

## Plasma TIMP1 Level Is a Prognostic Factor in Patients with Liver Metastases

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**Abstract.** *TIMP1 (tissue inhibitor of metalloproteinases 1) regulates extracellular matrix turnover and also promotes cell growth and has anti-apoptotic activity, which promotes malignant processes in tumor tissue. The aim of our study was to evaluate the relation of plasma TIMP1 protein levels with prognosis in patients with liver metastases, with particular regard to possible early-prediction of recurrence of the disease. Patients and Methods: We studied a group of 87 patients with metastatic liver disease (mostly from colorectal cancer) who underwent surgery for liver metastases, and assessed their preoperative plasma TIMP1 levels. These levels were evaluated according to prognosis. Furthermore, we measured plasma TIMP1 in the post-operative period and tried to relate the changes with the diagnosis of relapse. Results: We found preoperative plasma TIMP1 levels to be related to overall survival in the group of all patients with metastatic liver disease ( $p=0.0047$ ), with a higher level being associated with an adverse outcome; the cut-off value was set at 165 ng/ml. This applied to all patients, regardless of the type of surgery. Assessment of the post-operative dynamics of TIMP1 was not found to be statistically significant to indicate disease recurrence. Conclusion: We found there to be a relationship between higher plasma levels of TIMP1 and an adverse prognosis in patients with liver metastases. The assessment of plasma TIMP1 levels could help the detection of patients with worse outcome.*

Colonic and rectal cancer are among the most frequent types of malignant tumors worldwide. They account for about one

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*Key Words:* Tumor markers, liver metastases, TIMP1, prognosis, blood plasma.

million new cases per year (1) and are estimated as the third leading cause of malignancies-related death in the industrialized world (2).

Although there are several tumor markers routinely used in diagnosis and follow-up of colorectal cancer (CRC) (3), there is still an effort being made to search for new ones. We are interested in tissue inhibitor of matrix metalloproteinases 1 (TIMP1). Our aim was to evaluate the relation of plasma TIMP1 protein levels with prognosis in patients with liver metastases, with regard to possible early-prediction of recurrence of the disease, because it is known that repeated liver surgery can prolong a patient's survival to the same extent as the first operation, especially if the relapse is diagnosed at an early time (4-6).

TIMPs are natural inhibitors of matrix metalloproteinases (MMPs), the enzymes involved in extracellular matrix maintenance and remodeling. But TIMP1 not only acts as an inhibitor of MMPs, which would be expected to result rather in an anticancerogenic effect, but it also has an MMP-independent role, with a direct influence on cell growth, apoptosis and angiogenesis (7). These effects include promotion of cell division, cell growth and anti-apoptotic activity (8), but a growth-inhibitory effect was also reported, suggesting the influence of the cell environment on the behavior of the enzyme (9). The action of TIMPs is very complex and still not fully understood, although great progress in studying their mechanisms of action has been made during the last decade.

TIMP1 in CRC has been studied intensively and it has been shown that its mRNA expression is elevated in tumor tissues in comparison with corresponding normal tissues (10-12). It has also been reported to be increased in liver metastases (13, 14). Previously we published a study on a group of 40 patients with liver metastases of CRC and we reported a statistically significant relation between tissue TIMP1 mRNA levels and disease-free interval (DFI) (14), but the assessment of mRNA in tumor tissues is not very convenient for follow-up. Plasma measurement is simpler, sampling is easier and the results more reproducible. Blood

plasma or serum levels of the TIMP1 protein in CRC patients have also been the focus of many authors but they were estimated mostly in primary tumors, either as a detection marker (15), or in relation to patient outcome (16-19). However, only a limited number of studies evaluating plasma TIMP1 in metastatic liver disease are available (20). The aim of our study was to evaluate the relation of the TIMP1 protein in blood plasma with regard to prognosis in patients with liver metastases.

## Patients and Methods

**Patients.** We studied a group of 87 patients (54 men and 33 women) with metastatic liver disease who had undergone liver surgery at the Department of Surgery, University Hospital Pilsen, from 2002-2006 (median age=60.3 years, range=38-82 years, at the time of surgery). In all patients, the primary tumor had been removed previously. We divided the group according to the type of surgery: 39 patients were treated by radiofrequency ablation (RFA), 44 patients were treated by radical resection and four patients had another type of surgery. To characterize an influence of the type of surgical therapy on prognosis, we compared overall survival (OS) and DFI in a subgroup of patients treated by RFA and a subgroup with another type of surgery. We did not record any significant differences in OS between the groups based on different types of surgery. We found a significantly shorter DFI in patients treated by RFA in comparison with those treated by another type of surgery, mostly liver resection, regardless of TIMP1 level ( $p=0.0056$ ) (Figure 1). This finding is in accordance with the known fact that radical resection is the most effective treatment of liver metastatic disease (21).

Most patients' liver metastases originated from CRC (72 cases); 15 patients had liver metastases from other tumors: five from breast cancer, three from renal cell carcinoma, two from gall bladder cancer and single cases of lung cancer, pancreatic cancer, ovarian cancer and gastrointestinal stromal tumor (GIST); one tumor was of unknown origin (Table I). A total of 56 patients developed recurrence of the disease during the follow-up. The median time to disease relapse was 391 days. The median follow-up was 776.5 days. We assessed the preoperative plasma TIMP1 protein levels and correlated these levels with DFI and OS according to the type of surgery and the origin of the primary tumor. Furthermore, we measured plasma TIMP1 in the postoperative period and tried to relate the changes to the diagnosis of relapse. Unfortunately, it was not possible to follow the same sampling schedule for all patients, hence we were only able to include 29 patients for this part of the study (10 patients with recurrent disease and 19 in remission). We received informed consent from all participants. The study was approved by the local Ethical Committee.

**Blood plasma samples.** Blood plasma samples were collected preoperatively, one day before surgery and then within two weeks after the surgery, and subsequently according to the need of clinical examinations, approximately at 3-month periods. For the evaluation of postoperative plasma TIMP1 levels, we took into account the sampling which took place within four weeks before diagnosis of the relapse by imaging methods or after relapse, and compared it with the baseline, based on the values assessed not earlier than three months after surgery and not later than two months before the discovery of recurrent disease. The peripheral blood was drawn

Table I. Distribution of patients in subgroups according to the original tumor type and treatment.

| Patient group | Origin of metastases |                  | Treatment |                 |                        |
|---------------|----------------------|------------------|-----------|-----------------|------------------------|
|               | CRC                  | Other tumor type | RFA       | Liver resection | Other types of surgery |
| All (N=87)    | 72                   | 15               | 39        | 44              | 4                      |
| Men (N=54)    | 51                   | 3                | 21        | 29              | 3                      |
| Women (N=33)  | 21                   | 12               | 18        | 15              | 1                      |

CRC: Colorectal cancer; RFA: radiofrequency ablation.

Table II. Preoperative plasma tissue inhibitor of metalloproteinases 1 (TIMP1) levels in the individual subgroups of patients.

| Patient group             | Median (ng/ml) | Range (ng/ml) |
|---------------------------|----------------|---------------|
| All patients (N=87)       | 113.95         | 70.1-427.2    |
| CRC metastases (N=72)     | 114.00         | 70.1-344.4    |
| Non-CRC metastases (N=15) | 112.45         | 74.6-427.2    |
| RFA treatment (N=39)      | 117.65         | 70.1-427.2    |
| Radical resection (N=44)  | 113.40         | 73.4-274.3    |

CRC: Colorectal cancer; RFA: radiofrequency ablation.

using Vacuette® tubes (Greiner Bio-One, Kremsmünster, Austria) with EDTA as an anticoagulant. Plasma was separated by centrifugation at 1300 ×g and all specimens were immediately aliquoted and frozen. Samples were stored at -70°C. No more than one freeze-thaw cycle was allowed before an analysis.

**Quantitative estimation of the TIMP1 protein using enzyme-linked immunosorbent assay (ELISA).** Plasma TIMP1 levels were measured by ELISA technology using commercial kits: Quantikine Human kits (R&D Systems, Minneapolis, MN, USA). The internal quality control samples Quantikine Elisa Kit Controls (SET651-RD6 R&D Systems, Minneapolis, MN, USA) were measured in each assay for monitoring of plate-to-plate variation. The controls used were at three different levels, approximately 0.4 ng/ml, 2.3 ng/ml and 5.0 ng/ml, to ensure whole-calibration range stability. The samples were diluted 100-fold using Calibrator Diluent RD5P(1X) before the analysis, according to the instructions for use.

Preoperative plasma TIMP1 levels in the individual subgroups of patients are listed in Table II.

**Statistical analysis.** Statistical analysis was performed using the SAS 8.02 software (SAS Institute Inc., Cary, NC, USA). The statistical results in comparing groups were calculated using a Wilcoxon two-sample test. *p*-Values were considered statistically significant at the 0.05 level. Evaluation of prognostic significance and cut-off value were performed as analysis of maximum likelihood estimates. The optimal cut-off value was found in the most statistically significant result (with the lowest *p*-value) of maximum likelihood estimates analysis. The Kaplan-Meier survival distribution function based on this cut-off value was computed.

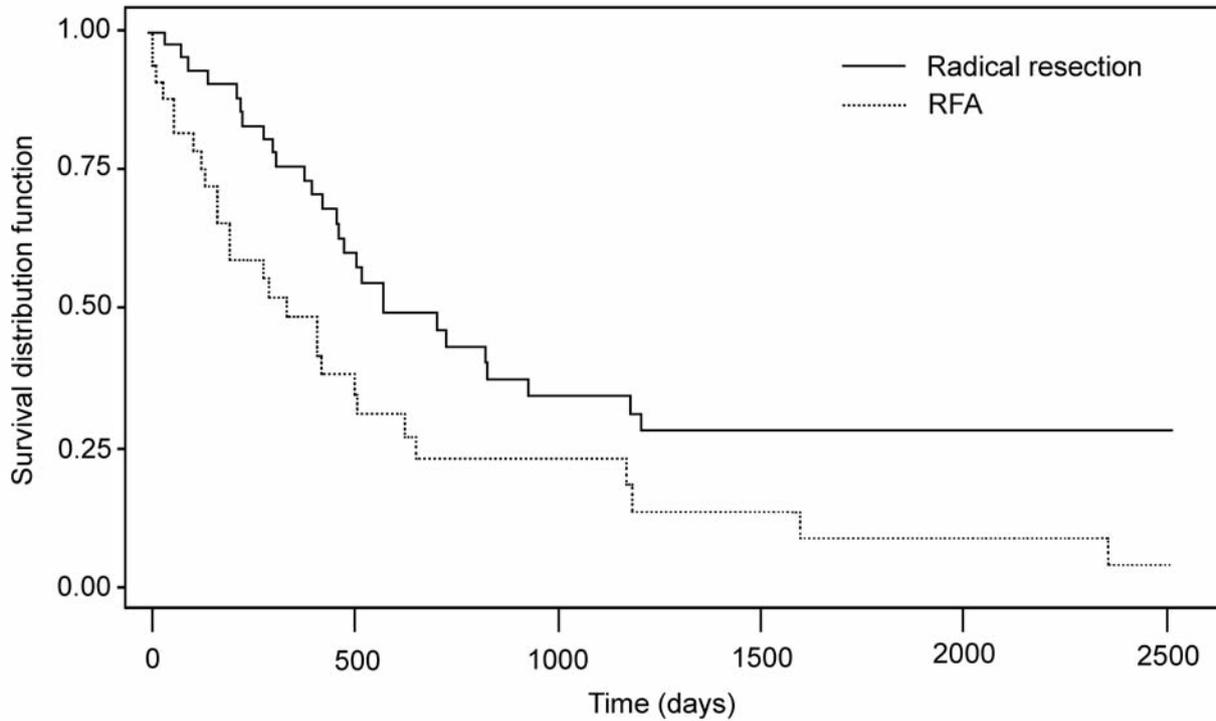


Figure 1. Influence of the type of therapy on the disease-free interval (DFI) in patients with metastatic liver disease (Kaplan–Meier DFI curve). There is a significant difference in DFI between patients treated by radiofrequency ablation (RFA) and patients treated by another type of surgery (mostly radical liver resection) (Wilcoxon  $p=0.0056$ , log-rank  $p=0.0109$ ).

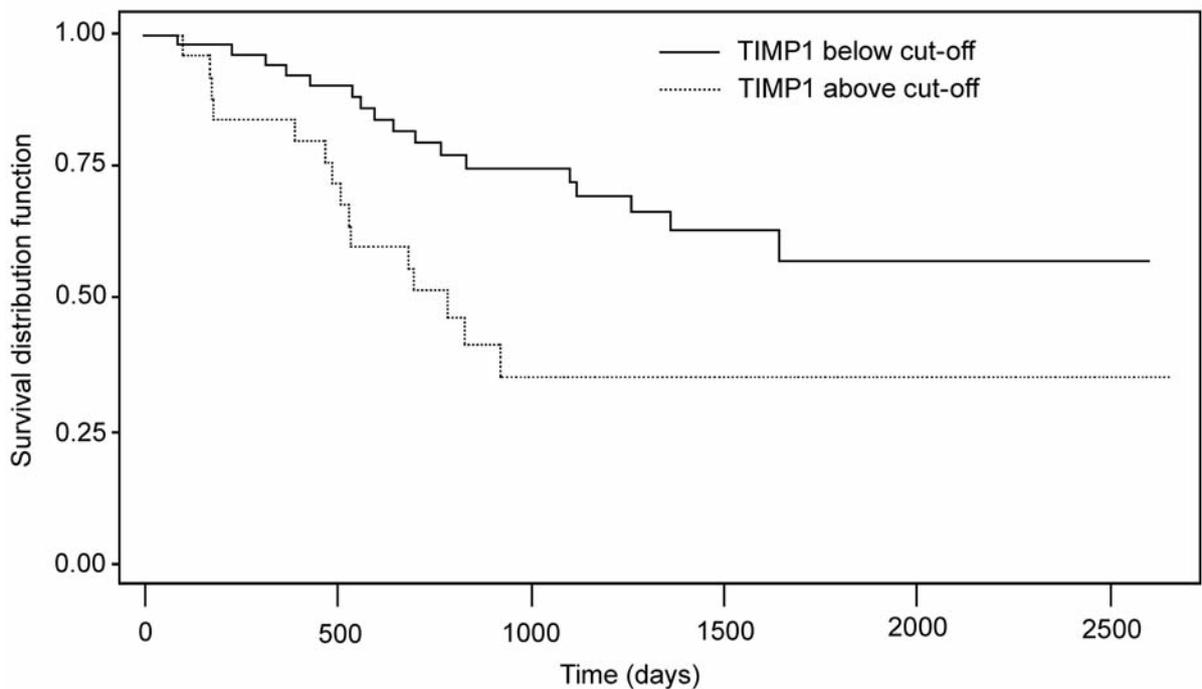


Figure 2. Relation of preoperative blood plasma level of tissue inhibitor of metalloproteinases 1 (TIMP1) protein to overall survival (OS) of patients with metastatic liver disease (Kaplan–Meier OS curve). There is a significant difference in OS between patients with plasma TIMP1 levels below and those above the cut-off value of 165 ng/ml (Wilcoxon  $p=0.0096$ , log-rank  $p=0.0047$ ).

Table III. Survival of patients with metastatic liver disease according to preoperative plasma tissue inhibitor of metalloproteinases 1 (TIMP1) higher and lower than the cut-off value.

| Marker | Number of patients | Patients below cut-off |                  | Cut-off (ng/ml) | Patients above cut-off |                  | Log-rank <i>p</i> -value | Wilcoxon <i>p</i> -value |
|--------|--------------------|------------------------|------------------|-----------------|------------------------|------------------|--------------------------|--------------------------|
|        |                    | N                      | Median OS (days) |                 | N                      | Median OS (days) |                          |                          |
| TIMP1  | 87                 | 61                     | 1318             | 165             | 26                     | 667              | 0.0096                   | 0.0047                   |

## Results

We found a statistically significant positive relation of preoperative plasma TIMP1 protein levels and OS in the whole group of patients with metastatic liver disease ( $p=0.0047$ ) and we searched for the optimal cut-off value; it was set at 165 ng/ml. We generated the Kaplan–Meier survival curve according to this cut-off value (Figure 2, Table III). We also recorded a statistically significant relation of preoperative plasma TIMP1 protein levels and OS in the group of patients with CRC metastases ( $p=0.0208$ ), a higher level being associated with an adverse outcome. We observed no relation of TIMP1 levels to OS in the group of patients with metastases from other tumor types. We recorded a significantly shorter OS in patients with a higher level of TIMP1 in a subgroup treated by RFA ( $p=0.0314$ ), as well as in the subgroup treated by liver resection ( $p=0.0457$ ). This also applies to the subgroup of patients with CRC metastases treated by liver resection ( $p=0.0322$ ), but not to patients with CRC metastases treated by RFA. No significant relations of preoperative plasma TIMP1 levels with OS were observed for the subgroup of patients with liver metastases of tumor types other than CRC (but there were only 15 patients in this subgroup), no matter what type of surgery was used for their treatment.

Concerning the DFI, we recorded a significantly shorter DFI with higher TIMP1 in a group of patients with metastases of other tumors ( $p=0.0415$ ), but no such significance was found for those with CRC metastases ( $p=0.6484$ ), or for the entire group of all patients ( $p=0.1259$ ). We did not record any significant relations of DFI to preoperative plasma TIMP1 levels according to the type of surgery performed.

One of the purposes of our study was to evaluate plasma TIMP1 levels for clinical use and to determine if the postoperative plasma TIMP1 levels can predict early-recurrence of the disease. Such a relationship was not found to be statistically significant (Wilcoxon test  $p=0.3783$ , Kruskal-Wallis test  $p=0.3586$ ). It should be noted that the number of patients whose data were available for this determination was limited (N=29).

## Discussion

The aim of our study was the evaluation of plasma TIMP1 levels as a prognostic marker with possible clinical use in patients with metastatic liver disease. Plasma TIMP1 levels have been intensively studied in primary CRC. Nielsen *et al.* concluded from a large population-based study that plasma TIMP1 levels could be of potential use for the detection of early-stages of CRC (15), and other authors also suggest its usefulness in this indication, including for the differentiation between carcinoma and adenoma (22, 23). Many studies have also been dedicated to the evaluation of this marker in relation to the staging of CRC, OS and DFI. Most reported significant correlation between plasma TIMP1 levels and many clinicopathological features, shorter OS and shorter DFI in patients with high levels of TIMP1 (18, 24-26), some suggesting TIMP1 to be an even stronger predictor of prognosis than the routinely used carcinoembryonic antigen (CEA) (16). Some of the investigators found only correlation with the advanced stage of CRC but not with prognosis (27). However, none of these studies dealt exclusively with patients with liver metastases. We discovered only one study which was focused on patients with metastatic liver disease (20) and surprisingly it did not describe any significant differences in plasma TIMP1 levels between patients with primary CRC without metastases and those with liver metastases, nor did it report any association with patient survival. In contrast, in the current study we found a statistically significant relation of preoperative plasma TIMP1 protein levels and OS in the whole group of patients with metastatic liver disease and in the subgroup of patients with CRC metastases. This relation was observed for the subgroup of patients treated by RFA, as well as for the subgroup of patients treated by radical liver resection. This indicates that the preoperative plasma TIMP1 level in patients with liver metastases is a prognostic factor independent of the type of surgical treatment. We did not record any statistical significance in DFI according to the levels of TIMP1 for patients with CRC liver metastases, but did so for patients with liver metastases of other tumor types. This may suggest that TIMP1 behaves differently depending

on the type of primary tumor and could also be helpful as a prognosticator in metastatic liver disease of different origins.

We also studied the dynamics of plasma TIMP1 after surgery for liver metastases. Although our results did not reach significant values, whether a substantial increase of plasma TIMP1 in the postoperative period could indicate forthcoming relapse, is still in question. This would be valuable information because repeated liver surgery can prolong a patient's survival to the same extent as the first operation, especially if the relapse is diagnosed at an early time. Unlike evaluation of the prognostic value of the preoperative TIMP1 levels (OS and DFI), where we had concise data from 87 patients, the assessment of the postoperative dynamics of TIMP1 levels and disease recurrence was limited by the number of patients with sufficient data for this evaluation (N=29). In this respect, we concur with conclusion of Waas *et al.* (20), who did not find any significant differences in postoperative changes of blood plasma TIMP1 between patients with relapse of liver metastases and those without relapse. Nevertheless, their study population was also quite small and there is no other study evaluating this parameter known to us, although there are some examining the changes of plasma TIMP1 in metastatic CRC during the treatment with different cytostatic chemotherapies (17, 28, 29).

## Conclusion

We found there to be a positive relationship between preoperative plasma TIMP1 levels in patients with liver metastases and OS. We set a cut-off value and reported that a preoperative plasma level of TIMP1 higher than 165 ng/ml is associated with an adverse prognosis. A significantly shorter OS was recorded for patients with a higher level of TIMP1 in a subgroup treated by RFA and in the subgroup treated by liver resection.

## Conflicts of Interest

The Authors declare that they have no conflict of interest.

## Acknowledgements

This study was supported by the SVV project of LF UK Plzen no. SVV-2012-264806 and the grant of the Ministry of Education, Youth and Sports of the Czech Republic no. CZ.1.07/2.3.00/20.0040 and grant IGA MZ CR 12025.

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*Received May 4, 2012*

*Revised July 30, 2012*

*Accepted August 2, 2012*