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Caspase-cleaved cytokeratin 18 fragment (M30) as marker of postoperative residual tumor load in colon cancer patients

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Abstract

Background: Soluble cytokeratin 18 (CK18; M65) and a caspase-cleaved fragment of CK18 (M30) have been used as biomarkers, corresponding to tumor cell death and apoptosis, respectively.
Methods: In the present study, M30 was quantified for the first time in serum samples of colon cancer patients pre- and postoperatively as well as during chemotherapy. Minimal residual disease (MRD) was assessed preoperatively by detection of pan-cytokeratin antibody A45-B/B3-positive cells in bone marrow aspirates.
Results: Out of 46 patients, those with colon tumors of stages I and IV had significantly elevated M30 serum concentrations compared to controls ($n = 23$). In 31 colon cancer patients, M30 determinations were performed prior to and seven days after tumor surgery. A group of 24 patients exhibited a significant decrease of M30 in response to tumor removal, in contrast to seven patients who revealed either persistent or higher M30 levels postoperatively. The frequency of MRD was not significantly different for patients with decreasing (4/24) and persisting (3/7) M30. However, M30 correlated significantly with the increased number of recurrences within 36 months in the group with persisting M30 (4/7 versus 2/24, $p = 0.032$; hazard ratio 8.3, $p = 0.016$). In a group of patients ($n = 10$) receiving capecitabine/oxaliplatin chemotherapy (CapOx), transient increases in M30 did not correlate with responses.
Conclusion: The data obtained within the present limited pilot study in colon cancer patients demonstrate that perioperative changes of M30 may indicate systemic residual tumor load and increased risk of recurrence warranting further evaluation of this marker of apoptosis in a larger prospective clinical trial.

Keywords: Colon cancer; Cytokeratin 18; Apoptosis; Caspase; Chemotherapy

Introduction

The majority of patients with colorectal cancer present with limited disease, however, despite radical surgery, about 30–50% of these patients develop metastatic disease.[1] The residual tumor load and the extent of pre- and perioperative tumor cell dissemination remains to be determined since appropriate detection systems are not available so far.[2] A host of diverse tumor markers has been investigated in order to detect residual disease and to aid in prognosis and selection of further therapy.[3]

Cytokeratins (CKs), proteins belonging to the intermediate filament (IF) family, are particularly useful tools for the surveillance of carcinomas.[4,5] The three most frequently applied CK markers used in the clinic so far, are tissue polypeptide antigen (TPA) measuring CKs 8, 18, and 19, and the more specific tissue polypeptide-specific antigen (TPS) and cytokeratin fragment 21-1 (CYFRA 21-1) recognizing CK18 and 19, respectively.[4] Further development of CK-based tumor markers have proceeded to the specific measurement of CK18 and a CK18 fragment, truncated at the N-terminus, from cancerous but not normal cells that is the product of caspase-mediated cleavage during apoptotic cell death.[6]

The levels of such CK18 fragments were significantly different for patients with either lung cancer, benign lung disease or healthy control subjects and predicted survival

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as well as response to therapy.[7] Similarly, patients with primary and recurrent breast cancer had higher M30 levels than healthy subjects, and M30 correlated with the number of involved organs and response.[5,8] In conclusion, assessment of CK18 and the caspase-cleaved CK18 fragment detected by M30 assay has been shown to discriminate patients with cancer from healthy controls, predict survival of the patients and prove efficacy of cytotoxic chemotherapy in different tumor entities.[5]

In the present study, the relevance of a CK18 fragment in colon cancer patients was evaluated using M30 ELISA assays. The different aspects investigated included comparison of preoperative serum concentrations of M30 with clinical parameters, perioperative changes of M30 serum concentrations as well as the influence of CapOx chemotherapy on this CK fragment in a subgroup of the colon cancer patients. Minimal residual disease (MRD) as negative prognostic factor was assessed by detection of cytokeratin-positive tumor cells in bone marrow aspirates.[9,10]

Patients and methods

Demographic data

A total of 56 patients with colorectal cancer were treated between January 2002 and December 2004 at the Donauspital, Vienna and had a follow-up period of more than 3 years. Forty-six patients underwent surgery for primary colorectal carcinoma and ten patients who received palliative chemotherapy for metastasis were included. None of the patients had received chemotherapy and/or radiotherapy prior to surgery. All patients were checked for infections by viral tests, blood count and chemistry, including determination of C-reactive protein. Twenty-three non-tumor patients admitted to the outpatient department for minor complaints served as controls. Bone marrow aspirates of cancer patients were obtained from both upper iliac crests (5 ml each) by needle aspiration. Collected blood was centrifuged at 2000 rpm for 10 min and stored at −20 °C. For patients with extended disease chemotherapy consisted of either capecitabine/oxaliplatin or irinotecan/irinotecan plus 5-fluorouracil (5-FU) in two cases. Capecitabine was given in a dose of 2 g/m^2 for d1–d14, oxaliplatin in a dose of 130 mg/m^2 on d1 and d22, irinotecan 125 mg/m^2 weekly, 5-FU, and leucovorin in doses of 1 g/m^2 on d1 and 500 mg/m^2 on d1, respectively. Written informed consent was obtained from all patients. The study was approved by the local ethics committee and the institutional review board.

Immunocytochemical analysis and scoring of MRD

Bone marrow aspirates were obtained from both upper iliac crests (5 ml each) by needle aspiration immediately prior to the operation under general anesthesia. Mononuclear cells of bone marrow aspirates were separated by Ficoll-Hypaque density gradient centrifugation. Cytospins containing 1×10^6 cells/slide were fixed in acetone and stained using pan-cytokeratin antibody A45-B/B3 (Micromet, Munich, Germany; final concentration 5 μg/ml; 20 min). Apoptotic tumor cells were detected in deparaffinized tissue sections following treatment of the slides with 100 μg/ml pepsin in 10 mM HCl for 30 min using the M30 Cytodeath antibody (Peviva, Bromma, Sweden; final concentration 0.5 μg/ml, 20 min). All other staining steps, including blocking and washes, were performed using the Idetect-Super-Stain-(alkaline phosphatase)-Fast-Red kit according to the manufacturer's instruction (ID Labs, London, ON, Canada) and mouse monoclonal isotype controls were included. For assessment of MRD at least 2×10^6 cells per specimen were screened blinded by two pathologists and a minimum of one tumor cell per 2×10^6 mononuclear cells was regarded as a positive result for A45-B/B3.

M30 and M65 ELISA

From all sera the concentrations of CK18-Asp396-NE and total CK18 were determined using the M30-Apoptosense® and the M65-ELISA assay® according to the manufacturer's instruction (Peviva, Bromma, Sweden), respectively. The coefficient of variance for the duplicate measurements of M30/M65 was <7.5%.

Statistical analysis

Statistical analysis of the control and tumor stage groups was done using ANOVA/Bonferroni correction and Dunnett's test (significance level $p < 0.05$), comparison of the distinct perioperative M30 groups by the Chi Square test, and risk of disease progression by Cox proportional hazards regression (SPSS software, SPSS, Chicago, IL, USA).

Results

Demographic data

For the forty-six patients with newly diagnosed colon cancer mean age was 69 ± 10 yrs (range: 43–87 yrs), with 15 female and 31 male patients. 16 patients were UICC stage I, 6 patients stage II, 12 patients stage III, 8 patients stage IV, and 4 patients had local relapses. 11/46 patients (24%) were positive for MRD (50% of the patients with stage IV cancer), exhibiting at least one tumor cell/2×10^6 mononuclear bone marrow cells. The patient subgroup (n = 31) for perioperative measurements of cytokeratins included the following tumor stages: 13 patients stage I, 3 patients stage II, 6 patients stage III, 5 patients stage IV, and 4 patients with local relapses. Out of this 31 colon cancer patients observed perioperatively, 7 (23%) were positive for MRD. None of the patients revealed signs of infection or inflammation.