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Tissue inhibitor of metalloproteinases-1 in the postoperative monitoring of colorectal cancer

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ABSTRACT

The aim of this study was to investigate whether the pre- and postoperative plasma levels of tissue inhibitor of metalloproteinases-1 (TIMP-1) were associated with outcome in colorectal cancer (CRC). Pre- and postoperative plasma TIMP-1 from 280 curatively resected CRC patients and carcinoembryonic antigen (CEA) in corresponding serum samples were measured and correlated with patient outcome (death, local recurrence (LR) and distant metastases (DM)). The results showed that the course of plasma TIMP-1 from pre- to postoperative levels correlated with patient outcome ($P = 0.005$). However, postoperative plasma TIMP-1 alone was strongly associated with patient outcome, high TIMP-1 predicting short survival ($P = 0.002$). Combining postoperative TIMP-1 and CEA demonstrated that high TIMP-1 and CEA levels predicted poor outcome ($P < 0.0001$); multivariate analysis identifying both parameters as strong prognostic factors for survival, LR and DM ($P < 0.0001$). In conclusion, postoperative plasma TIMP-1 predicts patient outcome both alone and in combination with CEA. Postoperative TIMP-1 may be a marker of residual disease after primary surgery for CRC.

1. Introduction

Colorectal cancer (CRC) affects approximately 217,000 individuals in the European Union. Eighty percent of patients with CRC present with potentially curable disease. Nevertheless, despite intended curative surgery, 50% of these patients experience relapse, which is invariably fatal.[1,2] Thus, hundreds of thousands of patients with resected CRC are candidates for surveillance, as it is a commonly held view that early detection of recurrence may allow for early intervention, with the aim of improving survival and quality of life. Major efforts have been put into the establishment of effective tests for detection of recurrent disease at a stage where intervention is not futile. A variety of methods has been employed for postoperative surveillance of CRC. Unfortunately, results from randomised studies of monitoring CRC patients have demonstrated only minimal efficacy.[2] Expert Panels of The American Society of Clinical Oncology (ASCO)[3] and the European Group of Tumour Markers (EGTM)[4] have recommended against the use of many of these tests. Nevertheless, it is recommended that postoperative measurement of serum carcinoembryonic antigen (CEA) is performed every 2–3 months for $\geqslant$2 years in patients with stage II or III disease where resection of liver metastases, if detected, is deemed possible.[3,4] Of note,

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however, is the fact that approximately 30% of all CRC recurrences do not result in elevated CEA serum levels.[5] Thus, it has been stressed by the ASCO and EGTM Expert Panels that new surveillance methods for the detection of CRC recurrences should be developed, evaluated and standardised.[3,4] This statement has gained further weight, as recent data have shown that chemotherapy of metastatic CRC can improve short-term survival and improve quality of life.[6]

Tissue inhibitor of metalloproteinases 1 (TIMP-1) is a 28 kDa glycoprotein demonstrated to form non-covalent 1:1 stochiometric complexes with the matrix metalloproteinases (MMP),[7] thereby inhibiting the matrix degrading properties of these endopeptidases,[8] which play a pivotal role in the growth and spread of cancerous disease. However, distinct from the anti-proteolytic property of TIMP-1, recent reports have ascribed different or even opposing functions to the enzyme inhibitor, such as stimulation of cell growth, inhibition of apoptosis and enhancement of tumourgenicity and carcinogenesis, indicating that the role of TIMP-1 in cancer progression may be multifunctional.[9–14] In accordance with a growth-promoting role of TIMP-1, high tumour tissue levels of TIMP-1 mRNA and protein have been reported in various cancer diseases, with high levels of TIMP-1 being associated with poor prognosis.[15–18]

Using a validated immunoassay for plasma TIMP-1, we have demonstrated that compared with blood donors, who have TIMP-1 levels distributed within a narrow range, both early and advanced stage CRC patients have significantly increased levels of plasma TIMP-1.[19,20] Furthermore, we have reported that preoperative plasma TIMP-1 is significantly correlated with survival of CRC patients; high plasma TIMP-1 independently predicting poor prognosis.[21,22] The present report aimed to test the usefulness of TIMP-1 levels in postoperative plasma samples as a monitoring marker in surveillance of CRC patients.

2. Materials and methods

2.1. Blood donors

Ethylenediaminetetraacetic acid (EDTA) plasma samples were analysed from a total of 808 blood donors for the establishment of the range of TIMP-1 in healthy individuals. A total of 391 males and 417 females were included. The median age was 42 years; however, the age range was wide (18–79 years) and included representative numbers of individuals at both extremes. All donors were apparently in good health, did not receive any medication and participated on a volunteer basis.

2.2. CRC patients

In the Danish part of a multicentre study with 20 participating hospitals, a prospective cohort of newly diagnosed CRC patients ($n = 740$) underwent elective large bowel resection for primary CRC in 1991–1993.[23] The diagnosis of CRC was histologically verified and its stage was established according to Dukes' classification.[24] No adjuvant chemotherapy or radiotherapy was administered following primary surgery, as these modalities were not part of the standard treatment regimen in Denmark at the time of the study. Patients were followed every 3 months in the outpatient clinic. The median observation time of the patients was 7.8 years, range 6.8–9.1. Time to first relapse (local or distant) and overall survival (death of all causes) was recorded.

Of the 740 CRC patients enrolled, curative resections were performed in 549 individuals with Dukes' stage A, B or C. Of these, 463 patients had preoperative blood samples collected, excluding 83 patients from two hospitals where blood samples were not collected or where EDTA tubes were not used. The 83 patients did not differ significantly in Dukes' stage, tumour localisation, age, gender or survival from the remaining 463 patients. Another 23 patients were excluded as they died within 6 months following curative surgery – of these 15 died within 1.5 months postoperatively. Of the remaining 440 patients with valid preoperative plasma samples, 325 had postoperative samples available for TIMP-1 determination. The reasons for this drop encompassed complete failure to collect postoperative blood samples, long waiting time for patients for sample collection, and failure of patients to attend for sample collection. Comparing the 325 patients available for the final analyses with the 115 patients without postoperative blood samples, it was found that the latter group was on average older and with more patients in Dukes' stage C; thus these patients had a higher risk of death during the observation period. There were no differences in gender or tumour localisation. A total of 325 corresponding pre- and postoperative EDTA plasma samples were subsequently analysed for TIMP-1. Of these, 280 patients had their postoperative blood sample taken within 2 months of the median elapsed time from operation to postoperative blood sampling (7 months 1st to 3rd quartile 6.7–7.2). A total of 276 corresponding postoperative serum samples were available for CEA analyses. During observation of the 280 patients, 135 patients died, 61 experienced local recurrence (LR) and 47 distant metastases (DM). The median (range) age of the 280 CRC patients was 68 (36–90) years. Table 1 summarises patient data.

2.3. Blood samples

Blood samples were obtained with informed consent from all patients and healthy donors in accordance with the Helsinki II declaration, and permission was granted by the Central National Ethical Committee, The Danish Board of Health and The Danish Data Protection Agency. Collection of all blood samples was performed strictly according to a previously

Table 1 – Patient characteristics of 280 patients included in the analysis

Parameter		Frequency (n)	%
Dukes' stage	A	44	16
	B	131	47
	C	105	38
Primary tumour localisation	Colon	155	55
	Rectum	125	45
Gender	Female	113	40
	Male	167	60

described protocol with minimal stasis to prevent platelet activation; the samples were processed, handled and stored (−80 °C) under identical conditions.[19] Corresponding plasma and serum samples were collected at one sitting to make marker measurement in plasma and serum directly comparable. Samples were collected preoperatively and planned 6 months postoperatively.

2.4. TIMP-1 ELISA

A previously described immunoassay was used for the quantitation of total TIMP-1 plasma levels.[19] In brief, the sandwich immunoassay consisted of a sheep polyclonal anti-TIMP-1 antiserum, a murine monoclonal anti-TIMP-1 IgG1 (MAC-15) and a rabbit anti-mouse-immunoglobulin/alkaline phosphatase conjugate (DakoCytomation, Glostrup, Denmark).[25–27] In the present study, the inter-assay coefficient of variation was 7.7%, determined using an internal, pooled plasma control included on each plate (plasma pool mean: 56.9 ng/ml, SD: 4.4 ng/ml, $n = 48$).

2.5. CEA EIA

A commercially available chemiluminescent EIA kit (Immulite CEA, DPC©, Los Angeles, United States of America (USA)) was used for CEA determination. According to the manufacturer's instructions, this assay has a detection limit of 0.2 ng/ml, recovery of approximately 100%, and intra- and interassay variations of 5% and 6%, respectively.[28] A predefined threshold for elevated serum CEA of 5.0 ng/ml was used.[29]

2.6. Statistical methods

The SAS® software package (version 8.2; SAS Institute, Cary, NC, USA) was used for management of patient data and for statistical analysis. For each patient, TIMP-1 levels were measured and entered into the database as pre- and postoperative data. TIMP-1 levels were scored as normal or elevated based on the age- and gender-adjusted 95th percentile of normal donors or by its actual level $\log_e$ transformed or as the ratio between the post- and preoperative TIMP-1 levels. Comparisons of preoperative TIMP-1 levels in Dukes' stages, primary tumour localisation, gender and age were carried out using a general linear model with TIMP-1 log transformed. The primary end-point was death of all causes and secondary endpoints were time to LR and DM. The Kaplan–Meier method was used to estimate survival probabilities. The Cox proportional hazard model was used for multivariate analysis, adjusting for the clinical baseline variables: age at operation, gender, Dukes' stage and primary tumour localisation (colon or rectum). All analyses were based on landmark time, that is, from the time of postoperative sampling. Only patients with a postoperative sample taken within 2 months of the median time from operation to postoperative sample (7 months) were included in the statistical analysis. The proportionality assumption was validated by graphical methods as well as by Schoenfeld residuals. The level of significance was set at 5%. For comparison of overall survival times, an expected survival curve for an age- and gender-matched background cohort was generated based on data drawn from the general Danish population.[30]

3. Results

3.1. TIMP-1 levels in healthy donors

The median (range) level of total plasma TIMP-1 in 808 healthy donors was 72 (30–229) ng/ml; 1st and 3rd quartiles: 62 and 86 ng/ml. As seen from Fig. 1, a significant association between plasma TIMP-1, age and gender was demonstrated, using linear regression on the log transformed TIMP-1 levels. Thus, mean TIMP-1 levels increased by 8% per decade ($P < 0.0001$) in both males and females. Furthermore, healthy male donors had approximately 7% higher TIMP-1 levels ($P < 0.0001$). Based on plasma TIMP-1 levels in the 808 healthy donors, age- and gender-adjusted 95th percentiles were calculated and subsequently used as cut-off points in the following analyses (Fig. 1).

3.2. Plasma TIMP-1 levels in CRC patients

The median (range) preoperative plasma TIMP-1 level in the 280 CRC patients was 113 (51–477) ng/ml. Forty-two percent ($n = 118$) of the 280 CRC patients had preoperative plasma TIMP-1 levels above the calculated cut-off point (age and gender adjusted) and were accordingly defined as having high preoperative TIMP-1 levels. Postoperatively, the median (range) TIMP-1 level was 102 (44–443) ng/ml. Twenty-eight percent ($n = 54$) of the 280 CRC patients were defined as having high TIMP-1 levels postoperatively. In a general linear model

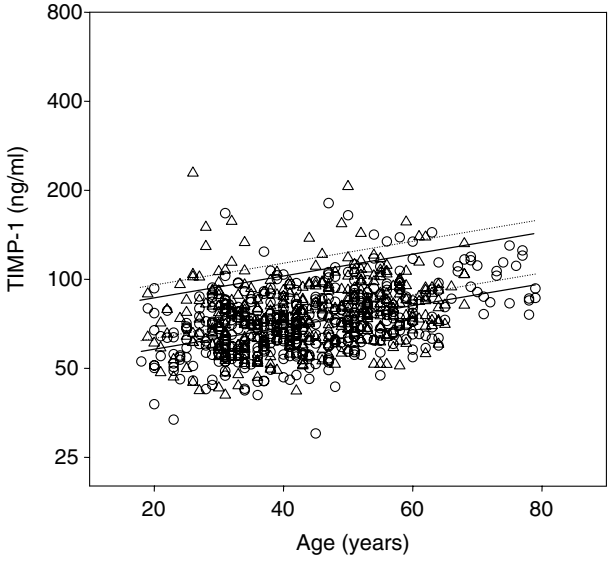

Fig. 1 – Plasma TIMP-1 levels and age in 808 healthy blood donors (males △, females ○). A small but significant relation between age and TIMP-1 level was found for both males and females, however, on average males had plasma TIMP-1 levels 7% higher than females. Lower and upper full lines: regression line and upper 95th percentile for females (F). Lower and upper dotted lines: regression line and upper 95th percentile for males (M).

including Dukes', gender, primary tumour localisation and age, only age was significantly associated with preoperative and postoperative TIMP-1 ($P < 0.0001$; Spearman rank correlation = 0.39 and 0.32). The measured plasma TIMP-1 levels of the CRC patients did not differ significantly between the participating CRC centres ($n = 20$; $P = 0.12$).

3.3. *Pre- and postoperative plasma TIMP-1 and patient outcome*

On the basis of pre- and postoperative plasma TIMP-1 levels being above or below the predefined cut-off points, the 280 CRC patients were stratified into four groups. Eighty-five percent (137/162) of the patients with low preoperative TIMP-1 levels remained low postoperatively (group I) and accordingly, 15 % (25/162) progressed to high postoperative TIMP-1 levels (group III). Fifty-four percent (64/118) of the patients with high preoperative levels had low postoperative TIMP-1 (group II) and accordingly, 46% (54/118) remained high 6 months after surgery (group IV). The pre- and postoperative TIMP-1 levels were moderately associated ($P < 0.0001$; Spearman rank correlation = 0.58) and postoperative TIMP-1 levels were significantly lower ($P < 0.0001$).

The course of TIMP-1 from pre- to postoperative levels was found to be associated with clinical outcome ($P = 0.005$). Corresponding hazard ratios (HR) and confidence intervals (95% CI) of groups II–IV, with group I as the baseline, demonstrated that while group II had a similar risk of death as group I (HR = 0.9; 95% CI: 0.6–1.4; $P = 0.69$), groups III and IV had significantly increased risks of death (HR = 2.3; 95% CI: 1.3–3.9; $P = 0.003$ and HR = 1.6; 95% CI: 1.0–2.4; $P = 0.045$, respectively) during observation. Thus, the course of plasma TIMP-1 from either low or high preoperative to high postoperative levels was associated with a significantly increased risk of mortality. Similar results were found when entering the end-points of LR and DM (data not shown).

From the analysis it appears that postoperative rather than preoperative TIMP-1 is determinant of patient outcome. Entering pre- and postoperative TIMP-1 levels in a Cox regression model demonstrated postoperative TIMP-1 as a significant covariate for patient survival (HR = 2.0; 95% CI: 1.3–2.9; $P = 0.0006$), whereas preoperative TIMP-1 was non-significant (HR = 0.8; 95% CI: 0.6–1.2; $P = 0.29$).

3.4. *Postoperative plasma TIMP-1 and Dukes' stage*

Survival analysis was performed including only postoperative plasma TIMP-1 and Dukes' stage in an additive model. It was found that high postoperative TIMP-1 was significantly associated with short patient survival when adjusting for Dukes' stage (HR = 1.8; 95% CI: 1.3–2.6; $P = 0.001$). Similarly, it could be demonstrated that patients with locally advanced stage CRC (Dukes' stage C) had a significantly increased risk of death compared with patients with early stage disease (Dukes' stage A + B) when adjusting for postoperative TIMP-1 (HR = 3.0; 95% CI: 2.1–4.2; $P < 0.0001$). This additive effect of postoperative TIMP-1 and Dukes' stage is illustrated in Fig. 2, showing survival of the 280 patients divided according to disease stage and postoperative TIMP-1 being either below or above the 95th percentile of healthy donors. Patients were

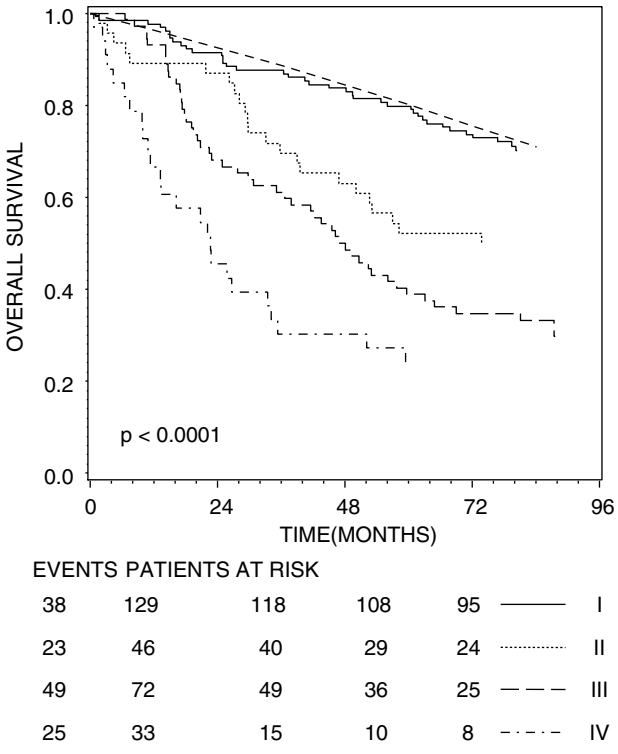

Fig. 2 – Postoperative plasma TIMP-1 levels, Dukes' stage and survival of 280 CRC patients. Patients were divided into four groups according to the combination of postoperative TIMP-1 and Dukes' stage: Group I low TIMP-1 + early stage disease (Dukes' stage A + B); group II high TIMP-1 + early stage disease; group III low TIMP-1 + locally advanced stage disease (Dukes' stage C); group IV high TIMP-1 + locally advanced stage disease. Straight dotted line: expected survival for an age- and gender-matched background Danish cohort. Number of events (death of all causes) and patients at risk during observation period are given below the figure.

stratified into four groups such that group I comprised patients with early stage disease and low postoperative TIMP-1 ($n = 129$), group II early stage disease and high postoperative TIMP-1 levels ($n = 46$), group III patients with locally advanced stage CRC and low postoperative TIMP-1 levels ($n = 72$) and group IV locally advanced stage CRC and high postoperative TIMP-1 levels ($n = 33$). As seen from Fig. 2, patients with early stage CRC with postoperative TIMP-1 above the cut-off point (group II) had a higher risk of death during observation compared with early stage CRC patients with low postoperative TIMP-1 (group I). In contrast, patients with locally advanced disease with low postoperative TIMP-1 (group III) had a risk of death similar to that of patients of group II. Locally advanced CRC in combination with elevated postoperative TIMP-1 (group IV) portended increased risk of death compared with locally advanced CRC and low postoperative TIMP-1. Thus, by stratifying CRC patients according to Dukes' stage and postoperative TIMP-1, patients with either low or high risk of death in early stage as well as in locally advanced CRC could be identified. For comparison, the expected overall survival of an age- and gender-matched Danish background

population was added to Fig. 2. As seen, the survival of early stage CRC patients with low postoperative TIMP-1 is similar to that of the Danish background population.

3.5. *Multivariate analyses for postoperative plasma TIMP-1*

With the end-point of death of all causes, multivariate Cox analysis was performed including postoperative TIMP-1 and other parameters, such as Dukes' stage, primary tumour localisation, patient age and gender. Scored as high or low according to the cut-off point, TIMP-1 was significantly associated with patient outcome such that high postoperative TIMP-1 predicted shorter survival independently of other parameters (HR = 1.7; 95% CI: 1.2–2.5; $P = 0.002$); see Table 2. Similar results were obtained for time to LR and DM (LR: HR = 1.8; 95% CI: 1.1–3.1; $P = 0.03$; DM: HR = 2.0; 95% CI: 1.1–3.7; $P = 0.02$, data for other parameters not shown).

3.6. *Postoperative serum CEA levels in CRC patients*

The median (range) CEA level in serum samples from 276 CRC patients was 2.0 (0.2–33.9) ng/ml. Using the predefined cut-off point of 5 mg/ml, 50 (16%) patients had elevated postoperative CEA.

3.7. *Postoperative TIMP-1 and CEA and patient outcome*

Fig. 3 illustrates survival of CRC patients (Dukes' stage A + B + C, 276 patients, 134 deaths) stratified into four groups according to their postoperative TIMP-1 and CEA levels being either above or below the predefined cut-off points. As seen from Fig. 3, the combination of postoperative TIMP-1 and CEA was highly associated with survival of CRC patients. Patients with high postoperative TIMP-1 levels had a HR of 1.5 (95% CI: 1.0–2.1; $P = 0.03$) compared with patients with low TIMP-1 levels for a given CEA level. Similarly, patients with high postoperative CEA had a HR of 3.0 (95% CI: 2.0–4.5; $P < 0.0001$) compared with patients with low CEA levels for a given TIMP-1 level. A weak but significant association between postoperative levels of TIMP-1 and CEA was found ($P < 0.0001$; Spearman rank correlation = 0.3).

Table 2 – Cox multivariate analysis of overall survival (135 deaths) of 280 CRC patients

Parameter	P-value	Hazard ratio (HR)	95% CI of HR
Postoperative TIMP-1 (high versus low)	0.002	1.7	1.2–2.5
Dukes' stage B (versus A)	0.03	2.1	1.1–4.1
Dukes' stage C (versus A)	<0.0001	5.6	2.9–10.6
Primary tumour localisation (rectum versus colon)	<0.0001	2.3	1.6–3.3
Patient age (years)	0.0002	1.03	1.02–1.05
Gender (male versus female)	0.01	1.6	1.1–2.3

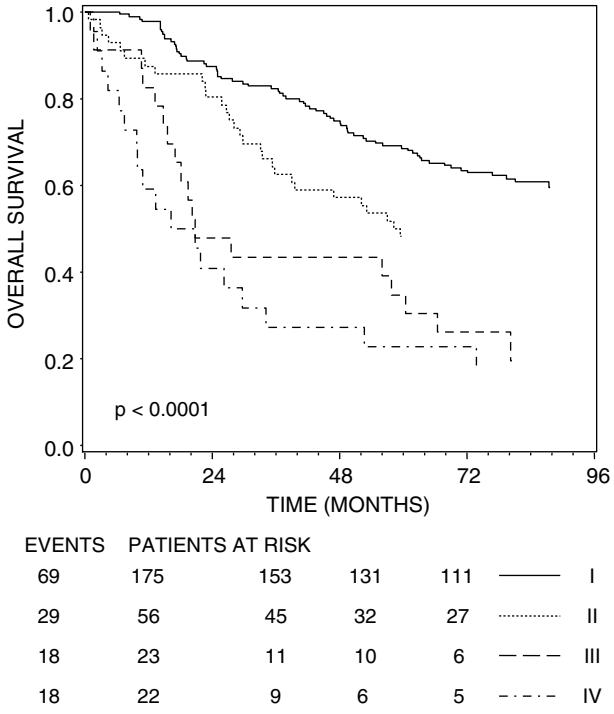

Fig. 3 – Postoperative plasma TIMP-1 levels, postoperative serum CEA and survival of 276 CRC patients. Patients were divided into four groups according to the combination of postoperative TIMP-1 and CEA levels (using the two predefined cut-off points): Group I low TIMP-1 + low CEA; group II high TIMP-1 + low CEA; group III low TIMP-1 + high CEA; group IV high TIMP-1 + high CEA. Number of events (death of all causes) and patients at risk during observation are given below the figure.

3.8. *Multivariate analyses for postoperative TIMP-1 and CEA*

Multivariate analyses were performed with the end-point of death of all causes, for postoperative TIMP-1 and CEA, including other recorded clinicopathological parameters. Scored as high or low according to the specified cut-off points, TIMP-1 and CEA were found to be independent predictors of CRC survival (TIMP-1: HR = 1.6; 95% CI: 1.1–2.3; $P = 0.02$; CEA: HR = 3.7; 95% CI: 2.5–5.6; $P < 0.0001$). Similar results were obtained for time to LR and DM including postoperative CEA (data not shown).

3.9. *Scoring TIMP-1 as a continuous variable*

Finally, instead of dichotomising TIMP-1 data according to the cut-off point, TIMP-1 was scored as the $\log_e$ of its actual postoperative level and thus treated as a continuous variable as we have done in previous survival studies of CRC patients.[21,30] Univariate survival analysis demonstrated postoperative TIMP-1 as a significant predictor of death (HR = 2.8; 95% CI: 1.8–4.3; $P < 0.0001$) and therefore patients with an increase of 1 in plasma TIMP-1 on the $\log_e$ scale had an estimated 2.8-fold increased risk of death over the observation period. Similarly, univariate analysis of preoperative TIMP-1 levels

Table 3 – Cox multivariate analysis of survival (135 deaths) of 280 CRC patients

Parameter	P-value	Hazard ratio (HR)	95% CI of HR
Postoperative TIMP-1 (log_e)	<0.0001	2.6	1.6–4.1
Dukes' stage B (versus A)	0.02	2.2	1.1–4.2
Dukes' stage C (versus A)	<0.0001	5.9	3.1–11.2
Primary tumour localisation (rectum versus colon)	<0.0001	2.3	1.6–3.3
Patient age (years)	0.004	1.03	1.01–1.05
Gender (male versus female)	0.03	1.5	1.0–2.1

demonstrated statistical significance (HR = 1.5; 95% CI: 1.1–2.0; $P = 0.02$). Adding the preoperative TIMP-1 level (log_e transformed) to the model did not improve the fit to the data significantly ($P = 0.46$), suggesting that the postoperative TIMP-1 level is sufficient for evaluation of prognosis. Corresponding results were found for time to LR (HR = 1.9; 95% CI: 1.0–3.7; $P = 0.06$) and time to DM (HR = 4.0; 95% CI: 2.0–8.0; $P = 0.0001$). Scoring preoperative and postoperative TIMP-1 levels by the ratio (log scale) demonstrated a significant effect ($P < 0.0001$; HR = 1.8; 95% CI: 1.3–2.4) with increased ratio having a poorer prognosis. However, this model formulation did not fit the data as well as the model entering the preoperative and postoperative TIMP-1 levels separately. Multivariate analysis established postoperative TIMP-1 as a significant predictor of survival (HR = 2.6; 95% CI: 1.6–4.1; $P < 0.0001$) independent of other covariates; see Table 3. Similar results were found for the end-points of LR and DM (LR: HR = 1.9; 95% CI: 1.0–3.8; $P = 0.05$; DM: HR = 4.1; 95% CI: 2.0–8.3; $P = <0.0001$). Including CEA in these analyses suggests that TIMP-1 ($P = 0.008$) is independent of CEA level as well as the baseline covariates for all end-points (data not shown).

4. Discussion

Preoperative plasma TIMP-1 has previously been shown to be strongly associated with CRC survival;[21,22] therefore, the present study was undertaken to clarify whether postoperative TIMP-1 in patients surgically treated for CRC would provide clinically useful information. The data showed that the course of plasma TIMP-1 from pre- to postoperative levels was significantly correlated with patient survival independently of other clinicopathological parameters. Furthermore, the course of plasma TIMP-1 from pre- to postoperative levels was also significantly and independently correlated with the risk of developing LR and DM. The analyses demonstrated that the postoperative plasma TIMP-1 level rather than the preoperative was determinant of patient outcome.

Including only postoperative data in the survival analyses, postoperative TIMP-1 provided significant prognostic information in the subgroups of CRC patients with early stage (Dukes' stage A + B) and locally advanced disease (Dukes' stage C). Of note, it was found that early stage CRC patients with low postoperative TIMP-1 had a survival comparable to an age- and gender-matched Danish background population and could as such be considered cured by the surgical intervention (= no residual disease). In contrast, early-stage CRC patients with high postoperative TIMP-1 had a significantly decreased chance of survival, similar to that of patients with locally advanced CRC and low postoperative plasma TIMP-1. An earlier study of preoperative plasma TIMP-1 levels in CRC including patients with metastatic disease demonstrated that stages A, B and C had similar TIMP-1 levels whereas stage D patients had significantly higher TIMP-1 levels.[21] Thus, it could be speculated that elevated postoperative TIMP-1 levels reflect the presence of residual cancerous disease. Further studies are needed to determine the clinically relevant cut-off point for stratification of patients.

In this study, 549 patients underwent curative resection of their tumours, but only 280 patients could finally be included in the analyses. Detailed information on this issue is given above, however, one problem was that although planned to be collected 6 months after surgery the timing of the postoperative samples varied substantially. Thus, we chose to analyse only those patients with time from operation to sampling close to 7 months in order to avoid bias. Therefore, we have no information on the optimal lead-time for postoperative sampling. Currently ongoing studies have been initiated to determine the optimal lead-time and the effect of adjuvant systemic therapy on postoperative TIMP-1 levels and to further evaluate the prognostic value of postoperative TIMP-1 in CRC since the reduction in number of patients is a weakness of the present study.

As CEA is the only serological tumour marker that has been recommended for use in CRC follow-up,[3,4] we included CEA measurements in the present study. We could confirm that high postoperative CEA levels as well as TIMP-1 predicted disease recurrence and death. However, it is noteworthy that only a weak association between postoperative CEA and TIMP-1 levels was found. Rather, an additive effect was obtained by combining CEA and TIMP-1 values in univariate survival analyses. Thus, additional statistically significant information on patient outcome was obtained by the combination of postoperative CEA and TIMP-1 data both regarding patient survival and occurrence of LR and DM. The data imply that adding TIMP-1 measurements to CEA may provide increased monitoring value in postoperative CRC surveillance.

In accordance with internationally accepted guidelines, any potential tumour marker must undergo strict pre- and peri-analytical validation including potential influence of sample collection, sample storage, assay performance, etc. Such studies have been performed for total TIMP-1 measurements in plasma.

Taken together, the present study implies that plasma TIMP-1 measurements have a prognostic value in CRC, not only when measured preoperatively, but also in the postoperative setting. The present study contains only a single postoperative TIMP-1 measurement for each patient and thus constitutes a static picture of postoperative serological TIMP-1 levels approximately 7 months after surgery; therefore, it could be speculated that repeated measurements of plasma TIMP-1 might prove beneficial for postoperative

CRC surveillance. In such a dynamic setting a more detailed picture of postoperative plasma TIMP-1 levels could be studied, both immediately following surgery and during outpatient follow-up. In order to test this hypothesis a prospective investigation is needed including the study of lead-time, optimal timing of blood sampling, repeated sampling and combination with other CRC tumour markers (e.g. CEA).

Conflict of interest statement

None declared.

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Hans Jørgen Nielsen is a Danish Cancer Society Professor of Surgical Oncology.

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