Chemoradiation-induced Changes in Serum CEA and Plasma TIMP-1 in Patients with Locally Advanced Rectal Cancer

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Abstract. *Background: Preoperative biomarkers serum CEA and plasma TIMP-1 have been shown to have prognostic and predictive value in patients with colorectal cancer. The aim of the present study was to evaluate the possible impact of chemoradiotherapy (CRT) on preoperative biomarker levels in patients with rectal cancer. Patients and Methods: Thirty-three patients with rectal cancer were prospectively included. The patients received CRT for 6-8 weeks. Blood samples were collected before CRT (pre-CRT) and preoperatively (post-CRT). Results: Median CEA was 3.5 (range 0.6-36.1) μg/l and 2.4 (range 0.0-10.2) μg/l (p=0.002) and median plasma TIMP-1 was 132.1 (range 77.8-342.7) μg/l and 140.0 (range 82.6-440.9) μg/l (p=0.04) in the pre- and post-CRT measurements, respectively. Conclusion: CRT induced a significant decrease in serum CEA and increase in plasma TIMP-1 levels. Therefore, the preoperative biomarker levels may be affected by treatments received before blood sample collection. Translation of results of preoperative biomarkers needs to take such facts into consideration.*

Colorectal cancer (CRC) is one of the most common malignancies of the aging population and the second leading cause of cancer-related death in the industrialized part of the world (1). Patients with rectal cancer (RC) comprise approximately 30%-35% of the CRC cases, and are known to have an increased risk of local recurrence, which may reduce disease-free survival (DFS) compared to patients with tumours of the colon (2). This fact appears to be due primarily to the surgical constraints imposed by tumour location in the rectum within the pelvis (3). Hence, management of RC is significantly different from that of the colon in terms of surgical techniques and adjuvant treatment modalities.

New advances, such as the standardized surgical technique total mesorectal excision, preoperative or postoperative radiotherapy, and neoadjuvant chemoradiation therapy (CRT), have reduced the previously high local recurrence rates and improved overall survival among patients with RC (4-7). Despite these advances, approximately 40% of patients who undergo resection with curative intent will still die from either local recurrence and/or distant metastasis (7).

Advances in the understanding of the biology of CRC, including RC, have opened many new research directions in the search for soluble biological markers that are useful in prediction of prognosis and response to a given treatment modality, and in selection of patients for such specific molecular targeted therapies. Hitherto, the only soluble biological marker that has been recommended by both the American Society of Clinical Oncology (8) and the European Group on Tumour Markers (9) is serum carcinoembryonic antigen (CEA). Its use is recommended primarily for monitoring recurrence, although a preoperative value may be useful for assessing prognosis (10, 11). CEA has not been recommended as a predictive marker in response to radiotherapy or CRT in patients with RC (8).

One of the new biological markers that may be of value is the 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 (12). Therefore, TIMP-1 should be advantageous for patients with malignant diseases, due to its inhibitory role on MMPs. Like other proteins identified with a specific action, TIMP-1 has other,

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MMP-independent, actions including stimulation of cell growth, regulation of angiogenesis and inhibition of apoptosis (12). Such actions may play detrimental roles for patients with malignancy, and potentially, changes in TIMP-1 expression may be of value in future identification of a clinical relevant biological marker.

Previous studies have shown that patients with CRC have significantly increased plasma TIMP-1 levels compared to healthy individuals (13-17). More importantly, TIMP-1 appears to identify patients with early-stage colon cancer with a sensitivity and specificity comparable with those obtained when detecting patients with late-stage cancer (16). It was recently shown that plasma TIMP-1 levels were significantly and independently associated with objective response, time to progression, and overall survival in patients with metastatic CRC receiving combination chemotherapy (18). It was also shown that plasma TIMP-1 levels strongly predict prognosis of patients with colorectal cancer independent of clinical parameters, including stage of disease (19). Combination of preoperative serum CEA and plasma TIMP-1 levels appears to improve the prognostic value compared to the use of either of the two biomarkers alone (20).

At present, the direct impact of a variety of treatment modalities on biomarker levels is not known. Therefore the aim of the present study was to evaluate the impact of neoadjuvant CRT on preoperative serum CEA and plasma TIMP-1 levels in patients with locally advanced RC.

Patients and Methods

From June 2006 to January 2008, a total of 33 patients (20 men and 13 women, median age 65 (28-91) years) with locally advanced primary RC were recruited into the study. The tumours were T3 or T4 with less than 5 mm to the mesorectal fascia (confirmed by magnetic resonance imaging) for tumours within the proximal two thirds of the rectum, and any T3-T4 within the distal one third of the rectum (21-23). Any nodal status (N0-2) was permitted, but patients with distant metastases detected on computerised tomography (CT) of the thorax and abdomen were not included. All patients had histologically confirmed primary adenocarcinoma of the rectum with the distal extent within 12 cm from the anal verge. Exclusion criteria were current infection, previous chemotherapy and/or radiotherapy, severe uncontrolled diabetes or any other serious medical condition that hindered treatment with CRT. In addition, patients who, in spite of neoadjuvant CRT, could not undergo subsequent resection were excluded. 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 patients.

The patients received CRT that consisted of 26 cycles of 2 Gy radiation 5 days a week for 6-8 weeks and weekly 1-2 hours infusion of folinic acid followed by 24 h infusion of 5-FU (2600 mg/m^2) for the same period. Before treatment initiation all patients underwent magnetic resonance imaging to evaluate the tumour location, size, extent and possible lymph node involvement. Blood samples were collected from an antecubital vein using a light tourniquet at the first visit before initiation of CRT treatment and again just before surgery, 6-8 weeks following the last CRT cycle. The samples were collected at room temperature using endotoxin-free tubes (Vacutainer® Becton-Dickinson, Mountain View, CA, USA). No additives were included in the tubes for serum, while EDTA was used as anticoagulation agent in tubes for plasma. The plasma samples were inverted at least five times, and all collected samples were kept at room temperature for maximum 1 h. Subsequently centrifugation was performed at 3000×*g* for 10 min at 21˚C. The serum and plasma supernatants were transferred to 2 ml cryo tubes (CM Lab A/S, Rødovre, Denmark) without disturbing the cell pellet, by leaving 5 mm serum or plasma over the buffy coat. The serum and plasma tubes were stored at –80˚C under constant surveillance until analyses.

CEA analysis. The serum CEA concentration in each sample was determined by a single measurement using the automated ADVIA Centaur analyzer (Siemens Healthcare Diagnostics Inc.). The CEA assay is a two-sited sandwich immunoassay using direct chemiluminometric technology. The first antibody, in the Lite reagent, is a purified polyclonal rabbit anti-CEA antibody labelled with acridinium ester. The second antibody, in the solid phase, is a monoclonal mouse anti-CEA antibody covalently coupled to paramagnetic particles. The cut-off limit was set to 5 μg/l. The dynamic range of the assay system was 0.5 μg/l to 150 μg/l. Concentrations above the upper limit were diluted. Concentrations below 0.5 μg/l were considered to be 0.0 μg/l. To ensure a stable analytical quality over time, two control samples were included in each assay run, each having different concentrations of CEA and used the Westgard rule (1-2SD) to accept or reject runs. The assay variability was below 7%.

TIMP-1 analyses. Plasma TIMP-1 levels were determined using an in-house TIMP-1 ELISA platform, which has been rigorously validated previously and demonstrates low intra- and inter-assay coefficients of variation (12). In brief, for measurements of TIMP-1 protein levels, microtitre plates were coated with a sheep polyclonal antibody and detection of bound TIMP-1 (free and complex-bound forms) was performed by use of a monoclonal antibody (MAC 15) and a secondary alkaline phosphatase-coupled antibody (DAKO A/S, Glostrup, Denmark). Readings of colour development were taken every 10 min for 1 h, and calculations of concentrations were based on included recombinant TIMP-1 protein standards. The cut-off level was identified in a previous study and set at 113.7 μg/l (24). Intra- and inter-assay variations were less than 10%.

Statistics. Serum CEA and plasma TIMP-1 levels are presented as the median and range. Pre-CRT and post-CRT measurements were compared using the paired Student's *t*-test with markers on the log scale. *P*-values less than 0.05 were considered significant. All calculations were performed using the statistical software SPSS version 17.0.1. (Chicago, IL, USA).

Results

Serum CEA. Ten patients (seven males and three females) had elevated pre-CRT serum CEA levels of more than 5 μg/l, compared to six patients (four males and two females) with elevated post-CRT serum CEA levels. Three out of these six patients had elevated pre-CRT levels, while the other three

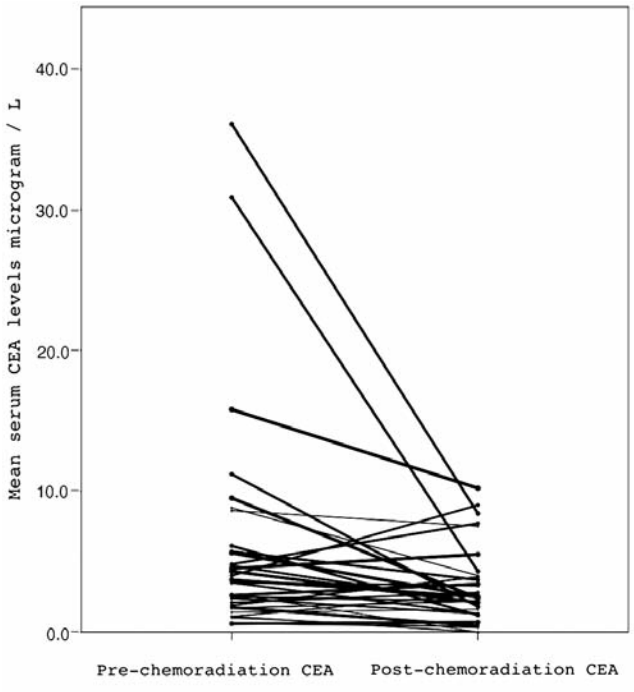

Figure 1. *Pre- and post-chemoradiation serum CEA levels.*

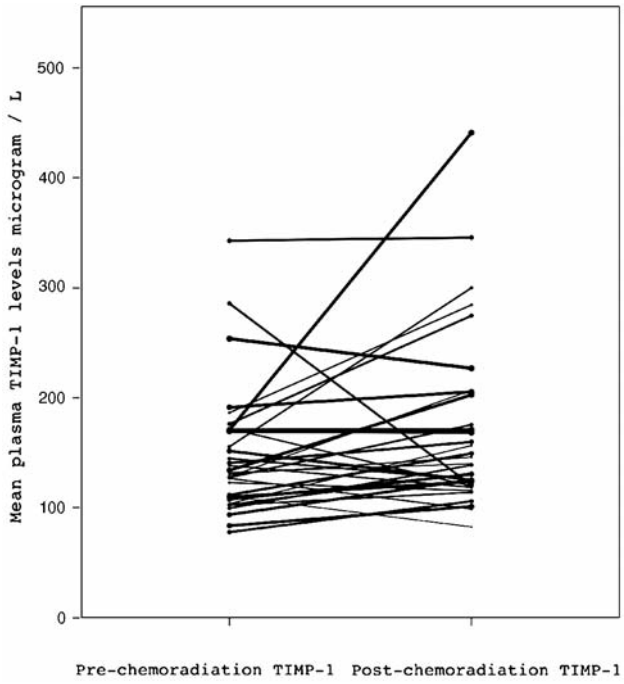

Figure 2. *Pre- and post-chemoradiation plasma TIMP-1 levels.*

patients had normal pre-CRT serum CEA levels. Median serum CEA levels were 3.5 μg/l (range 0.6-36.1) and 2.4 μg/l (range 0.0-10.2) in the pre- and post-CRT measurements, respectively (p=0.002, Figure 1). Thereby the median change in serum CEA from pre- to post-CRT was a 35% decrease (range –54% to –17%).

Plasma TIMP-1. Twenty-two patients (12 males and 10 females) had pre-CRT plasma TIMP-1 levels of more than 113.7 μg/l, while 29 patients (18 males and 11 females) had post-CRT levels above 113.7 μg/l after CRT. Plasma TIMP-1 levels declined in 10 patients, while 23 patients had higher post-CRT plasma TIMP-1 levels.

The median plasma TIMP-1 level was 132.1 (range 77.8-342.7) μg/l and 140.0 (range 82.6-440.9) μg/l in the pre- and post-CRT measurements, respectively (p=0.04, Figure 2). Thus, the median change in plasma TIMP-1 levels was 13% increase (range 1%-27%).

Discussion

The findings of the present study were a significant decrease in median serum CEA levels and a significant increase in median plasma TIMP-1 levels from pre- to post-CRT. Both preoperative serum CEA and plasma TIMP-1 levels have prognostic value for patients with CRC (16, 19, 25-28). Moreover, results from a recent study also indicate that the combination of serum CEA and plasma TIMP-1 may play a role in stage-independent selection of patients for adjuvant treatment modalities (24). Therefore, the results from the present minor pilot study may lead to considerations for future studies on the value of biomarkers in blood samples, which are collected just prior to surgery. It should be kept in mind that a major proportion of patients with locally advanced RC are offered preoperative radiotherapy or CRT, while studies on preoperative chemotherapy to patients with colon cancer are considered. Such approaches ought to lead to development of strict standard operative procedures for collection of blood samples from patients included in biomarker evaluation studies. Otherwise future implementation of biomarkers for prognostic, prediction and selection purposes will be difficult.

It is at present unknown why the median levels of serum CEA decrease as a response to CRT. The treatment may lead to apoptosis of malignant cells (29). In particular, radiation therapy appears to account for that, since it was recently shown that chemotherapy did not change the levels of serum CEA in patients receiving adjuvant treatment (30). Among the malignant cells, a variety may have synthesised and released CEA (31), and a reduction in the number of such cells may play a significant role in reducing the CEA levels.

Similarly, mechanisms leading to increased plasma TIMP-1 levels are not understood in detail. CRT may not only lead to reduction of the malignant cells within the tumour, but indeed radiotherapy also leads to inflammation and fibrosis of the treated tissues (32-37). It has been shown in a variety of studies that TIMP-1 is stored in granules of inflammatory cells and platelets (38), and such inflammatory cells may contribute to the increased plasma TIMP-1 levels after CRT, as also observed among patients with inflammatory diseases (37, 39). In addition, patients with fibrosis-associated diseases have increased plasma TIMP-1 levels compared with the levels among healthy individuals (32, 33).

In conclusion, this study show that CRT leads to changes in the serum and plasma levels of the two well-known biomarkers, CEA and TIMP-1. Therefore, such treatment may potentially also lead to changes in concentrations of other soluble biomarkers. However, this needs to be demonstrated in more detail. Future studies on development, evaluation and validation of biomarkers must be cautious in translation of the results when the variety of present treatment options is not accounted for. Whether the levels of pre-CRT serum CEA and plasma TIMP-1 may play a role in subsequent response of the treatment needs to be evaluated in sufficiently powered studies. In addition, the clinical value of the changes in the levels of the two biomarkers can only be evaluated in major studies including a sufficient number of patients.

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