrate antigen 19-9, *TK* thymidine kinase, *TPS* tissue polypeptide specific antigen, *PFS* progression free survival, *OS* overall survival

CEA plays a role in cell-to-cell adhesion and has a dominant effect in blocking cell differentiation [5], and it cooperates with Myc and Bcl-2 in cellular transformation [6]. Additionally, several proangiogenic effects of CEA have been described recently. Bramswig et al. reported that soluble CEA activates endothelial cells and tumor angiogenesis via paracrine manner. They observed that endothelial cell adhesion, spreading, migration, and proliferation were enhanced in the presence of CEA, and the CEA-induced endothelial cell activation was independent of the VEGF-VEGFR 1/2 system [7].

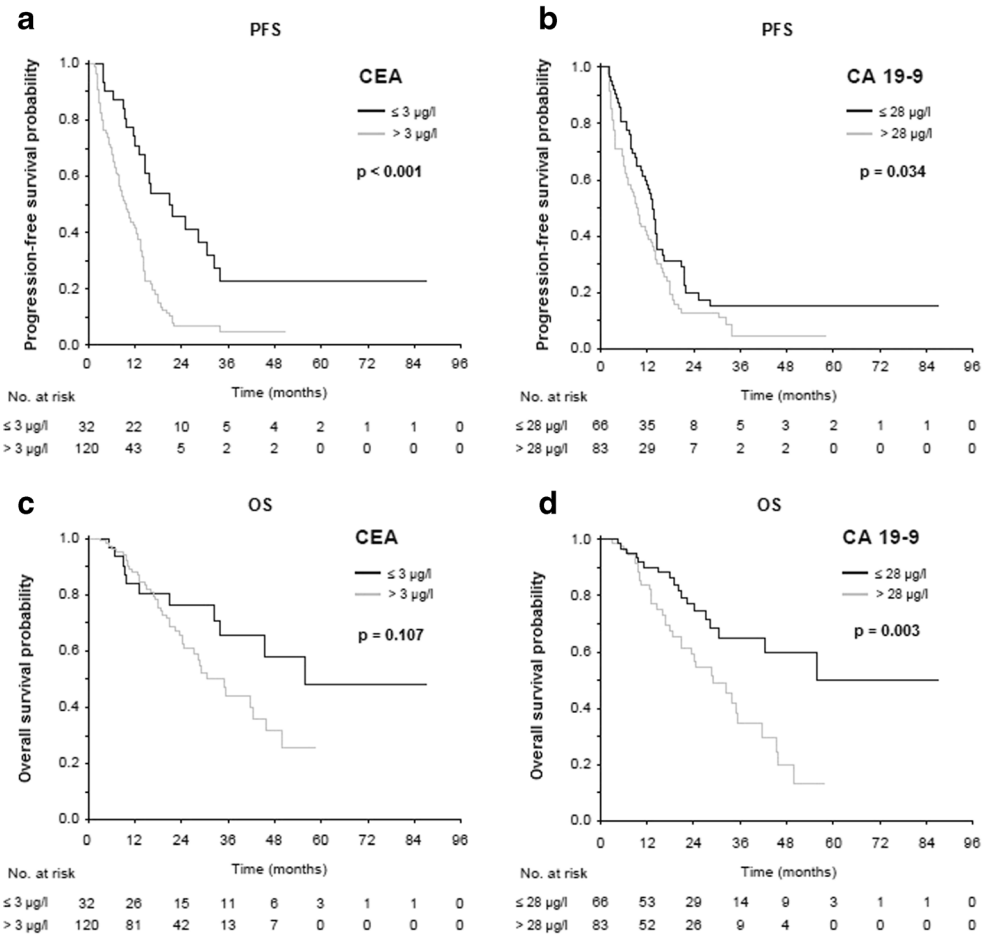

Fig. 1 Kaplan-Meier estimates of progression-free survival (PFS) and overall survival (OS) according to baseline levels of carcinoembryonic antigen (CEA) (**a**, **c**) and carbohydrate antigen 19-9 (CA 19-9) (**b**, **d**)

Table 3 Hazard ratios from univariate Cox proportional hazard model for progression-free survival and overall survival

Parameter	Category	*n*	Progression free survival		Overall survival	
			HR (95 % CI)	*p* value	HR (95 % CI)	*p* value
Sex	Males	104	0.99 (0.68–1.45)	0.955	1.07 (0.60–1.90)	0.832
	Females	48	1.00		1.00	
Age	≥65 years	48	0.70 (0.47–1.05)	0.088	0.63 (0.34–1.18)	0.146
	<65 years	103	1.00		1.00	
Number of metastatic sites	2 and more	67	1.75 (1.21–2.52)	*0.003*	1.20 (0.69–2.06)	0.520
	1	85	1.00		1.00	
Localization	Rectum	66	0.98 (0.68–1.41)	0.909	1.04 (0.61–1.77)	0.900
	Colon	86	1.00		1.00	
Primary metastatic	M1	101	1.05 (0.72–1.54)	0.805	1.17 (0.66–2.08)	0.585
	M0	51	1.00		1.00	
Lines of therapy	2nd and higher	21	1.80 (1.10–2.96)	*0.020*	2.03 (0.97–4.24)	0.061
	1st line	131	1.00		1.00	
CEA	>3 μg/l	120	2.70 (1.66–4.38)	*<0.001*	1.73 (0.88–3.41)	0.111
	≤3 μg/l	32	1.00		1.00	
CA 19-9	>28 μg/l	83	1.49 (1.03–2.16)	*0.035*	2.33 (1.32–4.12)	*0.004*
	≤28 μg/l	66	1.00		1.00	
TK	>8 U/I	78	1.07 (0.74–1.54)	0.736	1.46 (0.84–2.52)	0.181
	≤8 U/I	67	1.00		1.00	
TPS	>90 μg/l	101	1.06 (0.70–1.59)	0.797	0.85 (0.48–1.49)	0.563
	≤90 μg/l	42	1.00		1.00	

CEA carcinoembryonic antigen, *CA 19-9* carbohydrate antigen 19-9, *TK* thymidine kinase, *TPS* tissue polypeptide specific antigen, *HR* hazard ratio, *CI* confidence interval

This finding suggests that CEA bypass VEGF signaling and may result in resistance to VEGF-targeting drugs. However, the prognostic value of CEA in the setting of locoregional CRC or after metastasectomy has been previously clearly reported [21–25], it is controversial whether it is an independent prognostic parameter in metastatic CRC [26–28]. In our study, we observed a significantly shorter PFS (9.7 vs. 21.0 months; $p<0.001$) for patients with high pre-treatment CEA levels (>3 μg/l) compared to those with normal pre-treatment CEA levels (≤3 μg/l) although no association of CEA levels with OS was detected. The multivariate Cox proportional hazards model confirmed that high CEA was independently associated with shorter PFS (HR=2.80; $p<0.001$). Similar results have been recently published in a retrospective study by

Table 4 Hazard ratios from multivariable Cox proportional hazard model for progression-free survival and overall survival ($n=140$ [only patients in which all characteristics are known are included in the model])

Parameter	Category	*n*	Progression free survival		Overall survival	
			HR (95 % CI)	*p* value	HR (95 % CI)	*p* value
Number of metastatic sites	2 and more	60	2.00 (1.35–2.98)	*0.001*	1.70 (0.92–3.15)	0.092
	1	81	1.00		1.00	
Lines of therapy	2nd and higher	20	1.58 (0.91–2.72)	0.101	1.77 (0.79–3.96)	0.162
	1st line	121	1.00		1.00	
CEA	>3 μg/l	111	2.80 (1.65–4.76)	*<0.001*	1.52 (0.73–3.14)	0.259
	≤3 μg/l	30	1.00		1.00	
CA 19-9	>28 μg/l	78	1.37 (0.93–2.03)	0.111	2.25 (1.24–4.09)	*0.008*
	≤28 μg/l	63	1.00		1.00	
TK	>8 U/I	77	1.08 (0.72–1.62)	0.702	1.45 (0.79–2.66)	0.232
	≤8 U/I	64	1.00		1.00	
TPS	>90 μg/l	101	1.41 (0.91–2.18)	0.123	0.97 (0.52–1.80)	0.931
	≤90 μg/l	40	1.00		1.00	

CEA carcinoembryonic antigen, *CA 19-9* carbohydrate antigen 19-9, *TK* thymidine kinase, *TPS* tissue polypeptide specific antigen, *HR* hazard ratio, *CI* confidence interval

Prager et al., who reported significantly lower disease control rate (60.0 vs. 84 %; $p=0.0005$) and shorter PFS (6.4 vs. 8.5 month; $p=0.023$) with bevacizumab-based therapies in patients with high baseline CEA levels. Moreover, they also reported the lack of association between pre-treatment CEA levels and the treatment efficacy of cetuximab-based therapy [29]. In comparison with our study, they used different cutoff value (26.8 μg/l), which was obtained as a median value of pretreatment CEA levels. In order to facilitate the clinical interpretation of the results, we used the upper normal values for all the studied tumor markers as a cutoff.

CA 19-9 is an antigen expressed by the glycosylated extracellular MUC1 protein. CA 19-9 plays an important role in cancer invasion by enhancing cell adhesion and promoting angiogenesis indirectly [8]. In our study, we observed a significantly shorter PFS and OS for patients with high CA 19-9 (>28 μg/l), and the multivariate Cox proportional hazards model confirmed that high CA 19-9 was independently associated with shorter OS (HR=2.25; $p=0.008$) but not PFS (HR=1.37; $p=0.111$). The baseline serum level of CA19-9 has been previously reported as a significant prognostic indicator for mCRC (18), which is in agreement with our results. Interesting data on the predictive role of CA 19-9 have been provided by Formica et al. and Narita et al. [30, 31]. In observational studies, they found that only patients with high baseline levels of CA 19-9 benefited significantly from the administration of bevacizumab in comparison with chemotherapy alone [30, 31]. Narita et al. observed significantly longer OS for the group treated with bevacizumab and chemotherapy compared to those treated with chemotherapy alone among patients with high CA 19-9 levels (27.8 vs. 15.3 months; $p=0.0021$), however, the OS was not different with or without bevacizumab among patients with normal CA 19-9 levels (36.5 vs. 38.0 months; $p=0.9515$) [31]. Similarly to our study, they used normal values as a cutoff. All patients included in our study received treatment with bevacizumab; therefore, it is impossible to confirm or refute the results of these studies including also chemotherapy-only treated patients.

TK is an enzyme present in most cells, correlating with their proliferative characteristics. It has two isoforms, TK I and TK II that differ in chemical structure and biological function. TK I is expressed during cell division in the G_1 and S phase while it is absent in resting cells [9]. The association between TK I expression and angiogenesis in NSCLC has been recently reported by Brockenbrough et al. [32]. In our study, we did not demonstrate any significant association between PFS or OS and the baseline level of TK, although several authors have reported on the putative role of TK as a prognostic factor [33, 34].

The TPS assay detects the M3 epitope of cytokeratin 18 or of tissue polypeptide antigen. Cytokeratin 18 is an acid-type cytosolic protein expressed in simple epithelial cells and also by tumor cells [10, 11]. TPS has been shown to indicate tumor cell proliferative activity. TPS has been mostly studied as a biomarker for monitoring of treatment response to palliative chemotherapy in patients with gastrointestinal tumors including mCRC [35–37]. No impact of baseline level of TPS and survival has been detected in our study.

The principal limitations of the present study are its retrospective design and relatively small number of patients with resulting heterogeneity, especially regarding backbone chemotherapy regimens. Nevertheless, it is the largest study published so far to use a comprehensive tumor marker panel in patients with mCRC treated with bevacizumab.

In conclusion, the results of the conducted retrospective study suggest that the baseline level of CEA was independently associated with PFS in bevacizumab-based therapy. CA 19-9 was independently associated with OS in these patients. Both CEA and CA 19-9 are commonly used serum tumor markers which are simple and easy to detect, and thus, they are feasible for the use in the routine clinical practice. We have not demonstrated association of baseline levels of TK and TPS with patients'outcome. The differential association of CEA and CA19-9 with PFS and OS suggests that their levels reflect intrinsic molecular properties of the tumors rather than solely tumor volume. Prospective studies on the predictive role of serum tumor markers should be performed to confirm these results.

Acknowledgments The authors would like to thank all patients voluntarily taking part in the observational, population-based registry CORECT. This study is supported by the Ministry of Health, Czech Republic—conceptual development of research organization Faculty Hospital in Pilsen—FNPl, 00669806 and by the project CZ.1.05/2.1.00/03.0076 from the European Regional Development Fund.

Conflict of Interest JF has received honoraria from Astra Zeneca, Roche, and Novartis for consultations and lectures unrelated to this project. TB has received honoraria from Roche for consultations and lectures unrelated to this project. OF, VMM, LH, JK, ZB, VL, and OT declare that they have no actual or potential conflict of interest including any financial, personal, or other relationships with other people or organizations that could inappropriately influence this work.

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D (2011) Global cancer statistics. CA Cancer J Clin 61:69–90
2. Ferlay J, Parkin DM, Steliarova-Foucher E (2010) Estimates of cancer incidence and mortality in Europe in 2008. Eur J Cancer 46:765–81
3. Yamashita K, Watanabe M (2009) Clinical significance of tumor markers and an emerging perspective on colorectal cancer. Cancer Sci 100:195–9
4. Duffy MJ, Lamerz R, Haglund C, Nicolini A, Kalousová M, Holubec L et al (2014) Tumor markers in colorectal cancer, gastric cancer and gastrointestinal stromal cancers: European group on tumor markers 2014 guidelines update. Int J Cancer 134:2513–22

5. Benchimol S, Fuks A, Jothy S, Beauchemin N, Shirota K, Stanners CP (1989) Carcinoembryonic antigen, a human tumor marker, functions as an intercellular adhesion molecule. Cell 57:327–34
6. Screaton RA, Penn LZ, Stanners CP (1997) Carcinoembryonic antigen, a human tumor marker, cooperates with Myc and Bcl-2 in cellular transformation. J Cell Biol 137:939–52
7. Bramswig KH, Poettler M, Unseld M, Wrba F, Uhrin P, Zimmermann W et al (2013) Soluble carcinoembryonic antigen activates endothelial cells and tumor angiogenesis. Cancer Res 73:6584–96
8. Ballehaninna UK, Chamberlain RS (2012) The clinical utility of serum CA 19-9 in the diagnosis, prognosis and management of pancreatic adenocarcinoma: an evidence based appraisal. J Gastrointest Oncol 3:105–19
9. Zhou J, He E, Skog S (2013) The proliferation marker thymidine kinase 1 in clinical use. Mol Clin Oncol 1:18–28
10. Rydlander L, Ziegler E, Bergman T, Schöberl E, Steiner G, Bergman AC et al (1996) Molecular characterization of a tissue-polypeptide-specific-antigen epitope and its relationship to human cytokeratin 18. Eur J Biochem 241:309–14
11. Bodenmuller H (1995) The biochemistry of CYFRA 21-1 and other cytokeratin tests. Scand J Clin Lab Investig Suppl 221:60–6
12. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L et al (2000) New guidelines to evaluate the response to treatment in solid tumours. European organization for research and treatment of cancer, national cancer institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 3: 205–16
13. Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W et al (2004) Bevacizumab plus irinotecan, fluorouracil and leucovorin for metastatic colorectal cancer. N Engl J Med 250: 2335–42
14. Kabbinavar FF, Hambleton J, Mass RD, Hurwitz HI, Bergsland E, Sarkar S (2005) Combined analysis of efficacy: the addition of bevacizumab to fluorouracil/leucovorin improves survival in patients with metastatic colorectal cancer. J Clin Oncol 23:3706–12
15. Saltz LB, Clarke S, Díaz-Rubio E, Scheithauer W, Figer A, Wong R et al (2008) Bevacizumab in combination with oxaliplatin-based chemotherapy as first-line therapy in metastatic colorectal cancer: a randomized phase III study. J Clin Oncol 26:2013–9
16. Kozloff M, Yood MU, Berlin J, Flynn PJ, Kabbinavar FF, Purdie DM et al (2009) Clinical outcomes associated with bevacizumab-containing treatment of metastatic colorectal cancer: the BRiTE observational cohort study. Oncologist 14:862–70
17. Van Cutsem E, Rivera F, Berry S, Kretzschmar A, Michael M, DiBartolomeo M et al (2009) Safety and efficacy of first-line bevacizumab with FOLFOX, XELOX, FOLFIRI and fluoropyrimidines in metastatic colorectal cancer: the BEAT study. Ann Oncol 20:1842–7
18. Goede V, Coutelle O, Neuneier J, Reinacher-Schick A, Schnell R, Koslowsky TC et al (2010) Identification of serum angiopoietin-2 as a biomarker for clinical outcome of colorectal cancer patients treated with bevacizumab-containing therapy. Br J Cancer 103: 1407–14
19. Lambrechts D, Lenz HJ, de Haas S, Carmeliet P, Scherer SJ (2013) Markers of response for the antiangiogenic agent bevacizumab. J Clin Oncol 31:1219–30
20. Zhang W, Azuma M, Lurje G, Gordon MA, Yang D, Pohl A et al (2010) Molecular predictors of combination targeted therapies (cetuximab, bevacizumab) in irinotecan-refractory colorectal cancer (BOND-2 study). Anticancer Res 30:4209–17
21. Huh JW, Oh BR, Kim HR, Kim YJ (2010) Preoperative carcinoembryonic antigen level as an independent prognostic factor in potentially curative colon cancer. J Surg Oncol 101:396–400
22. Sun LC, Chu KS, Cheng SC, Lu CY, Kuo CH, Hsieh JS et al (2009) Preoperative serum carcinoembryonic antigen, albumin and age are supplementary to UICC staging systems in predicting survival for colorectal cancer patients undergoing surgical treatment. BMC Cancer 9:288
23. Park IJ, Choi GS, Lim KH, Kang BM, Jun SH (2009) Serum carcinoembryonic antigen monitoring after curative resection for colorectal cancer: clinical signifi- cance of the preoperative level. Ann Surg Oncol 16:3087–93
24. Peng Y, Wang L, Gu J (2013) Elevated preoperative carcinoembryonic antigen (CEA) and Ki67 is predictor of decreased survival in IIA stage colon cancer. World J Surg 37:208–13
25. Harrison LE, Guillem JG, Paty P, Cohen AM (1997) Preoperative carcinoembryonic antigen predicts outcomes in node-negative colon cancer patients: a multivariate analysis of 572 patients. J Am Coll Surg 185:55–9
26. Yuste AL, Aparicio J, Segura A, López-Tendero P, Gironés R, Pérez-Fidalgo JA et al (2003) Analysis of clinical prognostic factors for survival and time to progression in patients with metastatic colorectal cancer treated with 5-fluorouracil-based chemotherapy. Clin Colorectal Cancer 2:231–4
27. Wang WS, Lin JK, Chiou TJ, Liu JH, Fan FS, Yen CC et al (2002) CA19-9 as the most significant prognostic indicator of metastatic colorectal cancer. Hepatogastroenterology 49:160–4
28. Aggarwal C, Meropol NJ, Punt CJ, Iannotti N, Saidman BH, Sabbath KD et al (2013) Relationship among circulating tumor cells, CEA and overall survival in patients with metastatic colorectal cancer. Ann Oncol 4:420–8
29. Prager GW, Braemswig KH, Martel A, Unseld M, Heinze G, Brodowicz T et al (2014) Baseline carcinoembryonic antigen (CEA) serum levels predict bevacizumab-based treatment response in metastatic colorectal cancer. Cancer Sci 105:996–1001
30. Formica V, Massara MC, Portarena I, Fiaschetti V, Grenga I, Del Vecchio Blanco G et al (2009) Role of CA19.9 in predicting bevacizumab efficacy for metastatic colorectal cancer patients. Cancer Biomark 5:167–75
31. Narita Y, Taniguchi H, Komori A, Nitta S, Yamaguchi K, Kondo C et al (2014) CA19-9 level as a prognostic and predictive factor of bevacizumab efficacy in metastatic colorectal cancer patients undergoing oxaliplatin-based chemotherapy. Cancer Chemother Pharmacol 73:409–16
32. Brockenbrough JS, Morihara JK, Hawes SE, Stern JE, Rasey JS, Wiens LW et al (2009) Thymidine kinase 1 and thymidine phosphorylase expression in non-small-cell lung carcinoma in relation to angiogenesis and proliferation. J Histochem Cytochem 57:1087–97
33. Svobodova S, Topolcan O, Holubec L, Treska V, Sutnar A, Rupert K et al (2007) Prognostic importance of thymidine kinase in colorectal and breast cancer. Anticancer Res 27:1907–9
34. Treska V, Topolcan O, Stanislav K, Liska V, Holubec L (2009) Preoperative tumor markers as prognostic factors of colorectal liver metastases. Hepatogastroenterology 56:317–20
35. Glimelius B, Hoffman K, Einarsson R, Pählman L, Graf W (1996) Monitoring palliative chemotherapy in advanced gastrointestinal cancer using serial tissue polypeptide specific antigen (TPS) measurements. Acta Oncol 35:141–8
36. Kornek G, Schenk T, Raderer M, Djavarnmad M, Scheithauer W (1995) Tissue polypeptide-specific antigen (TPS) in monitoring palliative treatment response of patients with gastrointestinal tumours. Br J Cancer 71:182–5
37. Berglund A, Molin D, Larsson A, Einarsson R, Glimelius B (2002) Tumour markers as early predictors of response to chemotherapy in advanced colorectal carcinoma. Ann Oncol 13:1430–7