Changes in Soluble CEA and TIMP-1 Levels during Adjuvant Chemotherapy for Stage III Colon Cancer

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Abstract. Background: Tissue inhibitor of metalloproteinases-1 (TIMP-1) has been suggested to be a valuable marker in colorectal cancer (CRC), but the effects of chemotherapy on TIMP-1 levels are unknown. The present study evaluated the effect of chemotherapy on TIMP-1 levels in comparison with carcinoembryonic antigen (CEA) levels in patients with stage III colon cancer. Patients and Methods: Thirty patients who had been curatively resected for stage III colon cancer were included. The patients received 10-12 cycles of modified FOLFOX6 regimen. Blood samples were collected before and after the first and the second cycle and three months later. Results: No significant change could be detected in CEA levels while TIMP-1 raised significantly after the second cycle but returned to normal 3 months later. Conclusion: Plasma CEA levels are stable during adjuvant chemotherapy while the plasma level of TIMP-1 might be directly affected by chemotherapy represented by a transient rise about 2 weeks following the initiation of treatment.

Colorectal cancer (CRC) is one of the most common malignancies of the aging population and the third leading cause of cancer-related death in the industrialized part of the world (1). Approximately 80% of the patients diagnosed with primary CRC undergo intended curative resection, while 20% may be offered only palliative treatment. Despite curative surgery patients still have a significant and stage dependent risk of developing recurrent disease within the next 5 years. Towards improving that, much interest has been focused on enhancing the effect of adjuvant treatment modalities to eliminate microscopic disease and thus reduce the risk of recurrence. 5-Fluorouracil (5-FU) has been the main chemotherapeutic agent for decades. The introduction of oxaliplatin has further increased the efficacy of adjuvant treatment (2-4). However, the majority of the patients receiving adjuvant chemotherapy have no benefit from the treatment, either because they do not have residual disease or because the residual tumor cells are resistant to the administered drugs.

The prediction of response has therefore been the main concern for oncologists and gastroenterologists. Potentially, predictive biomarkers might be useful for identifying patients, who would benefit from a certain treatment modality and in addition protect those patients, who would not benefit, from ineffective treatment and thereby from the adverse effects induced by chemotherapy. Such patients might benefit from other treatment modalities.

Carcinoembryonic antigen (CEA) is still the only recommended biological marker in CRC. It is recommended for monitoring purposes during and after systemic therapy, as persistently raised values above baseline might suggest recurrent or progressive disease (5, 6). However the interpretation of CEA measurements into clinical decision-making has its limitations. CEA levels may rise in patients without recurrence, and may even be below the cut-off point of 5 μg/l in patients with disseminated disease. Therefore, additional biological markers are needed to improve the identification of patients at risk of recurrent disease after intended curative resection and to monitor such patients during and after adjuvant therapy.

One of the new biological markers that may be of value either as a single marker or in combination with CEA is soluble tissue inhibitor of metalloproteinases-1 (TIMP-1). TIMP-1 is a member of the natural matrix metalloproteinase (MMPs) inhibitor family. MMPs are zinc-dependent enzymes that collectively may degrade all components of the extracellular matrix (7). Presently, four members of the TIMP family have been identified (TIMP-1-4). The balance

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between MMPs and TIMPs regulates the extracellular matrix (ECM) turnover and remodeling during normal development and disease. In addition to its inhibitory role TIMP-1 also possesses specific effects as TIMP-1 may stimulate cell growth, participate in angiogenesis regulation and inhibit apoptosis (7). Thereby, TIMP-1 may have dual roles as a protective against tumor dissemination and as a promoter of tumor growth (8).

Previous studies have shown that patients with CRC have significantly increased plasma TIMP-1 levels compared to healthy individuals (9, 10). More importantly, TIMP-1 has been shown to detect early-stage colon cancer with sensitivity and specificity comparable to those of late stages (9). It has recently been shown that plasma TIMP-1 levels are significantly and independently associated with objective response, time to progression, and overall survival in patients with metastatic CRC receiving combination chemotherapy (11). In addition, it has been shown that plasma TIMP-1 levels predicted the prognosis of CRC patients independent of clinical parameters including Dukes’ stage (9, 12). On this basis, plasma TIMP-1 has been suggested as a biomarker to be used for monitoring CRC patients, both as a single entity and in combination with CEA (10).

While it is well established that CEA levels may change in individual patients during chemotherapy, this phenomenon has not been investigated for TIMP-1. Therefore, the aim of the present study was to evaluate the effect of adjuvant chemotherapy on TIMP-1 levels in comparison with CEA levels in stage III colon cancer patients.

**Patients and Methods**

Thirty patients (14 males and 16 females, median age 63) with primary colon cancer, who had been intended curatively resected for stage III disease (TNM stage: T1-4, N1-3, M0) and scheduled for adjuvant chemotherapy, were prospectively and consecutively included at the time of outpatient visit before the initiation of chemotherapy. Exclusion criteria were current infection, previous chemotherapy, severe uncontrolled diabetes or any other serious medical conditions that hindered treatment with chemotherapy. The study was performed according to the Helsinki II declaration and was approved by The National Ethics Committee (KF-01-164/03) and The Danish Data Protection Agency (2003-41-3312). Written informed consent was obtained from all the included patients.

The patients received 10-12 cycles of chemotherapy of a modified FOLFOX 6 regimen (Table I). Blood samples were collected from an antecubital vein using a light tourniquet just before and just after the first cycle (day 0 and day 2, respectively), just before and just after the second cycle (day 16 and day 18, respectively) and three months later, just before the seventh cycle (day 110). The samples were collected at room temperature using endotoxin-free tubes (Becton-Dickinson, Mountain View, CA, USA). No additives were included in the tubes for serum, while EDTA was used as anticoagulation agent in the tubes for plasma. The plasma samples were inverted at least five times, and all the collected samples were stored at room temperature for a maximum of two hours. Subsequent centrifugation was performed at 3000xg for 10 min at 21°C. The serum and plasma supernatants were transferred to 2 ml cryo-tubes (TechNunc; Roskilde, Denmark) without disturbing the cell pellet, by leaving 5 mm plasma over the buffy-coat. All the blood aliquots were stored at –80°C until analyses.

**CEA analysis.** The serum CEA concentration in each sample was determined by a single measurement using an automated ADVIA Centaur analyzer (Siemens Healthcare Diagnostics Inc, Deerfield, USA). The CEA assay is a two-sited sandwich immunoassay using direct chemiluminimetric technology. The first antibody, in the Lite reagent, is a purified polyclonal rabbit anti-CEA antibody labeled with acridinium ester. The second antibody, in the solid phase, is a monoclonal mouse anti-CEA antibody covalently coupled to paramagnetic particles. The measuring range of the assay system was 0.5 μg/L to 150 μg/L; samples with concentrations below 0.5 μg/L were considered 0 μg/L, while samples with concentrations above the measuring range were diluted and reanalyzed. To ensure a stable analytical quality over time, two control samples having different concentrations of CEA were included in each assay run. The Westgard rule (1-2SD) was used to accept or reject runs. The assay variability was <7%.

**TIMP-1 analysis.** Soluble TIMP-1 levels were determined in EDTA plasma by use of an in-house TIMP-1 ELISA, which previously has been rigorously validated and demonstrates low intra- and interassay coefficients of variation (CV) (7). In brief, for measurements of TIMP-1 protein levels, microtiter plates were coated with a sheep polyclonal antibody and detection of bound TIMP-1 (free and complex-bound forms) was carried out by the use of a monoclonal antibody (MAC 15) and a secondary alkaline phosphatase–coupled anti-mouse antibody (DAKO). Readings of color development were taken every 10 min for 1 h and the calculations of concentrations were based on included recombinant TIMP-1 protein standards. The TIMP-1 cut-off level was 113.7 μg/L (13). Intra- and inter assay variations were below 10%.

**Statistics.** A general linear model with repeated measurements was used to analyze the TIMP-1 and CEA levels at day 0, 2, 16, 18 and 110. TIMP-1 and CEA values were log transformed and estimates were obtained using generalized estimating equations with patient as the clustering variable.
The variables were summarized by mean and confidence intervals (CI). P-values lower than 0.05 were considered significant.

All the calculations were performed using the statistical software SPSS (SPSS Inc. Chicago, USA) version 16.0.1 or SAS (SAS Institute Inc., NC, USA) version 9.1.

**Results**

**CEA.** Three patients had elevated CEA levels of more than 5 μg/L before the initiation of treatment. Three patients on day 2, 3 patients on day 16, 1 patient on day 18 and 2 patients on day 110 had elevated CEA levels above the cut-off point. The CEA level was relatively stable during the study and none of the patients had sustained CEA elevation throughout the study. The mean CEA level before (day 0) and after the first cycle of treatment (day 2) was 1.66 μg/L and 1.16 μg/L respectively, (p=0.06). At the second cycle (day 16 and 18) the mean level was 1.71 μg/L and 1.69  μg/L respectively, (p=0.94).

At the three month visit (day 110) the mean CEA level was 1.61 μg/L before the infusion of chemotherapy, Figure 1.

**TIMP-1.** Twenty-five patients had an elevated TIMP-1 level above the cut-off value (113.7.0 μg/L) at day 0 (before the initiation of chemotherapy) In addition, 26 patients at day 2, 25 patients at day 16, 24 patients at day 18, and 17 patients at day 110 had elevated levels above the cut-off value. Ten patients had sustained elevation of TIMP-1 levels throughout the study. The mean plasma TIMP-1 level before (day 0) and after the first treatment cycle (day 2) was 150.70 μg/L and 153.53 μg/L respectively, (p=0.74).

Before and after the second cycle (day 16 and 18) TIMP-1 mean level was 166.28 μg/L and 190.81 μg/L respectively.

The level at day 18 was 15% higher than at day 16 (p=0.34) and was also significantly (26%) higher than pretreatment level, (p=0.038). At the third month before the seventh cycle (day 110) the mean level of plasma TIMP-1 was 145.20 μg/L, 31% lower than the level at day 18, (p=0.013), Figure 2.

**Clinical evaluation.** Clinical evaluation, endoscopy and thoraco-abdominal computer tomography examination 6 months following surgery revealed no signs of recurrence in any of the patients.

**Discussion**

There were two major findings of the present study. Firstly, the serum CEA levels were relatively stable during chemotherapy treatment. Secondly, the plasma TIMP-1 levels appeared to be influenced by the chemotherapy, observed as a significant, but transient increase in mean plasma TIMP-1 level at day 18, just after chemotherapy. The mean level of the plasma TIMP-1 decreased significantly from day 18 and returned to baseline levels at day 110 just before the seventh cycle of chemotherapy.

It was previously shown that elevated CEA levels above 5 μg/L in patients who subsequently underwent intended curative resection for primary CRC (n=181) returned to normal (below 5 μg/L) in 73% (n=133) of the cases, while sustained elevation was observed in 27% (n= 48) of the patients four weeks following surgery (14). Patients with sustained high postoperative CEA levels had a worse prognosis compared to the patients, in whom postoperative CEA levels returned to normal (14). Based on the current literature demonstrating a CEA surge induced by chemotherapy in patients with
metastatic CRC (15, 16), it was presumed that the adjuvant therapy given in the present study would also induce transient increase in CEA levels. However, a slight decrease in serum CEA was observed after the first cycle of chemotherapy, but the decrease was clinically irrelevant as the mean levels were far below the standard cut-off point of 5 μg/L. The differences in CEA reaction to chemotherapy in stage III and stage IV patients are poorly understood. It is plausible that some chemotherapeutic agents might enhance CEA-mRNA expression in tumor cells resulting in a transient rise in CEA followed by subsequent decline due to enhanced apoptosis. This is supported by a previous study showing that treatment of the human colon cancer cell line COLO201 with 5-FU increased the expression of CEAs-mRNA and the addition of a platinum to 5-FU enhanced this expression (17). The lack of an overt tumor burden in stage III compared to stage IV patients might also explain why such changes could not be reproduced in the current study.

The TIMP-1 protein is stored in granules in platelets and leukocytes and is released upon activation or due to disintegration of the cellular elements (18). It is also expressed by myofibroblasts in association with invading colon cancer cells (19). Previous studies showed that high preoperative plasma TIMP-1 levels could be maintained up to 3 months following curative resection for primary disease (13, 20). Therefore, stage III patients receiving chemotherapy, which is initiated approximately four weeks after intended curative resection, may still have plasma TIMP-1 levels in the range of preoperative levels. As shown in the present study, the mean plasma TIMP-1 levels increased significantly during chemotherapy. No clear correlation between this increase in TIMP-1 and patient outcome was found during short-term follow up, as radiological, endoscopic and clinical evaluations revealed no signs of recurrence six month after the operation in any of the patients.

The mechanism behind the observed increase in plasma TIMP-1 is still unclear, but may be related to cellular disintegration (tumour cells and/or blood cells) with subsequent release of soluble TIMP-1. The disintegration of platelets induced by chemotherapy (21, 22) and in particular to oxaliplatin is well-described (23-25) and might contribute to raising the TIMP-1 level. Whether leucocytes were disintegrated as well is unknown, but potential disintegration of neutrophils would also add to TIMP-1 levels. Another plausible explanation could be the up-regulation of TIMP-1 synthesis and release as a reaction to apoptosis induced by the chemotherapy. In support of this hypothesis, it has been shown previously that TIMP-1 possesses anti-apoptotic effects (26-28), which might be enhanced by administer chemotherapy. Whether this transient increase in TIMP-1 predicts favourable prognosis reflecting more effective cell killing or predicts a poor outcome reflecting enhanced TIMP-1 mediated resistance to apoptosis is still unclear.

It is concluded that chemotherapy (FOLFOX6 regimen) results in increasing mean plasma TIMP-1 levels. Whether CEA and TIMP-1 could be used to detect recurrence or metastases during chemotherapy could not be shown in the present limited study, which only evaluated the direct effect of chemotherapy on the kinetics of these two biomarkers. However, that question may be answered in subsequent well-powered prospective studies.

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References


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