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CLINICAL STUDY

Robotic stereotactic radiosurgery with CyberKnife – brain metastases and fibrinolysis

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ABSTRACT

OBJECTIVES: Deviations in haemostasis are found in about 50 % of patients with cancer and up to 90% of those with metastatic disease. Many studies investigate the dynamics of the processes of coagulation and fibrinolysis and their role as a predictor of therapeutic response, early relapse, or metastasis risk.

BACKGROUND: To investigate the serum levels of urokinase plasminogen activator (uPA) in patients with brain metastases treated with robotic stereotactic radiosurgery (SRS) with CyberKnife.

MATERIAL AND METHODS: Serum levels of urokinase plasminogen activator (uPA) were measured in 66 patients with solid tumours, divided into two groups, with oligometastatic disease and brain metastases. In this prospective longitudinal study, the serum levels of uPA were measured before starting the therapy and at the first, third, and sixth months after patients were irradiated with the CyberKnife system.

RESULTS: Analysis of serum uPA levels in the post-treatment period showed a statistically significant decrease between the baseline and the 6 months post-treatment time point in both patient groups. The baseline value of serum uPA in the group with lung cancer decreased by 62.7 %, and in the group with other types of cancer – by 60 %. Despite the significant reduction of serum uPA levels 6 months after the treatment, the levels remained significantly higher in both groups than in healthy controls.

CONCLUSION: Ongoing research on uPA and cancer will enrich our knowledge and expand the possibilities for clinical utilization of the marker in the oncology setting *(Tab. 2, Ref. 18)*. Text in PDF *www.elis.sk*

KEY WORDS: solid tumours, urokinase plasminogen activator, brain metastases, robotic stereotactic radiosurgery, CyberKnife.

Introduction

Historically, the concept for metastatic disease assumes that cancer is a systemic process even in its earliest stages (1). The dominant opinion for the initial therapeutic approach in patients with metastatic disease is a systemic treatment. With the progress of scientific knowledge, including the new insights into the cancer genome, this classical concept has been changed (2). The concept of oligometastatic disease (OMD) was introduced, which characterizes cancer disease with a limited number of metastases in one or more organs. This allows the successful application of locoregional treatment with a radical purpose (3). The rationale for a more aggressive local approach is that it is an "intermediate" condition in the disease's evolution, with a small number of metastases limited to a specific organ (4).

One of the fundamental processes in the stage of metastasis is haemostasis. A system that is anatomically and functionally associated with the vessel's wall includes the two basic subsystems – coagulation and fibrinolysis. It is characterized by procoagulant properties of tumour and endothelial cells, due to which neoangiogenesis and changes in haemostasis in malignant disease are inextricably linked. In addition to procoagulant substances, tumour cells express components of the fibrinolytic system – tissue plasminogen activator (t-PA), urokinase-type plasminogen activator (uPA), urokinase plasminogen activator receptors (uPAR) and their inhibitors – plasminogen activator inhibitor 1–3 (PAI 1–3) (5). Fibrinolytic system functions in the vascular space but also in the tumour tissue itself. Thus, in patients with malignancies, these are not simply uncontrollable changes in coagulation and fibrinolysis, but an expression of a new balance that favours tumour metastasis and neovascularisation. Deviations in haemostasis are found in about 50% of patients with cancer and up to 90% of those in the metastatic stage (1, 6). The dynamics of these changes during the treatment period and their role as a predictor of therapeutic response, early relapse, or risk of metastasis are investigated by many studies (7, 8).

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The question that arises is whether the treatment of OMD with modern methods of radiation oncology, particularly robotic stereotactic radiosurgery with the CyberKnife system, would impact fibrinolysis (4).

The aim of the study is to investigate the serum levels of urokinase plasminogen activator (uPA) in patients with brain metastases treated with robotic stereotactic radiosurgery (SRS) with CyberKnife system.

Material and methods

A total of 66 patients divided into two groups were enrolled in the study: 34 patients with non-small cell lung cancer (NSCLC), a second group included 32 patients with other types of primary cancer (OTPC). All patients had the oligometastatic disease (OMB), brain metastases detected by MRI, and have undergone SRS with CyberKnife system. The patients were treated at the Clinic of Radiation Oncology, UMHAT "Sv. Georgi" from 2017 to 2020. This study was approved by the Scientific Ethics Committee of Medical University-Plovdiv and was conducted following the Helsinki declaration. All patients participating in this study signed informed consent. Patients had to fulfil the following inclusion criteria: age ≥ 18 years; histologically verified oncological disease; ECOG / Performance status from 1 to 3 and OMD detected by MRI with localizations and sizes meeting the requirements for RSRS Cyberknife system treatment. The exclusion criteria were: ECOG / Performance status > 3; the history of heart failure NYHA grade III / IV; decompensated diabetes mellitus; acute viral/bacterial infection or exacerbated chronic inflammatory process in the last two weeks before taking the blood sample; chronic kidney disease with GFR < 60%, hepatic dysfunction with ASAT, ALAT > 5 times upper limit of normal.

Measurement of urokinase plasminogen activator (uPA)

Serum tests for uPA were run before therapy (baseline) and at 1-st, 3-rd, and 6-th month after treatment with RSRS Cyberknife system. Serum uPA levels were tested at the Central Clinical Laboratory at UMHAT "Sv. Georgi". An immune-enzyme method was used to quantify uPA level, using original ELISA kits MyBioSource, USA with analytical reliability: CV in series 5.2 %; in time 6.4 %; Sensitivity: 5.1 pg / mL and analyser: Multi parameters ELISA Reader- SIRIO, SEAC, Italy.

Statistical methods

The choice of statistical methods is consistent with the goals and objectives of the current research, the type of data, and the distribution of quantities. Data processing was carried out using the specialized software for social research IBM SPSS, version 26.0 (2018), a specialized program for medical analysis MedCalc version 19.0.7 (2020) and statistical program Minitab version 19 (2019), which allowed rapid and accurate preparation of the results for further research. The perceived critical level of significance in testing the null hypothesis H0 is $\alpha = 0.05$ (Z criterion = 1.96), with a 95 % guarantee probability (9, 10).

Results

The patients' mean age was 62 years, the youngest patient was 36 years, and the oldest 82 years old. A similar mean age was found in both groups, 59 years in the group of patients with NSCLC and 65 years in the OTPC group.

A significantly higher proportion of men (67 %, 44/66) than women (33 %, 22/66) was found ($p < 0.001$), probably associated with the predominant number of lung cancer patients. In both patient groups, the distribution by sex was similar, without significant difference ($p = 0.332$). In the NSCLC group, 70.6 % of patients were men and 29.4 % women, and in the OTPC group, 62.5 % were men and 37.5 % women.

Given the fact that serum urokinase plasminogen activator (uPA) levels showed an asymmetric distribution (Kolmogorov–Smirnov $p < 0.05$) in both patient groups and healthy control group, values were presented using median and interquartile range (Q1–Q3), and the dynamics were followed by the Friedman test and post hoc pairwise comparisons by the Wilcoxon test at a corrected error level of $\alpha = 0.012$.

In the group of patients with NSCLC, a steady downward trend in uPA levels was found, $p < 0.001$. The median level of uPA decreased from 436.95 pg/ml to 353.40 pg/ml at the first month after the treatment, with a significant difference of 83.55 pg/ml, $p = 0.001$. There was a new decrease in the median level of 274.85 pg/ml in the third month, with a significant difference of 78.55 pg/ml, $p = 0.003$. At the sixth month, the uPA decreased to 163 pg/ml, with a difference of 111.85 pg/ml, $p < 0.001$. The overall change in the uPA level showed a 62.7 % decrease from baseline (difference 273.95 pg/ml) (Tab. 1).

Tab. 1. Results of the intragroup comparisons of the serum levels of uPA in the NSCLC group.

Parameters	Timepoint				p
	Baseline (M0)	1st month (M1)	3rd month (M3)	6th month (M6)	
uPA (pg/ml)	**436.95**	**353.40**	**274.85**	**163.0**	0.000**[f]
Mediana (Q1–Q3)	(257.6–567.5)	(209.8–499.4)	(170.9–437.5)	(107.6–310.3)	
○ M0→M1					0.001**[w]
○ M1→M3					0.003**[w]
○ M3→M6					0.000**[w]
○ **M0→M6**					0.000**[w]

Q1–Q3 – Quarter 1 – Quarter 3; f – Friedman's test; w – Wilcoxon test; * statistical significance at error level $\alpha \leq 0.05 / 0.012$; ** statistical significance at error level $\alpha \leq 0.01$

Tab. 2. Results of the intragroup comparisons of the serum levels of uPA in the OTPC group.

Parameters	Timepoint				p
	Baseline (M0)	1st month (M1)	3rd month (M3)	6th month (M6)	
uPA (pg/ml)	**518.0**	**365.6**	**282.5**	**206.0**	0.000**[f]
Median (Q1–Q3)	(270.6–633.8)	(215.7–492.7)	(182.0–407.8)	(130.5–332.3)	
○ M0→M1					0.000**[w]
○ M1→M3					0.000**[w]
○ M3→M6					0.011*[w]
○ **M0→M6**					0.000**[w]

Q1–Q3 – Quarter 1 – Quarter 3; f – Friedman's test; w – Wilcoxon test; * statistical significance at error level $\alpha \leq 0.05 / 0.012$; ** statistical significance at error level $\alpha \leq 0.01$

In the group of patients with OTPC, significant dynamics in the level of uPA were also found, $p < 0.001$. The median level of uPA decreased from 518.0 pg/ml to 365.6 pg/ml at the first month after the treatment, with a significant difference of 152.4 pg/ml, $p = 0.001$. In the 3rd month, there was a new decrease in the median to 282.5 pg/ml, with a significant change of 83.1 pg/ml, $p < 0.001$. At 6th month, uPA decreased to 206 pg/ml, with a difference of 76.5 pg/ml, $p = 0.011 < 0.012$. The overall change in the uPA level showed a decrease of 60 % from baseline (difference of 312 pg/ml) (Tab. 2).

The analysis of serum uPA in the post-treatment period outlined a significant decrease in uPA levels between baseline and the 6th month time point for both patient groups. The baseline uPA value in the NSCLC group decreased by 62.7 % and in the OTPC group by 60 %. However, despite the statistically significant change, serum uPA levels at 6 months after the treatment remained significantly higher in both groups than in healthy controls.

Correlations were sought between changes in uPA serum levels and therapeutic effect. For the present analysis, the two patient groups were combined into one. The merging of the groups was motivated by similar trends found when monitoring the uPA levels. The analysis for potential relationship is based on the data from the 6-th month follow-up period because the most significant statistical changes in the uPA levels were detected at this time point. Spearman's rank-order correlation was applied, and no statistically significant correlation was found ($p = 0.085$).

Discussion

The plasminogen activator system is an extracellular proteolytic system responsible for a variety of physiological and pathological processes (11). It plays an important role in the processes of tumour progression and metastasis to distant organs. Its key components are uPA, its receptor (uPAR), and plasminogen activator inhibitors (PAI-1 and PAI-2). uPA/uPAR system may impact different processes involved in the tumour progression: tumorigenesis and suppression of apoptosis, regulation of the switch between dormancy and tumorigenicity, angiogenesis, proteolysis of the extracellular matrix, cell adhesion and migration, cell invasion, and metastasis (11). Various clinical studies have proven the role of fibrinolysis in many solid tumours with different localization, which shows its importance as a key component in tumour progression (12). Tissue overexpression of uPA/uPAR is found in breast, prostate, gastrointestinal, and lung cancer (6, 11, 13). Overexpression is associated with locally advanced or metastatic disease. It has been established as an independent predictive factor for disease-free and overall survival in patients with breast cancer, an important predictor for overall survival and metastasis in colorectal, lung, prostate, and ovarian cancers (13). Our previous studies showed a significant correlation between the fibrinolytic overactivity and the number of metastases, tumour volume, overall survival, and risk of progression in patients with breast cancer and ovarian cancer during chemotherapy (15).

In the literature, we did not find any data on modern ablative methods on fibrinolysis in patients with oligometastatic disease. The analysis of our results found a steady trend of the significant decrease in the level of uPA between baseline and the follow-up 6 months after the treatment in both patient groups. Despite the significant change, serum uPA levels at the 6-th month time point remained significantly higher than healthy controls uPA levels. A possible explanation for the observed differences may be related to the suppression of metastatic activity during RSRS Cyberknife system treatment.

Conclusion

The components of the PA system show altered expression patterns in several common malignancies. Urokinase plasminogen activator, for example, can be identified as an ideal diagnostic (16), therapeutic (17), and predictive (18) target to reduce cancer-associated morbidity and mortality. Ongoing research on tumorigenesis and the PA system will enrich our knowledge, including widening clinical application possibilities in oncology.

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