Cytokeratin Serum Biomarkers in Patients with Colorectal Cancer

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Abstract. *Background: Circulating cytokeratins have shown to be important for management of patients with lung cancer. Here we investigated their role for differential diagnosis, therapy monitoring and prognosis in colorectal cancer (CRC). Patients and Methods: Pretherapeutic levels of cytokeratin-19 fragments (CYFRA 21-1), carcino-embryonic antigen (CEA) and cancer antigen (CA) 19-9 were measured in 42 patients with CRC, 45 with benign colorectal diseases and 51 healthy controls. Furthermore, courses of CYFRA 21-1, tissue polypeptide antigen (TPA), tissue polypeptide specific antigen (TPS), M30-antigen, CEA and CA 19-9 were analyzed in prospectively collected sera of 15 patients with CRC during primary chemotherapy and were correlated with therapy response and overall survival (OS). Results: Similar to CEA and CA 19-9, CYFRA 21-1 was significantly elevated in serum from patients with CRC (median 2.1 ng/ml) as compared with healthy (1.2 ng/ml; p<0.0001) and benign gastrointestinal controls (1.7 ng/ml; p=0.0178) and showed stage dependency in CRC (p=0.0118). CYFRA 21-1 correlated with CEA in benign diseases and CRC but not with CA 19-9. The best discrimination between healthy controls and patients with CRC was achieved by combination of CYFRA 21-1 and CA 19-9 (area under the curve; AUC=86.7%), while the combination of CEA and CA 19-9 discriminated best between benign diseases and CRC (AUC=73.9%). In CRC patients during primary chemotherapy, levels of cytokeratins CYFRA 21-1, TPA, TPS, CEA and CA 19-9 tended to be higher in patients with poor response to therapy and with poor prognosis. Conclusion: Cytokeratins are elevated in patients with CRC and show some association with response to primary therapy and prognosis.*

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Colorectal cancer (CRC) is one of the four most common malignant diseases in the Western world; however, incidence and mortality rates have been declining during recent decades, mainly due to national screening programs leading to earlier diagnosis of CRC and better treatment possibilities – even for advanced-stage disease (1, 2). While in localized stages, surgery, and eventually adjuvant therapy, are the mainstays of the treatment, systemic chemotherapy is applied for more advanced stages, sometimes in combination with new biological treatments including antibody or tyrosine kinase inhibitor (TKI) therapies (3, 4). Along with the development of new effective drugs, there is a growing need for early indicators of therapy response and prognosis for better therapy stratification of the patients before and modification during therapy (5).

Carcinoembryonic antigen (CEA) has been used for years as a reliable and valid biomarker for differential diagnosis and monitoring purposes in patients with CRC (6-8). In addition to CEA, cancer antigen (CA)19-9 has revealed independent prognostic power for CRC (9, 10). For the early prediction of therapy response, mainly pathological criteria such as the KRAS status are used in clinical routine. These parameters have direct influence on the application of TK-inhibitors. Patients with KRAS mutations are not eligible for epidermal growth factor receptor (EGFR)-inhibiting cetuximab treatment (11).

Cytokeratin markers have been shown to be elevated not only in lung cancer but also in other malignant conditions and were suggested for use in differential diagnosis, therapy monitoring and prognosis in a variety of tumor diseases (12-15). The most frequently investigated markers are cytokeratin-19 fragments (CYFRA 21-1), tissue polypeptide antigen (TPA), tissue polypeptide specific antigen (TPS), and the apoptosis-specific M30 antigen (12-16). Here, we investigated their role in diagnosis, monitoring and prognosis of CRC.

Patients and Methods

Pretherapeutic levels of CYFRA 21-1, CEA and CA 19-9 were measured in 42 patients with CRC, 45 with benign gastrointestinal diseases and 51 healthy controls who were under the care of the Medical Faculty Pilsen, Czech Republic. Furthermore, courses of CYFRA 21-1, TPA, TPS, M30, CEA and CA 19-9 were analyzed in a pilot study in prospectively collected sera of 15 patients with CRC during primary chemotherapy and correlated with therapy response and overall survival (OS). The detailed patient characteristics are listed in Table I. Primary chemotherapy consisted of 5-fluorouracil, folinic acid and irinotecan according to the FOLFIRI regimen. The study was approved by the local Ethics Committee. Written informed consent was obtained from all patients.

Blood was drawn before the first, second and third cycle of chemotherapy. Blood was centrifuged and sera were stored at –80˚C until measurement. Concentrations of CYFRA 21-1 were measured on an ElecSys 2010 (Roche Diagnostics, Mannheim, Germany) at the Laboratory of the University Hospital Munich-Grosshadern, Germany. The concentrations of TPA, TPS and M30 were measured by ELISA (Peviva, Sweden) at the Laboratory of the University Hospital Pilzen, Czech Republic.

The clinical response to therapy was objectified by imaging techniques, mostly sonography or computed tomography, after three cycles of chemotherapy. The outcome was classified according to the World Health Organisation criteria: During palliative treatment, remission was defined as tumor reduction ≥50%, progression as tumor increase >25% or manifestation of new lesions, and stable disease as an intermediate status in between these modalities. Follow up of all patients was avaliable for at least three years. Overall survival of the patients after three years was taken as the endpoint for prognostic evaluations.

Differences in CYFRA 21-1, TPA, TPS, M30, CEA and CA 19-9 concentrations between patients groups, stage groups, response groups (progression (N=6) and no progression (N=9)) and survival (survivors N=6; non-survivors N=9) are shown graphically by whisker plots. Differences in patient groups were calculated by Mann-Whitney test, and between diverse stages by Kruskal-Wallis test. Numbers in response and survival groups were too small to be calculated statistically. Correlations between the markers were calculated by Spearman's rank correlation coefficent. Diagnostic discrimination of the markers were compared by areas under the curves (AUC) in receiver operating characteristic (ROC) curves. A *p*-value <0.05 was considered statistically significant. All tests done two-sided. Calculations were carried out by Graph Pad Prism 5 software (GraphPad Software, La Jolla, CA, USA).

Table I. *Clinical characteristics of the patients.*

	N	Gender (female/male)	Age (years)	
			Median	Range
Healthy controls	51	14/36	53.0	30-75
Benign gastrointestinal disease	45	25/20	60.0	22-84
Acute gastrointestinal disease	16	7/9	56.5	22-84
Chronic gastrointestinal disease	29	18/11	67.0	24-82
Colorectal cancer	42	15/27	67.0	30-89
Dukes A	5	2/3	60.0	54-89
Dukes B	8	4/4	75.0	68-84
Dukes C	12	4/8	59.5	30-87
Dukes D	17	5/12	67.0	48-81
	N	Percentage		
Patients during chemotherapy	15	8/7	64.0	53-71
Dukes C	5	33.3%		
Dukes D	10	66.7%		
Response at staging				
Remission	2	13.3%		
Stable disease	7	46.7%		
Progression	6	40.0%		
3-Year survival				
Survivors	6	40.0%		
Non-survivors	9	60.0%		

Results

Levels of CYFRA 21-1 were significantly elevated in patients with CRC (median 2.1 ng/ml) as compared with healthy (1.2 ng/ml; $p<0.0001$) and benign gastrointestinal controls (1.7 ng/ml; $p=0.0178$). Similarly, levels of CEA and CA 19-9 were higher in patients with CRC (medians 2.4 ng/ml and 18.7 U/ml) as compared with healthy (1.2 ng/ml and 9.9 U/ml; $p<0.0001$ and $p=0.0008$) and benign gastrointestinal controls (1.0 ng/ml and 10.0 U/ml; $p=0.0006$ and $p=0.0064$). Furthermore, there was a clear association with tumor stage for CYFRA 21-1 ($p=0.0118$), CEA ($p=0.0026$) and CA 19-9 ($p=0.0386$) (Figure 1). CYFRA 21-1 correlated with CEA in benign diseases (R=0.314; $p=0.0356$) and CRC (R=0.539; $p=0.0003$) but not with CA 19-9. CEA correlated with CA 19-9 only in patients with CRC (R=0.585; $p<0.0001$).

Best discrimination between healthy controls and patients with CRC was achieved by CYFRA 21-1 as a single marker (AUC=80.7%) and by the combination of CYFRA 21-1 and CA 19-9 (AUC=86.7%). Sensitivities at 95% specificity were 45.2% for CYFRA 21-1, 37.5% for CEA, 45% for CA 19-9 and 62.5% for the combination of CYFRA 21-1 and CA 19-9. Between patients with benign gastrointestinal diseases and with CRC, best discrimination was achieved by CEA as a single marker (AUC=71.8%) and the combination of CEA and CA 19-9 (AUC=73.9%). Sensitivities at 95% specificity were 26.2% for CYFRA 21-1, 32.5% for CEA, 30.0% for CA 19-9 and 37.5% for the combination of CEA and CA 19-9 (Figure 2).

In patients with CRC during primary chemotherapy, nine out of 15 showed no progression and were considered as responders while six had progressive disease in staging investigations after three cycles of chemotherapy. Six of the patients survived three years while nine died during that observation time. Baseline values of cytokeratins CYFRA 21-1, TPA, TPS and CEA before therapy cycles 1, 2 and 3 tended to be higher in patients with poor response to therapy, while levels of CA 19-9 and M30 were similar in both

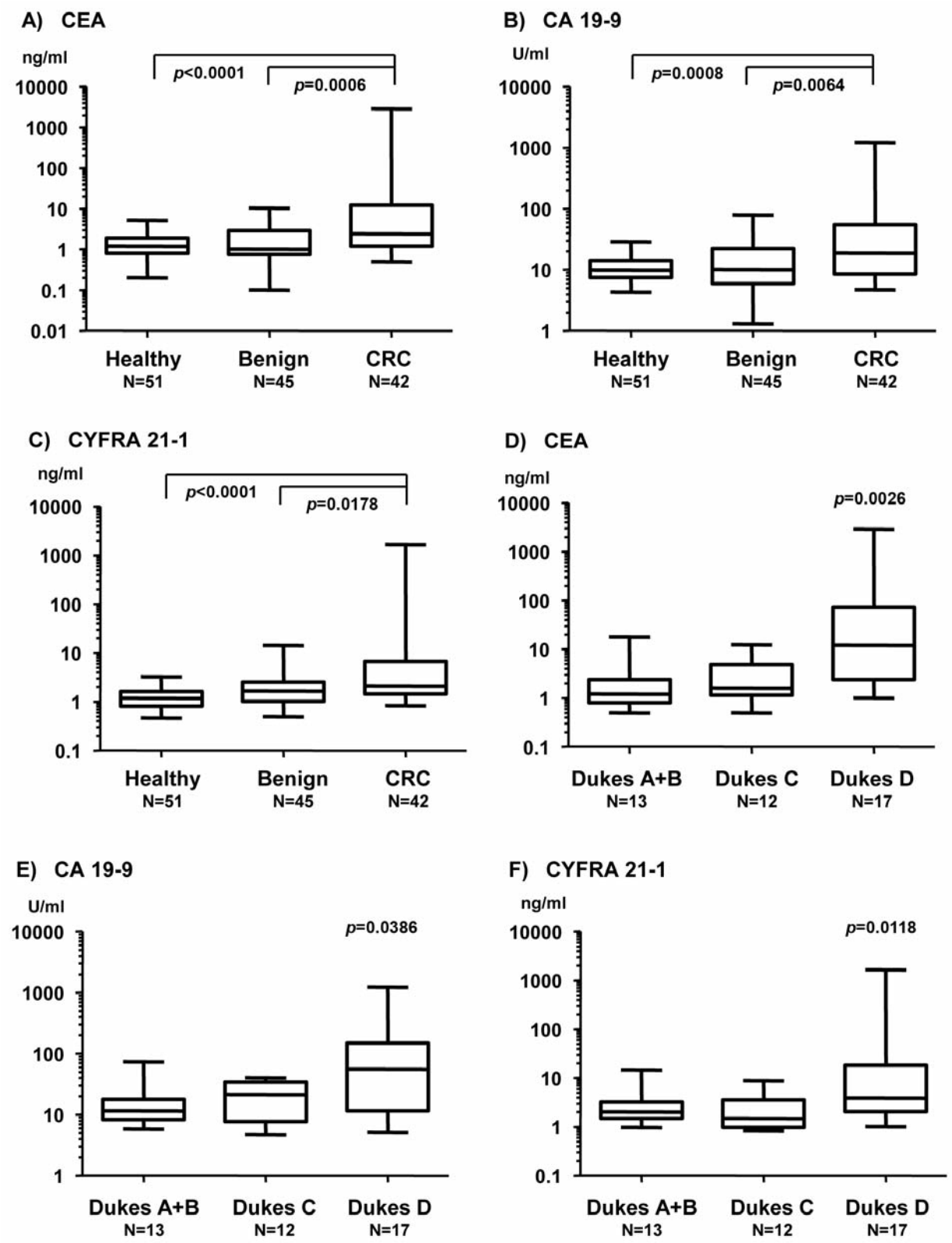

Figure 1. *Levels of carcinoembryonic antigen (CEA), cancer antigen (CA) 19-9 and cytokeratin-19 fragments (CYFRA 21-1) in patients with colorectal cancer (CRC), healthy controls and patients with benign gastrointestinal diseases. P-values were calculated by Mann-Whitney test (A-C). Dependency of CEA, CA 19-9 and CYFRA 21-1 on tumor stage. P-values were calculated by Kruskal-Wallis test (D-F).*

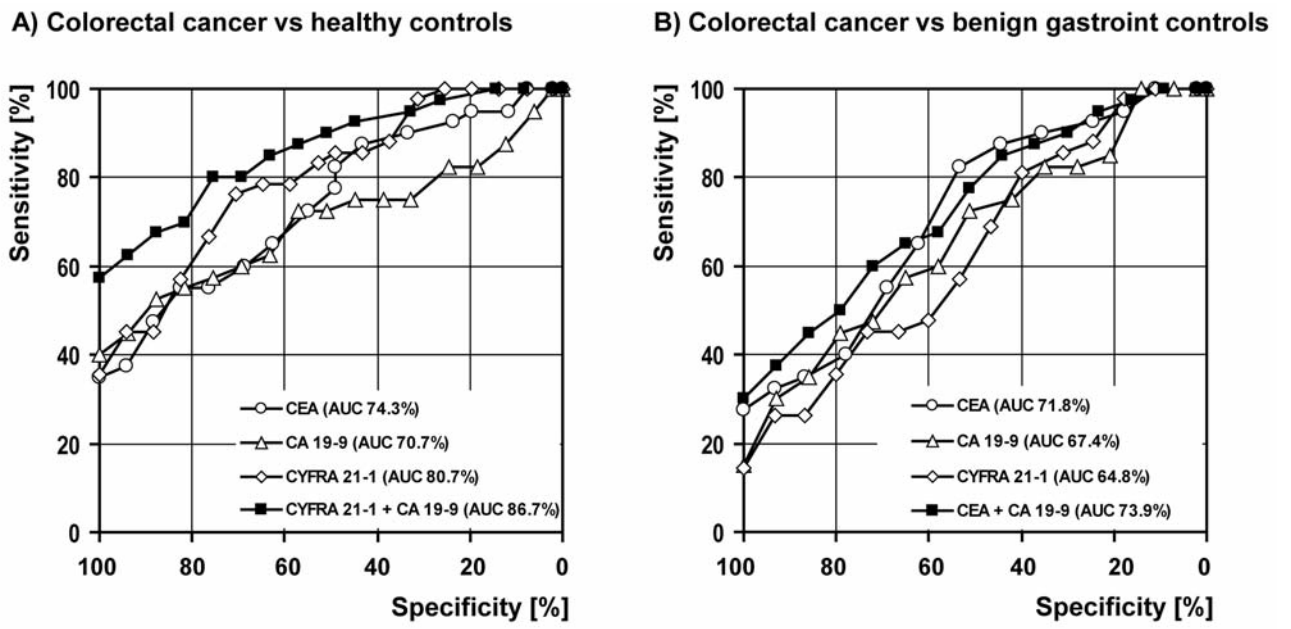

Figure 2. *Receiver operating characteristic (ROC) curves of carcinoembryonic antigen (CEA), cancer antigen (CA) 19-9 and cytokeratin-19 fragments (CYFRA 21-1) showing the discriminative power between patients with colorectal cancer (CRC) and healthy controls (A) as well as between patients with CRC and those with benign gastrointestinal diseases (B).*

response groups (Table II). Furthermore, baseline values of CYFRA 21-1, TPA, TPS, CEA and CA 19-9 tended to be higher in patients with poor survival, while differences in CEA and M30 were minimal in survivor groups (Table III).

Discussion

Cytokeratins are well recognized biomarkers in lung cancer and are recommended for use in differential diagnosis, prognosis and therapy monitoring, particularly of non-small cell lung cancer (NSCLC) (13, 17-19). In addition, CYFRA 21-1 has been found to be valuable for the early estimation of therapy response in NSCLC, as early as after one cycle of chemotherapy (20, 21). Beyond lung cancer, there are an increasing number of studies showing a remarkable sensitivity of CYFRA 21-1 but also of the other cytokeratin markers TPA and TPS in bladder, breast, ovarian and colorectal cancer (12-16, 22-24). This might be explained by a higher general cytokeratin release during cell death that might occur in tumor disease more frequently due to a high cellular turnover in some situations (14, 25). As cell death is further enhanced after application of cytotoxic therapies, there was a hope that more specific apoptosis-related markers, such as the M30 antigen, a cytokeratin-18 neoepitope that is uncovered after caspase cleavage at Asp396, would be helpful for therapy prediction and prognosis (14, 15, 26).

Although cytokeratin release depends strongly on tumor type and is characteristically lower in gastrointestinal tumors, we found a significant difference not only between patients with CRC and healthy controls, but also when patients with benign gastrointestinal diseases were considered as a control group. As known for other markers, such as CEA and CA 19-9, a dependency of CYFRA 21-1 on tumor stage was observed. This is quite remarkable as the median CYFRA 21-1 value of 2.1 ng/ml in patients with CRC was still in the reference range and the median of 3.9 ng/ml in Dukes D patients was only slightly elevated if the recommendations of the assay producer are taken as reference. Interestingly, CYFRA 21-1 correlated with CEA in benign disease but particularly in tumor disease that would hint to a potential association of CYFRA 21-1 with tumor mass in those patients. The high discriminative power of CYFRA 21-1 in ROC curves between patients with CRC and healthy controls is also remarkable, although the clinically more relevant discrimination between patients with CRC and benign controls was better by CEA and CA 19-9. Our results are in line with a recent comprehensive multimarker study showing similar absolute levels of CYFRA 21-1 in patients with CRC and controls and a similar power of discrimination (AUC=78%, sensitivity=35.5% at 95% specificity) (27). In that approach, the combination of CYFRA 21-1 with CEA, seprase, osteopontin, ferritin and anti-p53 yielded an overall sensitivity of about 70% at 95% specificity that was comparable with fecal immunochemical testing (27).

Concerning our pilot study on the relevance of cytokeratins for monitoring the response to therapy and prognosis, our results can only show tendencies due to the low number of patients enrolled. Nevertheless, the response was monitored

Table II. *Biomarkers in therapy prediction and monitoring of therapy response of patients with colorectal cancer (CRC) undergoing primary chemotherapy. Median values of carcinoembryonic antigen (CEA), cancer antigen (CA) 19-9 and cytokeratin-19 fragments (CYFRA 21-1) tissue polypeptide antigen (TPA), tissue polypeptide-specific antigen (TPS) and M30-antigen before therapy cycles 1, 2 and 3 (baseline values 1, 2 and 3) are given for patients with no progression (responders) and patients with progressive disease (non-responders) at staging after the third therapy cycle.*

Biomarkers	Baseline value 1		Baseline value 2		Baseline value 3	
	Responders	Non-responders	Responders	Non-responders	Responders	Non-responders
CEA (ng/ml)	1.9	50.0	2.1	40.0	1.9	41.7
CA 19-9 (U/ml)	12.6	18.6	11.1	21.1	31.6	17.7
CYFRA 21-1 (ng/ml)	1.3	2.1	1.3	2.4	1.5	1.7
TPA (ng/ml)	17	46	34	56	25	51
TPS (ng/ml)	32	35	35	76	28	61
M30 (U/ml)	101	131	162	140	161	165

Table III. *Biomarkers in estimating prognosis of patients with colorectal cancer (CRC) undergoing primary chemotherapy. Median values of carcinoembryonic antigen (CEA), cancer antigen (CA) 19-9 and cytokeratin-19 fragments (CYFRA 21-1) tissue polypeptide antigen (TPA), tissue polypeptide-specific antigen (TPS) and M30-antigen before therapy cycles 1, 2 and 3 (baseline values 1, 2 and 3) are given for patients who survived three years after chemotherapy and those who did not.*

Biomarkers	Baseline value 1		Baseline value 2		Baseline value 3	
	Survivors	Non-survivors	Survivors	Non-survivors	Survivors	Non-survivors
CEA (ng/ml)	1.8	3.0	2.0	3.2	1.9	3.4
CA 19-9 (U/ml)	11.6	23.9	11.1	26.1	20.3	22.7
CYFRA 21-1 (ng/ml)	1.3	2.1	1.3	2.4	1.4	1.7
TPA (ng/ml)	10	55	17	48	12	50
TPS (ng/ml)	17	49	18	70	22	60
M30 (U/ml)	120	122	153	143	210	161

homogeneously by computed tomography after three cycles of therapy and the survival was followed for three years in all patients. In responsive patients, median baseline values of cytokeratins before the various cycles were considerably lower than in non-responsive patients, once again at quite low absolute levels. The same applies to survivors when compared with non-surviving patients. As already found in earlier studies, CEA and CA 19-9 were also considerably different in response and prognostic groups (7-9). It will be interesting to include cytokeratin biomarkers together with established tumor markers CEA and CA 19-9 in future prospective clinical trials on patients with CRC to show their potentially additive value in response prediction and prognosis, as well as to identify the most meaningful cytokeratin biomarker.

Conclusion

Although the absolute values of cytokeratin biomarkers are only slightly elevated in CRC, they are able to differentiate between CRC and control groups and might bear predictive and prognostic potential in patients with CRC undergoing primary chemotherapy.

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