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NITRIC OXIDE – A BIOMARKER OF THE PROGRESS AND METASTASIS OF RENAL CELL CANCER INTO THE BRAIN

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Endogenous nitric oxide plays a pivotal role in cancer development. The purpose of the study was to investigate the role of the biomarker of nitric oxide metabolites in the progression and metastasis of renal cell carcinoma to the brain. To test our hypothesis, this case-control study consisted of the indicators of nitric oxide metabolites in the inactive parenchyma resected during operations for urolithiasis (the comparison group, n=13); 103 patients with renal cell carcinoma. To analyze the metabolites of nitric oxide, we used a technique that allows us to determine the content of nitrite and nitrate ions in the same sample. The content of stable metabolites of nitric oxide was expressed in μg/mg protein in tissues. Levels of nitric oxide metabolites were significantly higher (1.5-fold higher) in tumor tissue than in peri-tumor tissue. Nitric oxide exhibits a pro- or antitumorigenic effect depending on the level of NO and the stage of the tumor process. The level of nitric oxide in tumor tissue was an independent predictor of renal cell carcinoma progression and its metastasis to the brain. The content of nitric oxide in peri-tumor tissue has a high prognostic efficacy in renal cell carcinoma metastasis and as a protective factor that prevents tumor progression.

Key words: renal cell carcinoma, brain metastasis, nitric oxide.

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ОКСИД АЗОТУ – БІОМАРКЕР ПРОГРЕСУВАННЯ ТА МЕТАСТАЗУВАННЯ НИРКОВО-КЛІТИННОГО РАКУ В ГОЛОВНИЙ МОЗОК

Ендогенний оксид азоту відіграє ключову роль у розвитку раку. Метою дослідження було дослідити роль біомаркера метаболітів оксиду азоту в прогресуванні та метастазуванні нирково-клітинної карциноми в мозок. Для перевірки нашої гіпотези це дослідження типу «випадок-контроль» складалося з показників метаболітів оксиду азоту в інтактній паренхімі, резецованій під час операцій з приводу сечокам'яної хвороби (група порівняння, n=13); 103 хворих на нирково-клітинний рак. Для аналізу метаболітів оксиду азоту використовували методику, яка дозволяє визначити вміст нітрит- та нітрат-іонів в одному зразку. Вміст стабільних метаболітів оксиду азоту виражали в мкг/мг білка в тканинах. Рівні метаболітів оксиду азоту були значно вищими (у 1,5 рази вище) у пухлинній тканині, ніж у навколопухлинній тканині. Оксид азоту виявив про- або протипухлинну дію залежно від рівня NO та стадії пухлинного процесу. Рівень оксиду азоту в пухлинній тканині був незалежним предиктором прогресування нирково-клітинного раку та його метастазування в мозок. Вміст оксиду азоту в навколопухлинній тканині має високу прогностичну ефективність при метастазуванні нирково-клітинного раку та як протекторний фактор, що запобігає прогресуванню пухлини.

Ключові слова: нирково-клітинний рак, метастази в мозок, оксид азоту.

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Endogenous nitric oxide (NO) is a multifunctional inflammatory molecule and promotes inflammation under physiological condition. It is synthesized by 3 isoforms of NO synthase (NOS) [5]. The neuronal NOS and endothelial NOS constitutively catalyze the formation of NO, while the inducible NOS (iNOS) is being induced by inflammatory cytokines and produces larger, toxic amounts of NO [4]. All three forms of NOS are expressed by the kidney [8]. In contrast to conventional signaling molecules that act by binding to specific receptor molecules, NO exerts its biological actions via a wide range of chemical reactions [9]. The NO concentration and minor differences in the composition of the intracellular and extracellular environment determine the exact reactions attained. Under normal physiological conditions, cells produce small but significant amounts of NO, contributing to the regulation of anti-inflammatory effects and its antioxidant properties. However, in tissues with a high NO output, iNOS is activated, and nitration (addition of NO_2), nitrosation (addition of NO^+) and oxidation will be dominant [9]. NO plays a pivotal role in cancer development [6, 11, 12]. It has been detected that NOS expression is increased in various types of cancer, such as breast, cervical, brain, laryngeal, and head and neck cancer. NO exhibits a pro- or antitumorigenic effect. NO appears to enhance tumor growth and cell proliferation at measurable concentrations in different clinical samples from different cancer types [9]. On the one hand, excessive NO is toxic and can prevent

tumor growth by increasing the apoptosis rate of cells [1]. While on the other hand, NO is a mediator of signaling pathways, which promote cancer progression and metastasis. In summary, NO plays an important role in the complex interrelationships of ROS, inflammation, and cancer development and growth [4]. NO plays a significant role in the processes of angiogenesis and metastasis in cancer. NO, which acts as a vasodilator, aiding in the development of new blood vessels in tumors, crucial for providing nutrients and oxygen necessary for tumor growth [6, 8]. Brain metastases (BMs) are the most frequent cause of malignant tumor of the central nervous system (CNS), four times higher than primary tumors; about 20–40 % of patients with cancer will develop BM in their clinical course. Brain metastases (BrM) are a common complication, with a global prevalence of 7.3 % on initiation of systemic therapy for metastatic renal cell carcinoma (mRCC) and are associated with dismal overall survival [13].

The purpose of the study was to investigate the role of the biomarker of nitric oxide metabolites in the progression and metastasis of renal cell carcinoma to the brain.

Materials and methods. The studies were carried out on the basis of city and regional hospitals of the Luhansk region between 2015 and 2018. In accordance with the provisions of the Declaration of Helsinki by the World Medical Association of the last revision (1964–2013) informed consent for the use of biological material was obtained in all patients prior to inclusion in the study. Research permission was obtained from the Bioethics Committee of the Lugansk State Medical University (Rubizhne, Ukraine, number 25/2015). The patients' epidemiological data, laboratory examination, complications, clinical outcomes, CT imaging data, and treatment plans were extracted from medical records. The primary endpoint of this study was the advent of metastasis of renal cell carcinoma (RCC) to the brain.

To test our hypothesis, this case-control study consisted of the indicators of nitric oxide metabolites in the inactive parenchyma resected during operations for urolithiasis (the comparison group, n=13); 103 patients with kidney tumors. The clinical diagnosis in all patients was confirmed by morphological examination of the tumor according to the classification of kidney tumors of the World Health Organization (WHO/ISUP) [2]. According to the TNM classification, patients with malignant kidney tumors had stages of the tumor process: $T_1N_0M_0$ – 20 (19 %); $T_2N_0M_0$ – 58 (56 %); $T_2N_1M_1$ – 3 (3 %); $T_3N_0M_0$ – 17 (17 %); $T_3N_1M_0$ – 1 (1 %); $T_3N_0M_1$ – 2 (2 %); $T_4N_2M_0$ – 2 (2 %). The examined patients had metastases in the brain. We combined them into group $T_2N_0M_1 + T_3N_0M_1$.

Determination of nitric oxide metabolites in tissues.

To analyze the metabolites of nitric oxide, we used a technique that allows us to determine the content of nitrite and nitrate ions in the same sample [1]. For the determination of nitrites, diazotization was used taking into account the Gris reaction. Nitrates were reduced to nitrites, followed by diazotization. Thus, the total value of NO^{2-} and NO^{3-} in the sample (NO_x) was obtained. Nitrates and nitrites were determined spectrophotometrically at λ = 540 nm. The content of stable metabolites of nitric oxide was expressed in μg/mg protein in tissues.

Data Processing. Statistical and graphical analyses were done using STATISTICA 7.0 (StatSoft Inc. USA, version 7.0) and MedCalc Version 20.218 64-bit (MedCalc Software, Ostend, Belgium). Parametric data were summarized as mean (standard error) (Mean±SEM). Kolmogorov–Smirnov test was applied to examine the normality of data distribution. To examine group-wise differences, unpaired Student's t-test was used. Nonparametric results are expressed as median (Me) and standart deviation (SD). The difference between study groups was tested by a nonparametric Mann–Whitney U test was used. Receiver operating characteristics (ROC) curve analysis was performed to estimate optimal cut-off values, maximizing sensitivity and specificity according to the Youden index. The appearance of metastases analysis was performed using the Kaplan–Meier method; univariate and multivariate analyses were undertaken using log rank test and Cox's regression model, respectively. A *p*-value below 0.05 was considered statistically significant. The Cox proportional hazards regression model [10] was used to assess the effect of tissues NO levels on the metastasis of renal cell carcinoma to the brain in survival analysis.

Results of the study and their discussion. Descriptive statistics and results of comparison of patient groups according to the studied parameters included in the analysis of predictors of tumor progression and metastasis of renal cell carcinoma to the brain are presented in Table 1.

Levels of nitric oxide metabolites were significantly higher (1.5-fold higher) in tumor tissue than in peri-tumor tissue: $T_2N_0M_0$ – NO_3^- : 32.04±0.57 μg/per mg protein and 19.09±0.43 μg/per mg protein, p=0.0000001, respectively; NO_2^- : 2.65±0.09 μg/per mg protein, 1.5±0.05 μg/per mg protein, p=0.0000001, respectively; NO_x : 34.69±0.58 μg/per mg protein, 20.59±0.43 μg/per mg protein, p=0.0000001, respectively; $T_3N_0M_0$ – NO_3^- : 15.66±0.96 μg/per mg protein and 11.91±0.62 μg/per mg protein,

p=0,002438, respectively; NO_2^- : 1.27±0.05 μg/per mg protein, 1.08±0.009 μg/per mg protein, p=0.0000001, respectively; NO_x : 16.94±0.96 μg/per mg protein, 12.99±0.62 μg/per mg protein, p=0.0000001, respectively; $T_2N_0M_1$ +$T_3N_0M_1$ – NO_3^- : 41.48±1.29 μg/per mg protein and 27,69±2.37 μg/per mg protein, p=0,000919, respectively; NO_2^- : 3.9±0.1μg/per mg protein, 3.53±0.06 μg/per mg protein, p=0.0000001, respectively; NO_x : 45.37±1.27 μg/per mg protein, 31.21±2.37μg/per mg protein, p=0.000768, respectively.

Table 1

Levels of nitric oxide metabolites in tissues in renal cell carcinoma patients

Clinical groups	NO_3^-(μg/per mg protein) M ± SEM T-test Students		NO_2^-(μg/per mg protein) Me±SD, Mann-Whitney U Test		NO_x (μg/per mg protein) M ± SEM T-test Students	
Intact parenchyma (control), n=13	25.06±0.9		1.97±0.07		27.04±0.9	
Renal cell carcinoma	tumor	peritumor normal tissue	tumor	peritumor normal tissue	tumor	peritumor normal tissue
$T_2N_0M_0$, n=58	32.04±0.57	19.09±0.43	2.65±0.09	1.5±0.05	34.69±0.58	20.59±0.43
p level	p^1=0.000001	p^1=0.0000001 p^4=0.0000001	p^1=0.0000001	p^1=0.0000001 p^4=0.0000001	p^1=0.0000001	p^1=0.0000001 p^4=0.0000001
$T_3N_0M_0$, n=17	15.66±0.96	11.91±0.62	1.27±0.05	1.08±0.009	16.94±0.96	12.99±0.62
p level	p^1=0.0000001 p^2 = 0.0000001	p^1=0.0000001 p^2 = 0.0000001 p^4=0.002438	p^1=0.0000001 p^2=0.0000001	p^1=0.0000001 p^2 = 0.0000001 p^4=0.0000001	p^1=0.0000001 p^2 = 0.0000001	p^1=0.0000001 p^2 = 0.0000001 p^4=0.001550
$T_2N_0M_1$ +$T_3N_0M_1$, n=5	41.48±1.29	27.69±2.37	3.9±0.1	3.53±0.06	45.37±1.27	31.21±2.37
p level	p^1=0.0000001 p^2 = 0.000014 p^3=0.0000001	p^1=0.215670 p^2 = 0.0000001 p^3=0.0000001 p^4=0.000919	p^1=0.00023 p^2=0.0000001 p^3=0.00008	p^1=0.0002 p^2 = 0.0000001 p^3=0.000076 p^4=0.0000001	p^1=0.0000001 p^2 = 0.0000001 p^3=0.0000001	p^1=0.051229 p^2 = 0.000002 p^3=0.000076 p^4=0.000768

Notes: NO_2 – data are Me ± SD Intergroup by the Mann–Whitney U test NO_3, NOx – data are Means ± SEM for Gaussian variables Intergroup by the T-test Students p^1 – significant differences between control group and test groups p^2 – significant differences between patients with $T_2N_0M_0$, and other test groups p^3 – significant differences between patients with $T_3N_0M_0$, and $T_2N_0M_1$ +$T_3N_0M_1$ test groups p^4– significant differences between patients with tumor, and peri-tumor normal tissue test groups

The level of all nitric oxide metabolites in tumor tissue is higher in the $T_2N_0M_0$ group compared to normal parenchyma tissue. For example, NO_x : 34.69±0.58 μg/per mg protein and 27.04±0.9 μg/per mg protein, p=0.0000001, respectively. As the tumor progresses, the level of nitric oxide metabolites in the tumor decreases more than 2-fold: NO_x : $T_3N_0M_0$ – 16.94±0.96 μg/per mg protein and $T_2N_0M_0$ – 34.69±0.58 μg/per mg protein. With metastasis of renal cell carcinoma to the brain, the level of nitric oxide metabolites in the primary tumor increases sharply (45.37±1.27 μg/per mg protein): 1.5-fold compared to the $T_2N_0M_0$ group (34.69±0.58 μg/per mg protein) and 2.5-fold compared to the $T_3N_0M_0$ group (16.94±0.96 μg/per mg protein).

Analysis of the ROC curve in patients with renal cell carcinoma is shown in Fig. 1.

Since NOx is a summary indicator of NO_2^- and NO_3^-, ROC analysis was performed based on this index. According to the receiver operating characteristic curve, the area under ROC curve (AUC) values of NO_x to predict tumor stage progression and its metastasis was greatest in all groups patients with renal cell carcinoma: $T_2N_0M_0$ – tumor tissue – 0.941, optimal cut-off values of NO_x – >29.89 μg/per mg protein; p<0.0001; $T_2N_0M_0$ – peri-tumor tissue AUC=0.919, optimal cut-off values of NO_x – ≤ 24.74 μg/per mg protein; p< 0.0001; $T_3N_0M_0$ – tumor tissue AUC=1.000, optimal cut-off values of NO_x – ≤ 23.79 μg/per mg protein; p< 0.0001; $T_3N_0M_0$ – peri-tumor tissue AUC=0.081, optimal cut-off values of NO_x – ≤ 16.13 μg/per mg protein; p< 0.0001; $T_2N_0M_0$ –$T_2N_0M_1$+$T_3N_0M_1$ – tumor tissue AUC=0.966, optimal cut-off values of NO_x – > 41.54 μg/per mg protein; p< 0.0001; $T_2N_0M_0$ –$T_2N_0M_1$+$T_3N_0M_1$– peri-tumor tissue AUC=0.976, optimal cut-off values of NO_x – >24.74 μg/per mg protein; p< 0.0001. The best cutoff value of NO_x for predicting the metastasis of renal cell carcinoma to the brain was >22.57, with a sensitivity of 100.0 % and a specificity of 100.0 % and AUC=1.000 in group $T_3N_0M_0$ –$T_2N_0M_1$+$T_3N_0M_1$ – tumor tissue and in group $T_3N_0M_0$ –$T_2N_0M_1$+$T_3N_0M_1$ – peri-tumor tissue AUC=0.981, optimal cut-off values of NO_x – >15.77 μg/per mg protein; p< 0.0001.

The Kaplan–Meier survival curves (Fig.2), after classifying the patients on the basis of Youden cut-offs obtained by ROC curves, showed a decrease in the time of RCC progression to stage $T_3N_0M_0$ within 5-year survival, the Mean of which was 4.19±0.1 years (95 % CI for the mean 3.99 to 4.39), while in the observation group it was 4.9±0.1 years (95 % CI for the mean 4.7 to 5.07). The hazard ratio (HR) was (HR = 0.13; 95 % CI 0.06 to 0.28, p = 0.0001). Thus, the optimal cut-off values of NOx – < 23.79 μg/per mg protein in tissue reduces the risk of RCC progression to stage $T_3N_0M_0$ by 87 % (100 × (1 − HR) %).

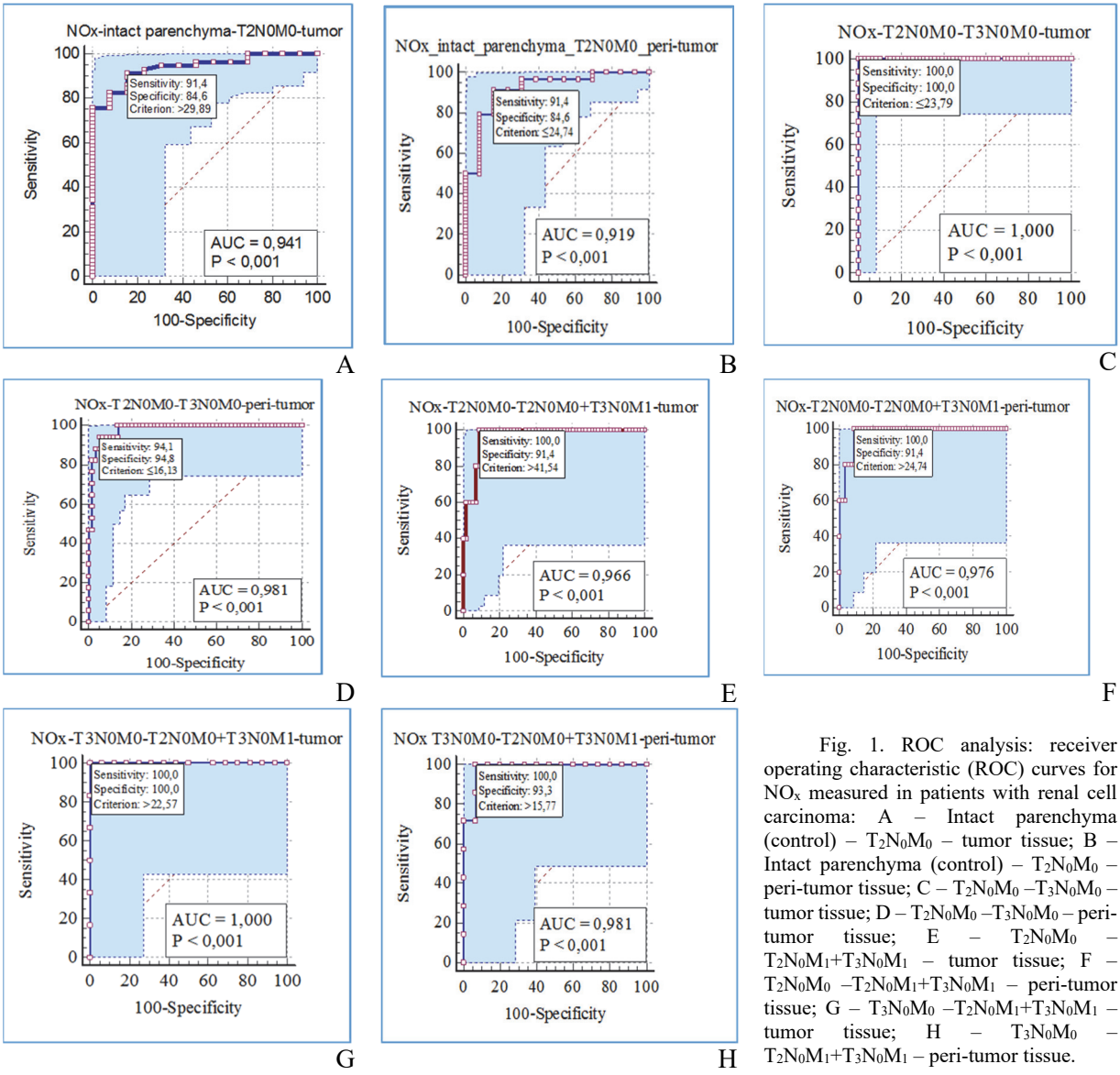

Fig. 1. ROC analysis: receiver operating characteristic (ROC) curves for NO_x measured in patients with renal cell carcinoma: A – Intact parenchyma (control) – $T_2N_0M_0$ – tumor tissue; B – Intact parenchyma (control) – $T_2N_0M_0$ – peri-tumor tissue; C – $T_2N_0M_0$ –$T_3N_0M_0$ – tumor tissue; D – $T_2N_0M_0$ –$T_3N_0M_0$ – peri-tumor tissue; E – $T_2N_0M_0$ – $T_2N_0M_1$+$T_3N_0M_1$ – tumor tissue; F – $T_2N_0M_0$ –$T_2N_0M_1$+$T_3N_0M_1$ – peri-tumor tissue; G – $T_3N_0M_0$ –$T_2N_0M_1$+$T_3N_0M_1$ – tumor tissue; H – $T_3N_0M_0$ – $T_2N_0M_1$+$T_3N_0M_1$ – peri-tumor tissue.

Note: Here and in the following figures: p<0.001 – calculated by univariate logistic regression analysis.

In peritumor tissues $T_3N_0M_0$ the time of RCC progression to stage $T_3N_0M_0$ within 5-year survival also decreased: the Mean was 3.6±0.25 years (95 % CI for the mean 3.06 to 4.06). The hazard ratio (HR) was (HR = 0.017; 95 % CI 0.005 to 0.05, p = 0.0001). The optimal cut-off values of NO_x – > 16.13 µg/per mg protein in tissue reduces the risk of RCC progression to stage $T_3N_0M_0$ by 98 %. The time of RCC metastasis in the brain for NO_x measured in patients with renal cell carcinoma in group $T_2N_0M_0$ – $T_2N_0M_1$+$T_3N_0M_1$ – tumor tissue Mean =3.9±0.4 years (95 % CI for the mean 3.1 to 4.7). The hazard ratio (HR) was (HR = 0.005; 95 % CI 0.0006 to 0.037, p = 0.0001). The optimal cut-off values of NO_x – < 41.54 µg/per mg protein in tissue reduce the risk of RCC progression to stage $T_3N_0M_0$ by 99 %. In group $T_2N_0M_0$ –$T_2N_0M_1$+$T_3N_0M_1$ – peri-tumor tissue the time of RCC metastasis in the brain Mean =4.0±0.4 years (95 % CI for the mean 3.2 to 4.8). The hazard ratio (HR) was (HR = 0.006; 95 % CI 0.0007 to 0.04, p = 0.0001). The optimal cut-off values of NO_x – < 24.74 µg/per mg protein in tissue reduce the risk of RCC progression to stage $T_3N_0M_0$ by 99 %. The time of RCC metastasis in the brain for NO_x measured in patients with renal cell carcinoma significantly decreased in group $T_3N_0M_0$ –$T_2N_0M_1$+$T_3N_0M_1$ – tumor tissue Mean =2.6±0.2 years (95 % CI for the mean 2.1 to 3.08). The hazard ratio (HR) was (HR = 0.0024; 95 % CI 0.00022 to 0.026, p = 0.0001). The optimal cut-off values of NOx – < 23.79 µg/per mg protein in tissue reduce the risk of RCC progression to stage $T_3N_0M_0$ by 99.8 %. In group $T_3N_0M_0$ –$T_2N_0M_1$+$T_3N_0M_1$ – peri-tumor tissue the time of RCC metastasis in the brain Mean =3.0±0.23 years (95 % CI for the mean 2.56 to 3.45). The hazard ratio (HR) was (HR = 0.0013; 95 % CI 0.00034 to 0.0049, p = 0.0001). The optimal cut-off values of NO_x – < 15.77 µg/per mg protein in tissue reduces the risk of RCC progression to stage $T_3N_0M_0$ by 99.9 %.

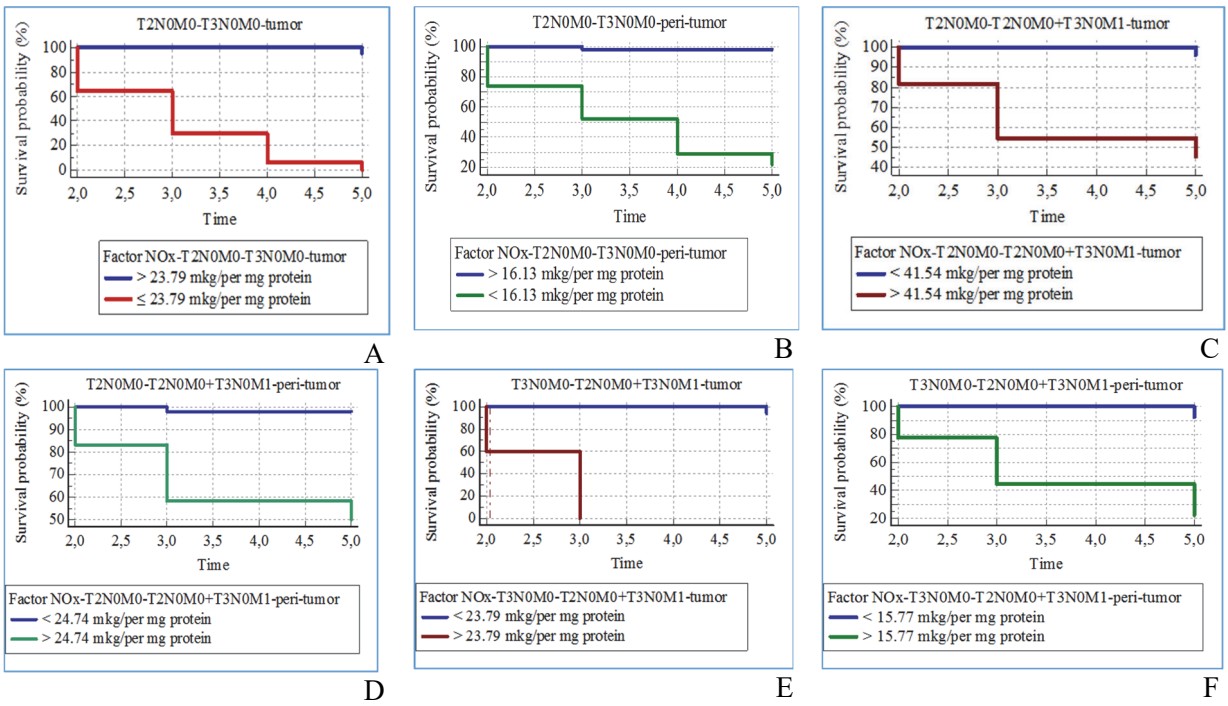

Fig. 2. Kaplan–Meier curves of the time of RCC stage progression and its metastasis for NO_x measured in patients with renal cell carcinoma: A – $T_2N_0M_0$ –$T_3N_0M_0$ – tumor tissue; B – $T_2N_0M_0$ –$T_3N_0M_0$ – peri-tumor tissue; C – $T_2N_0M_0$ –$T_2N_0M_1$+$T_3N_0M_1$ – tumor tissue; D – $T_2N_0M_0$ –$T_2N_0M_1$+$T_3N_0M_1$ – peri-tumor tissue; E – $T_3N_0M_0$ –$T_2N_0M_1$+$T_3N_0M_1$ – tumor tissue; F – $T_3N_0M_0$ – $T_2N_0M_1$+$T_3N_0M_1$ – peri-tumor tissue with different cut-off values of the of indexes investigated. p value by Long-rank test. Note: Here and in the following figures: p<0.0001 – calculated by univariate logistic regression analysis.

Next, we performed a Cox proportional hazards regression analyses of predictors for progression RCC stage and its metastasis. In univariate analysis, NOx was significantly associated with an increased risk of time progression of RCC stage and its metastasis in all observation groups (Table 2).

Table 2

Unadjusted and adjusted hazard ratios (HR) for respective univariate Cox proportional hazard models for progression and metastasis of renal cell carcinoma to the brain

Covariate	Harrell's C-index	b	SE	Wald	p	Exp(b) (Hazard Ratio)	95 % CI of Exp(b)
$T_2N_0M_0$ – tumor tissue	0.792	0.28	0.045	38.66	<0.0001	1.32	1.21–1.44
$T_2N_0M_0$ – peri-tumor tissue	0.482	-0.10	0.034	8.53	= 0.0035	0.904	0.85–0.97
$T_3N_0M_0$ – tumor tissue	0.729	-0.38	0.086	19.35	<0.0001	0.69	0.58–0.81
$T_3N_0M_0$ – peri-tumor tissue	0.762	-0.39	0.071	31.13	<0.0001	0.67	0.58–0.77
$T_2N_0M_0$ –$T_2N_0M_1$+ $T_3N_0M_1$ – tumor tissue	0.966	0.45	0.14	10.33	=0.0013	1.58	1.19–2.08
$T_2N_0M_0$ – $T_2N_0M_1$+$T_3N_0M_1$ – peri-tumor tissue	0.815	0.24	0.057	17.28	<0.0001	1.27	1.13–1.42
$T_3N_0M_0$ –$T_2N_0M_1$+$T_3N_0M_1$ – tumor tissue	0.850	0.45	0.24	3.55	=0.0595	1.56	0.98–2.49
$T_3N_0M_0$ –$T_2N_0M_1$+$T_3N_0M_1$ – peri-tumor tissue	0.639	0.17	0.056	8.66	=0.0033	1.18	1.06–1.32

In Cox regression (as in logistic regression), the null hypothesis (the predictor has no relationship with the dependent variable, i.e. its regression coefficient is not significantly different from zero) is tested using the Wald criterion. If the regression coefficient is significantly different from zero, then the independent variable makes a significant contribution to the predictive ability of the model, which is what our results show. Coefficient Exp(B), which shows how many times the risk of an outcome occurring changes if the value of the predictor changes by one. If the value of Exp(B) or the risk ratio is greater than one, then the positive value of this factor will be a factor associated with the risk of developing the outcome, if less than one, then it will be associated with an increase in survival time (that is, it will act as a protective factor with respect to the outcome). The Cox model shows that NO_x is a significant predictor of $T_2N_0M_0$ tumor staging in tumor tissue (p<0.0001), with Exp(b) (Hazard Ratio) =1.32; Harrell's C-index =0.729, as values close to 1, which indicate high performance of the Cox-model. In the $T_3N_0M_0$ – tumor tissue group, NO_x, as follows from the Cox model, acts as a protective factor that prevents tumor progression, since Exp(b) (Hazard Ratio) < 1 and is equal to 0.69; Harrell's C-index =0.792, as values close to 1, which indicate high performance of the Cox-model. The most adequate Cox-model (p=0.0013) (Harrell's C-index

=0.966) showed nitric oxide as a predictor of RCC brain metastasis in group $T_2N_0M_0$ –$T_2N_0M_1$+$T_3N_0M_1$ – tumor tissue (Exp(b) (Hazard Ratio) > 1 and is equal to 1.58). In the surrounding tissues in the $T_2N_0M_0$ – $T_2N_0M_1$+$T_3N_0M_1$ – peri-tumor tissue group, nitric oxide also acted as a predictor of RCC brain metastasis (Exp(b) (Hazard Ratio) > 1 and is equal to 1.27; Harrell's C-index =0.815; p<0.0001).

NO derived from cancer cells may promote cancer cell invasion, proliferation, and angiogenesis [4]. Our data showed that at stage $T_2N_0M_0$ RCC, there is hyperproduction of nitric oxide in tissues (cut-off values >29.89 μg/per mg protein) and, as follows from the Kaplan–Meier method and Cox analysis, indicates a predictive role of nitric oxide in the development of RCC and stage progression. The increase in NO level, which determines the dynamics of neoplastic development, may be related to the intensification of its synthesis [3, 6].

At stage $T_2N_0M_0$ of RCC, the role of nitric oxide in tumor progression changes: the low concentrations of NO_x (cut-off values of NOx – < 23.79 μg/per mg protein in tissue) reduces the risk of RCC progression on stage $T_3N_0M_0$ and as follows from the Cox model, acts as a protective factor that prevents tumor progression. The function of NO in carcinogenesis is multifaceted, as it can both promote and inhibit tumor progression depending on various conditions. In renal carcinoma cells, it has been shown that increased iNOS expression is characterized by antitumor properties, namely inhibition of their proliferation [3, 4].

But already at stage $T_2N_0M_1$ with metastasis to the brain, the content of nitric oxide in the tissues of the primary tumor in the kidneys increases sharply (cut-off values of NO_x – > 41.54 μg/per mg protein), which, according to the COX analysis, indicates a predictive role of nitric oxide in metastasis. The predictive role of nitric oxide in metastasis is preserved at stage $T_3N_0M_0$ of RCC, but at a lower level of nitric oxide production in the primary tumor (>23.79 μg/per mg protein).

Many authors have shown differences in the activity and expression of nitric oxide synthase between neoplastic tissue and normal body tissue [7, 9, 11, 12]. In our study, we also conducted a comparative analysis of nitric oxide content in renal cell carcinoma tumor tissue and peri-tumor tissue. The content of nitric oxide in the peri-tumor tissue of renal cell carcinoma was 1.5–2.5 times lower than in the tumor tissue in all observation groups. The dynamics of changes in the content of nitric oxide in the peri-tumor tissue were the same as in the tumor tissue in all observation groups. As follows from the Cox model, the low concentrations of NO_x in peri-tissue reduce the risk of RCC progression at stage $T_2N_0M_0$ and $T_3N_0M_0$ and act as a protective factor that prevents tumor progression. In metastasis, the diagnostic value of nitric oxide content in the peri-tumor tissue was also established. Moreover, nitric oxide in the peri-tumor tissue in metastasis manifests as a predictor of the process, as follows from the Cox model.

Conclusions

1. Nitric oxide exhibits a pro- or antitumorigenic effect depending on the level of NO and the stage of the tumor process.

2. The level of nitric oxide in tumor tissue was an independent predictor of RCC progression and its metastasis to the brain.

3. The content of nitric oxide in peri-tumor tissue has a high prognostic efficacy in RCC metastasis and as a protective factor that prevents tumor progression.

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ROUTINE BIOCHEMICAL DIAGNOSIS AND PROGNOSIS OF COVID-19 SEVERITY IN THE CONDITIONS OF THE REGIONAL HOSPITAL

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The purpose of the study was to evaluate routine laboratory indicators of biochemical blood analysis as markers of potential risk and factors of infection with COVID-19 and the severity of respiratory failure in both women and men. This single-center retrospective study was conducted with the participation of 57 COVID-19-positive individuals hospitalized at the Rivne Regional War Veterans Hospital in October-November 2021. Blood urea levels were higher in respiratory failure group 1 than in respiratory failure group 2 in both women and men. The dynamics of creatinine levels in women and men with COVID-19 in all groups were the same. as well as blood urea levels. The level of total protein in women with COVID-19 decreased with the progression of respiratory failure to stage II, while in men, on the contrary, it increased. Female COVID-19 patients with grade I respiratory failure have higher total protein than male grade I respiratory failure, and female patients with grade 2 respiratory failure have lower total protein levels in women than in men. The dynamics of the level of albumin in women and men with COVID-19 in all groups was the same as the level of total protein. Based on ROC analysis, Kaplan-Meier method; univariate analysis using the log-rank test and the Cox regression model found that blood urea and creatinine levels are biomarkers of the development of respiratory failure (I or II) within 30 days after hospitalization in patients with COVID-19, both women and men.

Key words: COVID-19, biochemical indicators, ROC analysis.

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РУТИННА БІОХІМІЧНА ДІАГНОСТИКА ТА ПРОГНОЗ ВАЖКОСТІ COVID-19 В УМОВАХ ОБЛАСНОЇ ЛІКАРНІ

Метою дослідження було оцінити рутинні лабораторні показники біохімічного аналізу крові як маркерів потенційного ризику та факторів інфікування COVID-19 та тяжкості дихальної недостатності як у жінок, так і у чоловіків. Це одноцентрове ретроспективне дослідження проводилося за участю 57 COVID-19-позитивних осіб, госпіталізованих до Рівненського обласного госпіталю ветеранів війни у жовтні-листопаді 2021 року. Рівні сечовини крові були вищими в групі дихальної недостатності 1, ніж у групі дихальної недостатності 2, як у жінок, так і у чоловіків. Динаміка рівня креатиніну у жінок і чоловіків із COVID-19 у всіх групах була такою ж, як і рівень сечовини крові. Рівень загального білка у жінок із COVID-19 знижувався з наростанням дихальної недостатності до II стадії, а у чоловіків, навпаки, зростав. Рівень загального білка у хворих на COVID-19 жінок з дихальною недостатністю I ступеня вищий, ніж у чоловіків з дихальною недостатністю I ступеня, а у пацієнтів з дихальною недостатністю 2 ступеня рівень загального білка у жінок нижчий, ніж у чоловіків. Динаміка рівня альбуміну у жінок і чоловіків з COVID-19 в усіх групах була такою ж, як і рівень загального білка. На підставі ROC-аналізу, метода Каплана-Мейєра; однофакторного аналізу з використанням логарифмічного рангового тесту та регресійної моделі Кокса встановлено, що рівні сечовини крові та креатиніну є біомаркерами розвитку дихальної недостатності (I або II) протягом 30 днів після госпіталізації у хворих на COVID-19, як жінок, так і чоловіків.

Ключові слова: COVID-19, біохімічні показники, ROC-аналіз.

The study is a fragment of the research project "Development of new highly economical methods of biomarker diagnostics and prediction of the course and complications of COVID-19 and community-acquired pneumonia in military personnel and civilians", state registration No. 0123U10124; "Development and implementation of innovative technologies for the diagnosis of oncogynecological and oncourological diseases based on liquid biopsy data of extracellular DNA and stem cells", state registration No. 0123U101248.

The scientific community is in urgent need for reliable biomarkers related to coronavirus disease 2019 (COVID-19) disease progression, in order to stratify high-risk patients. The rapid disease spread necessitates the immediate categorization of patients into risk groups following diagnosis, to ensure optimal resource allocation [11]. Timely detection of COVID-19 patients at high risk of death and supportive care