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CLINICAL STUDY

Matrix metalloproteinases and their inhibitors in correlation to proliferative and classical tumour markers during surgical therapy of colorectal liver metastases

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Abstract: *Objectives:* Classical and proliferative tumour markers and matrix metalloproteinases and their tissue inhibitors reflect the features of malignancy and are useful in prediction of prognosis in patients with colorectal liver metastases. There is very limited information about their physiological functions during regeneration and healing of liver parenchyma after any type of liver surgery for malignancy.
Methods: The presented study included the patients, who underwent following surgical procedures for CLM, benign liver lesions and inguinal hernias: Group A: 22 patients with inguinal hernias, Group B: 26 patients with benign liver lesions, Group C: 30 patients with colorectal liver metastases (CLM) who were treated by radiofrequency ablation, Group D: 41 patients with CLM who underwent a radical surgical therapy – resection, and Group E: 22 patients with inoperable CLM who underwent an explorative laparotomy without any surgical procedure.
Results: The preoperative and postoperative serum levels of CEA, CA 19-9, TK, TPA, TPS, MMP-2, MMP-9, TIMP-1, and TIMP-2 were statistically analyzed and compared within the groups to estimate the influence of a surgical procedure type. These results reflect the influence of surgical procedure on the serum levels of studied tumour markers during operation.
Conclusions: It was the first description using these types of comparison to all metalloproteinases, their inhibitors, and proliferative and classical tumour markers. It could help us to estimate the critical relations of these tumour markers in prognoses of disease free survival or overall survival in patients after a surgical procedure for CLM *(Tab. 5, Ref. 26)*. Full Text in PDF *www.elis.sk.*
Key words: liver surgery, MMPs, TIMPs.

Abbreviations: CA 19–9 – carbohydrate antigen 19–9, CEA – carcinoembryonic antigen, CLM – colorectal liver metastases, DFI – disease free interval, MMP – matrix metalloproteinase, RFA – radiofrequency ablation, ROC – receiving operative curves, TIMP – tissue inhibitor of metalloproteinases, TPA – tissue Polypeptide Antigen, TPS – tissue Specific Polypeptide Antigen, TK – thymidine kinase.

Colorectal liver metastases (CLM) are the main secondary malignancy of liver, which liver surgery is focused on today (1). The main problem is not the operative technique or preoperative detection of malignant lesions but the preoperative judgment of planned operative procedure with regard to patients´ benefit, which is described by disease free survival, survival rate and quality of life (2, 3). These parameters are confronted with all accessible treatment strategies: surgical vs oncological, radical vs. palliative vs. symptomatic. The remaining and still open question discussed preoperatively with every patient is the early recurrence, which could shorten all the named parameters and so move our patients, with regard to their benefit, from radical operation to palliative therapy. The underwent surgical procedures with their complications, duration of hospital stay and followed rehabilitation, morbidity and mortality have in the case of early recurrence a very poor benefit! The question for today is: Is there any possibility to predict the early recurrence with a high accuracy?

Classical and proliferative tumour markers and matrix metalloproteinases and their tissue inhibitors reflect the features of malignancy and are useful in prediction of prognosis in patients with colorectal liver metastases (4). There is very limited information about their physiological functions during regeneration and healing of liver parenchyma after any type of liver surgery for malignancy. The aim of the study was to analyze the preoperative and postoperative serum levels of CEA, CA 19–9, TK, TPA, TPS, MMP–2, MMP–9, TIMP–1, and TIMP–2 and to analyze the influence of surgical procedure on serum levels of studied tumour markers during an operation. The authors aimed at studying the

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relation between the tumour markers (MMP, TIMP, classical and proliferative tumour markers) and the prediction of recurrence and survival rate after a liver surgery for CLM.

Material and methods

The presented study included patients who were operated on at the Department of Surgery, University Hospital Pilsen, Charles University Prague between 11/2002 and 12/2004 and underwent surgical procedures for CLM, benign liver lesions, and inguinal hernias.

The patients were divided into four groups for statistical analyses as follows:

Group A: patients with inguinal hernias who were treated with classical hernioplasty with usage of their own tissues without opening the abdominal cavity (laparotomy or laparoscopy), without usage of mesh – prolene or goretex, and without any complication. The patients included into this group did not suffer from malignant or degenerative diseases in anamnesis. They had also no extensive polymorbidity including liver diseases and no inflammatory diseases six month before the operation. This group was designed as manifestation of physiological function and expression of the studied tumour markers under primary healing of a wound. 22 patients (11 men and 11 women) were included with the mean age of 56 years (range 16–78 years).

Group B: patients with benign liver lesions (cysts, focal nodular hyperplasia, hemangiomas, adenomas, etc.). This group had 26 patients (8 men and 18 women) with the mean age of 54 years (range 33–83 years). The performed surgical procedures involved enucleation of lesion, fenestration of cysts, resection of maximally four segments.

Group C: 30 patients (22 men and 8 women) with CLM who were treated by radiofrequency ablation (RFA) with the mean age of 64 years (range 48–78 years).

Group D: 41 (29 men and 12 women) patients with CLM who underwent a radical surgical therapy – resection. The mean age in this group was 61 years (range 45–82 years).

Group E: 22 patients (10 men and 12 women) with inoperable CLM, those who underwent explorative laparotomy without any surgical procedure. The average age in this group was 63 years (range 29–85 years).

The serum samples were obtained before (maximally 14 days before) and after the operation (maximally 14 days after). The performed surgical therapy was absolutely independent on the cohort membership – the patients with CLM were put in study groups retrospectively.

All the blood samples for assessment of tumour markers, matrix metalloproteinases and their tissue inhibitors were taken under standard conditions from the cubital vein during the morning hours. The serum for assessment of routine tumour markers acquired through centrifugation was stored until laboratory analysis at the temperature of –20 °C. The serum for assessment of matrix metalloproteinases and their tissue inhibitors acquired through centrifugation was stored until laboratory analysis at the temperature of –75 °C. Tumour markers were assessed at the Dept. of Nuclear Medicine, Faculty Hospital Pilsen with commercial laboratory kits, in accordance with the manufacturers' recommendations. The following tumour markers were assessed: carcinoembryonic antigen (CEA– ng/mL, IRMA, Immunotech, CR), carbohydrate antigen 19–9 (CA 19–9– IU/L, Shering-CIS France), cytokeratines: tissue specific polypeptide antigen (TPS- kIU/L, IRMA, IDL Sweden), tissue polypeptide antigen (TPA-kIU/L, IRMA, DiaSorin, Italy). Thymidine kinase (TK-IU/L) was measured by radioenzymo analyses (REA) using Immunotech (Prague) assay kits. Matrix metalloproteinases (MMP–2– ng/mL, MMP–9–ng/mL) and their tissue inhibitors (TIMP–1– ng/mL, TIMP–2–ng/mL) were assessed by ELISA methods (Chemicon – Millipore, USA). Serum levels of tumour markers were correlated with the clinical diagnoses of the patients.

Statistical analysis was performed by the statistical software CRAN. The statistical description parameters were used: mean, median, standard deviation, interquartile interval, minimum value, and maximum values. Non-parametrical Kruskal–Wallis and Wilcoxon tests were disposed for statistical comparison of distribution of particular parameters in the studied groups regard the distribution of these values.

Results

The basic descriptive statistic of studied tumour markers for particular subgroups is presented in Tables 1–4. The *group A* was used as the control group – patients with the inguinal hernias. The optimal value of normal serum levels was taken as the 95th percentile of particular serum level values (Tab. 1). In the all following groups there were, in comparison with the group A, changes of serum levels evoked either by a diseases or a surgical procedure.

Group B The comparison of the control group (A) with the group of benign liver lesions demonstrated differences of preoperative serum levels of MMP–2 and –9 and TIMP–2 (p- value<0.016,

Tab. 1. Basic descriptive statistics: control group A (patients with inguinal hernias, n=22).

Marker	Control group A – inguinal hernias, n=22			
	preoperative	preoperative	postoperative	postoperative
	median	min.– max.	median	min.–max.
CEA (ng/mL)	0.65	0.1–5.5	0.65	0.1–4.7
CA 19-9 (IU/L)	6.75	0.8–51.2	6.25	0.8–48.4
TPS (kIU/L)	42.5	0–283	36	0–283
TPA (kIU/L)	31.5	0–234	29.5	0–145
TK (IU/L)	5.45	2.3–26.9	4.6	1.7–17.2
MMP-2 (ng/mL)	398.7	293–669	388	264.3–640
MMP-9 (ng/mL)	74.7	18–270.8	137.4	97–383.1
TIMP-1 (ng/mL)	109.05	65.6–225.5	116.5	66.8–233.7
TIMP-2 (ng/mL)	18.9	16.2–113.2	18.5	15.9–94.1

Tab. 2. Basic descriptive statistics: group B (patients with benign liver lesions, n=26).

Marker	Group B – benign liver lesions, n=26			
	preoperative	preoperative	postoperative	postoperative
	median	min. – max.	median	min. – max.
CEA (ng/mL)	1.05	0.3–1124	0.7	0.2–389.9
CA 19-9 (IU/L)	9.85	0.8–34.5	11.2	0.8–249
TPS (kIU/L)	34	10–261	66	10–225
TPA (kIU/L)	16	10–101	44.5	10.1–91
TK (IU/L)	6	3.1–30	12	1.8–97.3
MMP-2 (ng/mL)	481	288–938	476	231–860
MMP-9 (ng/mL)	160.75	45.8–457	174.9	28.6–91.2
TIMP-1 (ng/mL)	106	59.5–195.2	126.9	82.4–371.1
TIMP-2 (ng/mL)	41.85	22.1–81	42.6	20–78.5

Tab. 3. Basic descriptive statistics: group C (patients with colorectal liver metastases (CLM) who were treated by radiofrequency ablation (RFA), n=30).

Marker	Group C: CLM – RFA, n=30			
	preoperative	postoperative	preoperative	postoperative
	median	min.–max.	median	min.–max.
CEA (ng/mL)	19.5	1.0–3070	13.75	0.4–3586
CA 19-9 (IU/L)	15.15	0.80–1869	20.25	0.80–1869
TPS (kIU/L)	62.5	10–453	143	13–476
TPA (kIU/L)	70	10–220	96	10–430
TK (IU/L)	7.4	2.3–34.1	8.7	1.4–42
MMP-2 (ng/mL)	506	267–759	447.5	56.9–4000
MMP-9 (ng/mL)	101.15	38.6–379.2	106.65	36.7–600
TIMP-1 (ng/mL)	111.55	72.2–344.4	167.7	87.2–319.4
TIMP-2 (ng/mL)	34.95	20.3–67.6	31.5	20–81.2

Tab. 4. Basic descriptive statistics: group D (patients with colorectal liver metastases (CLM) who underwent radical surgical resection, n=41).

Marker	Group D: CLM – liver resections, n=41			
	preoperative	preoperative	postoperative	postoperative
	median	min.–max.	median	min.–max.
CEA (ng/mL)	23.4	0.3–44528	4.5	0.2–327
CA 19-9 (IU/L)	19	0.8–6985	32.8	0.8–445
TPS (kIU/L)	91	10.1–6641	98	10.1–2372
TPA (kIU/L)	99.5	10.1–2087	84	10.1–2069
TK (IU/L)	6.4	3–14.2	10.4	4.2–67
MMP-2 (ng/mL)	473	287–1000	501	284–1694
MMP-9 (ng/mL)	120	30.1–397.4	119.7	36.7–266
TIMP-1 (ng/mL)	134.5	73.8–498.1	165.2	63.7–314.5
TIMP-2 (ng/mL)	28.3	17.2–83	28.7	18.9– 83

Tab. 5. Basic descriptive statistics: group E (patients with inoperable colorectal liver metastases /CLM/, who underwent explorative laparotomy, n=22).

Marker	Group E: CLM – explorative laparotomy, n=22			
	preoperative	preoperative	postoperative	postoperative
	median	min. – max.	median	min. – max.
CEA (ng/mL)	12.85	0.4–7722	33.9	0.5–5431
CA 19-9 (IU/L)	112.25	3.5–20949	124.8	10– 8953
TPS (kIU/L)	380	17–4973	326.5	29–1629
TPA (kIU/L)	281	10–1710	266.5	45–527
TK (IU/L)	9.35	3.9–278	10.15	4.7–406.7
MMP-2 (ng/mL)	534	290–690	542	258–658
MMP-9 (ng/mL)	120.8	45.4–278.5	132	61.8–233.8
TIMP-1 (ng/mL)	169.5	82.8–275.9	206.4	133–277.6
TIMP-2 (ng/mL)	44.05	29.8–74.8	52.3	30.6–68.3

0.0002, 0.0001 respectively) and postoperative serum levels of TPS, TK, MMP–2 and TIMP-2 (p-value<0.018, 0.0012, 0.013 and 0.0001respectively), statistically significant.

Group C The comparison of the control group with the group of patients who underwent a radiofrequency ablation of CLM displayed differences of preoperative serum levels of TPA, TPS, MMP–2 ,TIMP–2, CEA and CA 19–9 (p-value<0.0018, 0.0246, 0.007, 0.001, 0.001 and 0.0219 respectively) and postoperative serum levels of TK, TPA, TPS, TIMP–1 and –2, CEA and CA 19–9 (p-value<0.0082, 0.0018, 0.0001, 0.002, 0.001 a 0.001, 0.0007 respectively), statistically significant.

Group D The comparison of the control group with the group of patients who underwent a radical surgical procedure for CLM demonstrated differences of preoperative serum levels of TPA, TPS, MMP–2 and –9, TIMP–1 and –2, CEA and CA 19–9 (p-value<0.0007, 0.0007, 0.02, 0.003, 0.04, 0.001, 0.001 and 0.0012 respectively) and postoperative serum levels of TK, TPA, TPS, MMP–9, TIMP–1 and –2, CEA and CA 19–9 (p-value<0.0001, 0.0014, 0.0001, 0.001, 0.003, 0.001, 0.001 and 0.008 respectively), statistically significant.

Group E The comparison of the control group with the group of patients who underwent the explorative laparotomy for inoper-

able CLM showed differences of preoperative serum levels of TK, TPA, TPS, MMP–2 and -9, TIMP–1 and -2, CEA and CA 19–9 (p-value<0.0032, 0.0001, 0.0001, 0.004, 0.007, 0.0007, 0.001, 0.0011 and 0.0001 respectively) and postoperative serum levels of TK, TPA, TPS, MMP–2, TIMP–1 and –2, CEA and CA 19-9 (p-value <0.0119, 0.0016, 0.0052, 0.008, 0.0003, 0.0002, 0.0008 and 0.0001 respectively), statistically significant (Tab. 5).

Discussion

Matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) have been implicated not only in tumour invasion but also in tissue remodelling, especially in extracellular matrix rebuilding and in regeneration processes and inflammatory responses (5). The levels of MMPs and TIMPs have been also proved to be correlated with tumour aggressiveness and progression (6, 7, 8). The activity of MMP–2 and –9 and TIMP-1 is overexpressed in tumour mass of colorectal cancer contrary to surrounding healthy colon tissue (9). Contrary TIMP–2 levels are controversial. Baker detected significantly higher levels in normal colon tissue whereas Murashige did not (9, 10). The upregulated levels of MMPs and TIMPs also correlate with the aggression or recurrence of the metastatic process in colorectal cancer, especially in colorectal liver metastases (CLM) (11, 12). The elevation of MMP–9 is associated with an invasiveness of colorectal cancer. A successive increase of tissue expression of MMP–2, –7 and -9 relates with an increase of malignancy – from mucous lesions and adenomas to carcinomas (13).

MMP–2 and –9 have been shown to be overexpressed by the stroma surrounding the tumour (14, 15). This could be the explanation of malignancy invasiveness mechanism. The same mechanism of extracellular matrix (basement membrane) degradation occurs at initiation of angiogenesis, which is crucial for growth of tumour mass (13, 26).

Expression of TIMP–1 is associated with the invasiveness or extension of colorectal cancer (16, 17). TIMP–1 is produced by fibroblast-like cells of the invading cancer. The tumour surrounding mucosa is almost without production of TIMP–1. TIMP–1 is not secreted by benign or malignant cells, cells of vessels and muscle cells (16). MMPs reflect not only the penetration of malignancy to surrounding healthy tissue through the increased destruction of matrix and new synthesis of tumour stroma, but also influence the tumour growth secondary by releasing of cytokines (transforming growth factor alpha and Insulin like growth factor – II) bound in extracellular matrix in an inactive form and activated just through the releasing (18). MMPs participate in other processes influencing the primary tumour growth, angiogenesis and invasion, intra- and extravasation of metastatical cells and growth of metastatic process (19). The synchronous determination of the levels of MMP–9 in portal and peripheral blood is useful for selecting colorectal cancer patients at high risk of hepatic recurrence (20). TIMP–1 and –2 and MMP–2 were detected during tissue regeneration in rats while MMP–3, –9, –10, –13 and –14 were not (21).

The role of classical tumour markers is generally accepted in prediction of relapse or survival rate of patients after liver surgery for colorectal cancer (22, 23). The serum levels of proliferative tumour markers (TPS, TPA, TK) have been used as good prognostic factors for stating the tumour aggression and recurrence of CLM after a liver surgery. Their relation to survival rate is unsure (24, 25).

The authors aimed at studying the relation between the tumour markers (MMP, TIMP, classical and proliferative tumour markers) and prediction of recurrence and survival rate after a liver surgery for CLM. The behavior of particular tumour markers was observed in benign liver lesions during the surgical treatment and in healthy patients without any malignancy or complicated comorbidity undergoing classical hernioplasty for groin hernias without abdominal cavity affecting. The comparison of separate tumour markers in the groups of patients and the relations among them should clear up their role in recurrence and mechanism of tumour progression. The aim was not to study each tumour marker individually but in relation to other tumour markers, especially with their other supposed functions and mechanism of behavior. Last but not least, the factor taken into account was to study all these tumour markers in the same patients and so uncover their relation in each patient. Only CLM patients were enrolled into this study for the elimination of influence of diverse malignant diseases (primary or secondary) with different behavior.

Group A The assessed serum levels of studied tumour markers in the control group A were used for analyses of other groups of patients with benign or malignant liver diseases. The surgical procedure itself (incisional groin hernia repair) had to serve as the physiological background of normal wound healing. We studied the influence of serum levels of tumour markers to differentiate the changes during the more extensive surgical procedures in liver parenchyma. Some elevations or declines in serum levels of studied markers during the operation in the group A were interpreted through the pleiotropic function of the given tumour markers during the first period of wound healing and regeneration of non-liver tissues.

Group B The differences of preoperative and postoperative changes between the group of benign liver lesions and the control group could be explained by the activity of benign lesion tissue, which could show some features of precanceroses and sometimes there could be malignant inversion (liver adenomas) or increased proliferative activity (focal nodular hyperplasia) detected. The influence of large active surface of endothelium in hemangiomas, especially cavernomatous, is also worth considering. The differences between the groups A and B are incomparable with increases recorded in the groups with liver malignancy. In case of benign liver diseases we could also hypothesize about the of liver parenchyma activity on the immediate surroundings of benign lesion. This could be influenced by an insufficient biliary drainage and underlying ischemia in consequence of pressure by the benign lesion (cystis, hemangioma). This could contribute to tissue remodelling or changes in function and elevation of tumour markers in comparison with the control group. These first two discussed groups (A and B) served as the essential groups to eliminate the influence of changes of serum levels during surgical procedures, which reflected the physiological function of metalloproteinases, their inhibitors and other studied tumour markers. These differ-

ences in test grave our hypothesis that also in benign liver lesions the serum levels of studied tumour markers could be regulated up.

Group C The statistical analysis of the studied tumour markers in the group C (radiofrequency of CLM) brought very valuable results. These were influenced by the retained destructed tumour tissue, which could release tumour markers in blood circulation not only during its thermic destruction but also in the postoperative period during remodelation of this destructed lesion and creation of scars. Almost unchanged serum level of CEA is crucial for this hypothesis. In the studied metalloproteinases, their inhibitors and proliferative tumour markers, there was an absolute elevation of their serum levels detected in the postoperative period. This could be explained by their releasing from tissue depots or by fibrogenesis and proliferation in scaring lesion and their immediate surroundings.

Group D The classical tumour markers reacted upon elimination of malignant lesion by a marked decrease of their serum levels. This confirms the relation of classical tumour markers to the volume of tumour mass. The metalloproteinases and their inhibitors or proliferative tumour markers, which reflect aggression, invasiveness and advanced stage of CLM, were elevated after a radical liver surgery (24). In the case of resection, we could also hypothesize the persisting activity of the tumour markers in serum. We could not exclude either participation of these tumour markers in regeneration and remodelling of liver parenchyma after resection as a reaction on changes in functional reserves of remnant liver parenchyma. The liver parenchyma in the immediate surrounding of resection surface could also increase expression of these tumour markers as a reflection of its remodelling because of change in its blood supply and biliary drainage.

Group E Most of the studied tumour markers continue in the same expression of their serum activity after the explorative laparotomy for inoperable CLM. This could be explained by the minimal influence of performed laparotomy, which is a standard component of other surgical procedures (RFA, liver resections). We could confirm that the changes in groups C and D resulted, due to surgical procedure, in liver parenchyma. The group of patients with the performed explorative laparotomy serves as another comparative group, this time with tumour without any intervention (25).

These results reflect the influence of the type of surgical procedure on serum levels of studied tumour markers during operation. The authors tried to negate the influence of physiological activity of these tumour markers, which is supposed to result from their pleiotropic functions in regeneration and remodelling of healing tissues. It was the first description using the comparison of all metalloproteinases, their inhibitors, proliferative and classical tumour markers. It could help us to find the critical relations of these tumour markers to prognosis of disease free survival or overall survival in patients after a surgical procedure for CLM.

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