Plasma TIMP-1 levels and treatment outcome in patients treated with XELOX for metastatic colorectal cancer

C. Frederiksen*, C. Qvortrup2,3†, I. J. Christensen4, B. Glömelius5,6, Å. Berglund5,6, B. V. Jensen7, S. E. Nielsen8, N. Keldsen9, H. J. Nielsen1, N. Brünner10 & P. Pfeiffer2,3

1Department of Surgical Gastroenterology, Hvidovre University Hospital, Hvidovre; 2Department of Oncology, Odense University Hospital, Odense; 3Institute of Clinical Research, University of Southern Denmark, Odense; 4The Finsen Laboratory, Rigshospitalet, Copenhagen Biocenter, Copenhagen, Denmark; 5Department of Oncology, Radiology and Clinical Immunology, Uppsala University, Uppsala; 6Department of Oncology and Pathology, Karolinska Institutet, Stockholm, Sweden; 7Department of Oncology, Herlev University Hospital, Herlev; 8Department of Oncology, Hillerød Hospital, Hillerød; 9Department of Oncology, Herning Hospital, Herning; 10Institute of Veterinary Disease Biology, Faculty of Life Sciences, University of Copenhagen, Frederiksberg, Denmark

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Background: The aim was to evaluate the association between plasma tissue inhibitor of metalloproteinase-1 (TIMP-1) and serum carcinoembryonic antigen (CEA) levels and outcome in patients with metastatic colorectal cancer (mCRC) receiving XELOX (combination chemotherapy with capecitabine and oxaliplatin) as first-line treatment.

Patients and methods: One hundred and twenty patients were included. Blood samples were collected before treatment and 3 weeks later before the next treatment cycle. Plasma TIMP-1 and serum CEA levels were correlated to treatment outcome.

Results: No significant associations between baseline TIMP-1 or CEA levels and best response to treatment or progression-free survival (PFS) could be demonstrated. In contrast, high baseline plasma TIMP-1 levels were associated with poor overall survival (OS), P = 0.008, hazard ratio (HR) = 1.80 [95% confidence interval (CI): 1.17–2.78]. Furthermore, increase in TIMP-1 levels from baseline to immediately before the second cycle of chemotherapy had a significant negative effect on survival (P = 0.03, HR = 1.30, 95% CI: 1.02–1.65) while a decrease in TIMP-1 was significantly associated with a higher objective response rate (P = 0.03).

Conclusions: Both high baseline and subsequent increase in TIMP-1 levels were associated with shorter OS in patients with mCRC receiving XELOX as first-line treatment, whereas baseline TIMP-1 levels were not associated with response or PFS following XELOX treatment.

Key words: biomarkers, metastatic colorectal cancer, overall survival, progression-free survival, response, XELOX

introduction

Each year, 1 million new cases of colorectal cancer (CRC) are diagnosed worldwide [1] and ~50% of these patients will develop metastatic disease (metastatic colorectal cancer (mCRC)) and subsequently die of the disease. For several years, first- and second-line chemotherapy with 5-fluorouracil (5-FU) and folinic acid (FA) in combination with either irinotecan (FOLFIRI) [2] or oxaliplatin (FOLFOX) [3] has been standard therapy. More recently, these combinations are used together with either epidermal growth factor receptor inhibitors or anti-angiogenic drugs [4] in patients with mCRC, improving median overall survival (OS) for patients to 20–22 months compared with 12 months with 5-FU/FA alone [5].

Capcitabine is an oral prodrug of 5-FU administered in combination with oxaliplatin (XELOX); it has an efficiency equivalent to FOLFOX [6].

Unfortunately, not all patients will benefit from treatment and some may obtain benefit from one drug combination but not from another combination. Identification of new molecular markers that can be used to select one treatment combination over another is therefore urgently warranted.

Several biological markers have been proposed as potential biomarkers in CRC patients. So far, only carcinoembryonic antigen (CEA) has been clinically implemented as a monitoring marker [7]. However, present data are insufficient to recommend routine use of CEA alone for predicting response to chemotherapy [7].

There has been major progress in understanding tumour formation and the metastatic processes [8] in which the activity of proteases and their inhibitors has become evident [9]. Tissue inhibitor of metalloproteinase-1 (TIMP-1) may play a specific role in promoting cancer progression. Several studies have...
demonstrated an association between high plasma TIMP-1 levels and poor prognosis in patients with CRC [10–12]. Recently, an association between plasma levels of TIMP-1 and efficacy of 5-FU combined with irinotecan (FOLFIRI/FLIRI) was demonstrated in patients with mCRC [13]. The study showed that baseline plasma TIMP-1 levels were significantly associated with response rate (RR), time to progression and OS with low levels being associated with better patient outcome. The biological explanation for these relationships has been suggested to be an inhibition by TIMP-1 of chemotherapy-induced apoptosis [14–16]. However, at present, no data are available on the associations between plasma TIMP-1 levels and response to chemotherapy regimens that include oxaliplatin in patients with mCRC.

The aim of the present study was to evaluate the association between baseline levels of plasma TIMP-1 and serum CEA and the association between changes in levels of these two biomarkers and clinical outcome in mCRC patients receiving XELOX as first-line therapy.

**materials and methods**

This study was a part of a Nordic randomised study comparing two different administration schedules of XELOX (XELOX30 or chronomodulated XELOX90)—the XELOX III study. A detailed report of eligibility criteria, baseline characteristics, treatment schedules and outcome was reported in the clinical part of the study [17]. From April 2004 to September 2005, 141 patients with measurable mCRC were randomly assigned to one of two administration schedules. As part of the study, patients had blood samples collected and stored according to a predefined standard operative procedure (SOP). Seven of nine participating hospitals collaborated in the blood sampling part of the XELOX III study as two of the centres participating in the clinical part of the XELOX III did not have the facilities to obtain and store the blood samples according to the SOP. Therefore, 120 patients were included in the present study. No significant differences in RR, progression-free survival (PFS) or OS were found between the two administration schedules [17]. Thus, we regarded the patient population as one group.

The study was approved by the Ethics Committees in each country and carried out in accordance with the Helsinki Declaration and according to Good Clinical Practise Guidelines. The REMARK recommendations were followed whenever applicable [18].

**blood sampling**

Samples available for the present study were obtained at baseline (before start of chemotherapy) and 3 weeks after the first treatment, i.e. immediately before the second XELOX treatment.

Blood samples were drawn into silicone-coated endotoxin-free tubes with EDTA as an anticoagulant for plasma and without additives for serum (Vacutainers®; Becton-Dickinson, Mountain View, CA). Within 2 h, the supernatants were separated from the blood cells by centrifugation at 3500 g for 10 min at room temperature and stored at −80°C until all samples had been collected from the patients included.

**TIMP-1 and CEA analysis**

Total TIMP-1 levels (free and in complex with matrix metalloproteinase) were determined using the MAC15 antibody in-house validated kinetic enzyme-linked immunosorbent assay (ELISA) as previously described [19]. Duplicate measurements were carried out and the mean values were used for statistical analysis. The mean intra-assay coefficient of variation (CV) was 5.1% (range 1.5%–9.8%) and the inter-assay CV was 6.7%.

Serum CEA analyses were measured using the principle of solid-phase ELISA from IBL Immuno Biological Laboratories [20]. Duplicate measurements were carried out and mean CEA values were used for statistical analysis. The mean inter-assay CV was 5.1%.

Samples from each patient were analysed simultaneously on one ELISA plate to minimise inter-assay variations.

**response and survival**

Response was calculated according to the RECIST version 1.0 [21] as assessed by the investigators. PFS was defined as the time from inclusion to progressive disease (PD) or death of any cause. OS was defined as the time from inclusion to death of any cause. OS was updated on 1 April 2009. We grouped patients in two groups—good responders and poor responders—according to the best response obtained to treatment. Good responders included complete response + partial response and poor responders included stable disease and PD. Non-assessable patients were excluded.

**statistical analyses**

Descriptive statistics for TIMP-1 and CEA were presented by their medians and ranges. Tests for location were carried out using the Wilcoxon rank sum test and the Spearman rank correlation for measures of association. Estimation of probability of response was carried out using logistic regression analysis with TIMP-1 and CEA scored by the actual value on the log scale (natural). The results were presented by odds ratios (OR) and the area under the receiver operating characteristic curve (AUC) as a measure of discrimination. PFS and OS were analysed using the Cox proportional hazards model. TIMP-1 and CEA were scored as in the logistic regression analysis. Results were presented with the hazard ratios (HRs) and 95% confidence interval (CI). Model assessment was done using conventional techniques. The change in TIMP-1 levels compared with the baseline level was evaluated using a standard error estimated from 23 normal individuals [22]. That study showed that a variation over time in TIMP-1 levels in healthy individuals with two measurements from the same individual would be significantly different if a 31% decrease or a 45% increase was seen. Graphical representations of survival probabilities were presented by Kaplan–Meier curves dichotomising continuous variables by the median for TIMP-1 and by the internationally accepted cut-off value of 5 ng/ml for CEA. P values ≤5% were considered statistically significant. By reviewing the literature, a change in CEA concentration of 50% was estimated as significant. Statistical calculations were made using SAS (v9.1; SAS Institute, Cary, NC).

**results**

TIMP-1 results were available in 120 patients and CEA results in only 115 of these patients due to lack of a serum sample in five patients. Patient characteristics are shown in Table 1. The median baseline plasma TIMP-1 level was 208 ng/ml (range 53–735 ng/ml) and the median serum CEA level was 10.5 ng/ml (range 0.4–1260 ng/ml). Seventy-four (64%) patients had a CEA level above the cut-off value of 5 ng/ml. The rank correlation between TIMP-1 and CEA was $r = 0.36$.

No associations between levels of TIMP-1 or CEA to location of primary tumour, age, gender or number of organs involved could be demonstrated (data not shown). However, weak but statistically significant associations were found between TIMP-1 and platelet counts ($r = 0.28$, $P = 0.002$) and white blood
55 (55%) had been classified as good responders and 46 (45%) as poor responders. Twenty-two patients (23%) had a decrease of CEA dichotomised by 5 ng/ml showed that neither TIMP-1 (P = 0.26, HR = 1.25, 95% CI: 0.85–1.82) nor CEA (P = 0.74, HR = 1.07, 95% CI: 0.71–1.61) was significantly associated with PFS.

However, high baseline levels of TIMP-1 (continuous variable) were significantly associated with a shorter OS (P = 0.008, HR = 1.80, 95% CI: 1.17–2.78). The Kaplan–Meier estimate (Figure 1A) with plasma TIMP-1 dichotomised by its median (208 ng/ml) showed that patients with levels below the median had a significantly longer OS than patients with plasma TIMP-1 levels above the median (P = 0.009, HR = 1.65, 95% CI: 1.13–2.41).

Likewise, a significant difference between baseline levels of CEA (continuous variable) and OS was demonstrated (P = 0.03, HR = 1.09, 95% CI: 1.01–1.19). When CEA was dichotomised by its cut-off value (5 ng/ml), the Kaplan–Meier estimate (Figure 1B) showed that a CEA level >5 ng/ml was significantly associated with a shorter OS (P = 0.04, HR = 1.53, 95% CI: 1.02–2.30).

Multivariable analysis of OS only including the baseline clinical characteristics showed that only number of organs involved was retained in the final model (data not shown). When we included blood measurements in the analysis of OS, plasma TIMP-1 retained significance, whereas platelet counts, WBC and alkaline phosphatase were not significant in this model (data not shown). The same analyses for CEA demonstrated that alkaline phosphatase was significant, whereas neither CEA nor WBC or platelet counts showed significance.

changes from baseline levels of TIMP-1 and CEA
Subsequently, the association between the relative difference of plasma TIMP-1 and serum CEA values, respectively (difference between baseline value and value just before the second cycle of treatment), and best objective response to treatment, PFS and OS was analysed. A significant association was found between changes in plasma TIMP-1 levels and objective response (P = 0.03, AUC = 0.62); a decrease of >31% in plasma TIMP-1 levels exhibited an increase in the odds for a good response, with an OR of 0.62 (95% CI: 0.40–0.94). Five patients (5%) had an increase in TIMP-1 level of >45%, all five (100%) were non-responders. Twenty-two patients (23%) had a decrease of >31%, of these 16 (73%) were responders and 6 (27%) were non-responders. Changes in plasma TIMP-1 had a non-significant effect of PFS (P = 0.09, HR = 1.14, 95% CI: 0.98–1.33).

Furthermore, changes in plasma TIMP-1 levels were significantly associated to OS. A decrease of >31% was significantly associated with longer OS (HR = 0.84, 95% CI: 0.71–0.99, P = 0.03). In contrast, an increase in plasma TIMP-1 levels of >45% compared with baseline levels had a significant negative effect on OS (HR = 1.30, 95% CI: 1.02–1.65, P = 0.03).

In addition, when analysing the actual value of plasma TIMP-1 levels as a continuous variable after the first cycle of chemotherapy, a significant association with OS (P = 0.0001, HR = 2.89, 95% CI: 1.68–4.96) was observed.

Changes in CEA levels were not significantly associated with best objective response (P = 0.39, AUC = 0.58). The OR for an

Table 1. Baseline characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) median (minimum/maximum)</td>
<td>65 (36–80)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>69</td>
</tr>
<tr>
<td>Female</td>
<td>51</td>
</tr>
<tr>
<td>Tumour marker median (minimum/maximum)</td>
<td></td>
</tr>
<tr>
<td>TIMP-1 (ng/ml)</td>
<td>208 (53–735)</td>
</tr>
<tr>
<td>CEA (ng/ml)</td>
<td>32 (0–8920)</td>
</tr>
<tr>
<td>Performance status WHO</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>62 (52%)</td>
</tr>
<tr>
<td>1</td>
<td>53 (45%)</td>
</tr>
<tr>
<td>2</td>
<td>4 (3%)</td>
</tr>
<tr>
<td>Site of primary tumour</td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td>72 (60%)</td>
</tr>
<tr>
<td>Rectum</td>
<td>48 (40%)</td>
</tr>
<tr>
<td>Prior adjuvant therapy</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>99 (83%)</td>
</tr>
<tr>
<td>Yes</td>
<td>21 (17%)</td>
</tr>
<tr>
<td>Number of organs involved</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>38 (32%)</td>
</tr>
<tr>
<td>2</td>
<td>48 (40%)</td>
</tr>
<tr>
<td>3</td>
<td>33 (28%)</td>
</tr>
</tbody>
</table>

WHO, World Health Organization.
increase of 50% was 0.92 (95% CI: 0.75–1.12). In contrast, changes in serum CEA levels as compared with baseline were significantly associated with short OS ($P$ = 0.049, HR = 1.04, 95% CI: 1.00–1.07) and a shorter PFS ($P$ = 0.005, HR = 1.04, 95% CI: 1.01–1.07); both HRs were calculated for a 50% increase.

Multivariate analysis including CEA showed that CEA did not contribute significantly to the analysis of best objective response ($P$ = 0.43). The same analysis for OS showed that CEA was not significant ($P$ = 0.08). In contrast, the analysis of PFS showed serum CEA being significant ($P$ = 0.007) while TIMP-1 was not ($P$ = 0.06). Therefore, the combination of changes in TIMP-1 and CEA did not significantly improve prediction.

**discussion**

The present study evaluated whether the concentration of plasma TIMP-1 and serum CEA measured at baseline and again early in treatment had any association with clinical outcome in patients with mCRC treated in a multicentre randomised study with XELOX as first-line therapy.

It was shown that neither baseline plasma TIMP-1 nor baseline serum CEA levels were correlated to best response or PFS. However, high baseline levels of plasma TIMP-1 were statistically significantly associated with shorter OS.

Correlations between changes in plasma TIMP-1 (from pretreatment level to just before the second cycle of treatment) and best objective response, PFS and OS have not been investigated previously in patients with mCRC. The present study showed that plasma TIMP-1 levels at the second cycle of chemotherapy were significantly associated with shorter OS and best objective response but not PFS. Of specific interest was that an increase from baseline levels to the second cycle of chemotherapy of >45% in plasma TIMP-1 levels had a significant negative effect on OS. On the other hand, patients who exhibited a reduction in plasma TIMP-1 from the baseline level had a better OS, and these patients were more likely to be classified as good responders indicating that plasma TIMP-1 may be useful as a monitoring marker during treatment. However, this has to be tested and validated in future studies.

The statistically significant association with OS, but not PFS, indicates that TIMP-1 is a prognostic but not a predictive marker in patients receiving XELOX. The fact that plasma...
TIMP-1 has value in the prognostic evaluation of CRC patients in agreement with previous findings demonstrating that high levels indicate poor survival of patients with curatively resected CRC or with mCRC [10, 11, 23, 24]. In contrast to the present findings, a previous study described a significant association between baseline plasma TIMP-1 levels and best objective response, PFS and OS in mCRC patients treated with 5-FU and irinotecan [13], suggesting that low baseline plasma TIMP-1 levels could be used to identify patients who would benefit the most from this treatment combination.

The mechanism of high plasma TIMP-1 levels and poor survival as well as poor response to some forms of chemotherapy is not known in detail. However, the majority of conventional chemotherapeutic drugs act by killing cancer cells through induction of apoptosis. Preclinical studies have demonstrated an anti-apoptotic effect of TIMP-1 [15, 25]. Recently, Davidsen et al. [16] showed that TIMP-1-deficient cancer cells, as compared with TIMP-1 wild-type cancer cells, had increased sensitivity to apoptosis induced by a variety of chemotherapeutic drugs. From these studies it seemed that the ability of TIMP-1 to protect tumour cells from apoptosis was independent of the class of chemotherapy used, but the findings of the present study may suggest otherwise. The difference between the chemotherapy regimens in the present study (oxaliplatin based) versus the study by Sorensen et al. [13] (irinotecan based) raises the possibility that the anti-apoptotic effect of TIMP-1 is associated with specific types of chemotherapy. This hypothesis is supported by very recent findings that lack of tumour cell TIMP-1 immunoreactivity predicts sensitivity to anthracycline-containing therapy but not to cyclophosphamide, methotrexate and 5FU in adjuvant treatment of high-risk breast cancer patients [26]. It is of interest to note that while irinotecan is a topoisomerase I inhibitor, anthracyclines are topoisomerase II inhibitors and our current hypothesis is that the TIMP-1 protein specifically interacts with topoisomerase inhibitors, while the exact mechanism for this interaction is still unknown. In further support of this hypothesis is that human breast cancer cells overexpressing TIMP-1 gain resistance to topoisomerase I and II inhibitors [27].

Multicentre randomised phase III studies have not detected any differences in terms of RRs, PFS or OS in mCRC patients treated with FOLFOX compared with FOLFIRI [28]. Based on our present and recent results [13], it can thus be hypothesised that determination of plasma or tumour tissue TIMP-1 content could be used to select patients for one or the other treatment regimens, i.e. patients with high TIMP-1 levels could be offered oxaliplatin-based regimens as first-line treatment, while mCRC patients with low plasma TIMP-1 levels could be offered irinotecan-based treatment as first-line treatment. However, this hypothesis needs to be tested properly before being used in daily routine. Our research group are planning to start a prospective study randomising patients with mCRC and no prior adjuvant chemotherapy to receive either standard chemotherapy plus biological therapy or biomarker-guided chemotherapy plus biological therapy. In addition to TIMP-1, we will include DNA excision repair protein, thymidylate synthetase and topoisomerase 1.

In order to visualise the findings behind this hypothesis, we constructed a common Kaplan–Meier plot including non-dichotomised PFS curves from patients in our prior study [13] and patients in the present study (Figure 2A) and Kaplan–Meier plots in which TIMP-1 was dichotomised by its respective median values (Figure 2B and C). As shown in Figure 2A, the estimate for PFS of the two patient cohorts first deviates after 8 months of treatment where it reaches a significant difference in favour of irinotecan-containing arms. However, when dichotomising the patients according to the median TIMP-1 level, patients with plasma TIMP-1 below the median value had a better PFS when treated with FOLFIRI/FLIRI compared with those treated with XELOX. In contrast, there was no significant difference in PFS in patients with plasma TIMP-1 levels above the median treated with either FOLFIRI/FLIRI or XELOX. It should be stressed though that comparing clinical studies as we have done might introduce significant bias and the data presented should therefore be treated with caution.

According to the American Society of Clinical Oncology [7] and the European Group of Tumour Markers [29] guidelines, CEA is the marker of choice for monitoring patients with mCRC. However, this use of CEA has serious limitations. The sensitivity and specificity of CEA as a monitor marker of response are limited [30] and not solely dependent on the tumour but also on the site of metastasis as well as on other non-cancer-related parameters [7]. In the present study, we found no association between baseline serum CEA level and response to treatment or PFS. However, changes in CEA from baseline to just before the second series of chemotherapy showed that a 50% increase in serum CEA correlated significantly with shorter PFS (P = 0.005, HR = 1.04, 95% CI: 1.01–1.07). Furthermore, a study evaluating tumour markers as early predictors of response to treatment (first-line single-agent 5 FU) showed that CEA had a very low sensitivity (13%) [30].

In conclusion, the present study could not show any correlation between baseline plasma TIMP-1 levels and the likelihood of tumour response or PFS but only with OS in patients with mCRC receiving XELOX as first-line treatment. However, the present study, together with our previous study [13], raises the hypothesis that plasma TIMP-1 can be used to guide treatment of the individual mCRC patient, i.e. whether the patient should receive irinotecan- or oxaliplatin-based chemotherapy as first-line treatment. If a predictive test could be used to guide selection between these two drug combinations for first-line treatment of an individual mCRC patient, it is anticipated that the RR would increase—thus likely leading to longer PFS and improved quality of life for the individual patient. The risk of such an approach is considered minimal since at present there are no guidelines, besides the different toxicity profiles determining which of the two chemotherapeutic regimens that should be used as first-line treatment of mCRC.

funding
Figure 2. (A) Kaplan–Meier curves for the progression-free survival (PFS) of patients by treatment trial. Patients were either receiving FLIRI/FOLFIRI (combination chemotherapy with 5-fluorouracil, folinic acid and irinotecan) [22] or XELOX (combination chemotherapy with capecitabine and oxaliplatin) as first-line treatment of advanced colorectal cancer (CRC). Data were available for 90 patients in the FLIRI/FOLFIRI trial and 111 patients in the XELOX trial. The number of patients at risk is shown below the graph. (B) Kaplan–Meier curves for the PFS of patients by treatment trial. Patients were either receiving FLIRI/FOLFIRI [22] or XELOX as first-line treatment of advanced CRC. Data were available for 45 patients in the FLIRI/FOLFIRI trial and 56 patients in the XELOX trial. The number of patients at risk is shown below the graph. (C) Kaplan–Meier curves for the PFS of patients with high plasma tissue inhibitor of metalloproteinase-1 levels (above the median) by treatment trial. Patients were either receiving FLIRI/FOLFIRI [22] or XELOX as first-line treatment of advanced CRC. Data were available for 45 patients in the FLIRI/FOLFIRI trial and 55 patients in the XELOX trial. The number of patients at risk is shown below the graph.
acknowledgements

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disclosure

The institutions of NB, IJC and HJN possess patents and patent applications on TIMP-1. The clinical part of the study received support from Roche and Sanofi-Aventis.

references