OXIDATIVE STRESS LEVEL AND ANTHROPOGENIC LOAD INDEX AS PROGNOSTIC CRITERIA OF DISEASE OUTCOME IN PATIENTS WITH OROPHARYNGEAL CANCER

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The aim of the investigation was to assess the role of ecological problems and oxidative homeostasis parameters in life expectancy prognosis of oncology patients in the territories with different anthropogenic load level.

Materials and Methods. A group of oncology patients included 80 males with oropharyngeal tumors (III–IV stage tumors of oral and oropharyngeal cavity), mean age being 58.63±0.68. According to clinical and morphological characteristics of the disease, and social and adaptation criteria all the patients were referred to a homogeneous group. A control group consisted of 10 men from different territories with no oncology in past history.

Results. Pro-, antioxidant system components were found to be the markers of latent inhomogeneity in a group of patient with III–IV stage oropharyngeal tumors. Oxidative stress level in blood of patients with similar clinicopathologic and anatomical characteristics appeared to be different. Regression analysis findings showed the life expectancy in patients with III–IV stage oropharyngeal tumors to be associated with anthropogenic load index of residence place, red blood cell superoxide dismutase activity, maximum chemiluminescence intensity, TNF-α concentration, ketondinitrophenylhydrazine level in induced oxidation and malondialdehyde concentration in blood plasma.

Conclusion. The study has demonstrated for the first time the relationship of environmental factor and oxidative homeostasis parameters, and their integrated effect on life expectancy of oncology patients.

Key words: oropharyngeal tumors; oxidative stress; protein oxidative modification.

Currently, the problems of oncologic ecology and personalized medicine are being discussed with interest [1, 2]. The first direction is associated with the study of carcinogen, anthropogenic load and lifestyle effect on malignant transformation, and the second — on the search of molecular and biochemical markers for disease outcome prognosis and trial of medical therapy for oncology patients.

Biomedicine is armed with extensive knowledge of the role of free-radical oxidation products as essential mediators of intercellular interactions realizing adaptive mechanisms in particular ecological conditions [3], as well as in etiopathogenesis of malignant tumors [4–6]. It has been reported that in uterine cancer formation there is the increase of lipid peroxidation products in patients' blood, and these products react with thiobarbituric acid [7], in cervical cancer — decreased content of reduced glutathione, vitamins A and E, as well as superoxide dismutase (SOD) activity [8]. Patients with non-metastatic breast cancer presenting clinically homogeneous group were found to have prognostic variability based on the interpretation of a complex of blood serum biochemical parameters including autocrine growth factor level, high-density lipoprotein cholesterol concentration, relative stearic acid content, total cholesterol concentration in blood serum [9]. Different survival rate after the treatment of breast cancer is explained by genetic polymorphism of antioxidant enzymes [10].

Our team has previously found [11] that among patients with locally advanced oropharyngeal squamous cell carcinoma referred to one prognostic group there is latent heterogeneity related to blood plasma chemiluminescence intensity and malondialdehyde (MDA) content. The necessity of further investigation of the problem motivated the study of free-radical activity of blood proteins and lipids in oncology patients living in the territories with different anthropogenic load, as well as defining the role of ecological problems of oxidative homeostasis parameters when prognosticating their survival.

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The aim of the investigation was to assess the role of ecological problems and oxidative homeostasis parameters in life expectancy prognosis of oncology patients in the territories with different anthropogenic load level.

Materials and Methods. A group of oncology patients included 80 males with oropharyngeal tumors (III–IV stage tumors of oral and oropharyngeal cavity) from different districts of Nizhny Novgorod region admitted to Nizhny Novgorod Regional Oncologic Dispensary. Morphological type of tumors was squamous and non-squamous cell cancer. Mean age was 58.63±0.68. The main criteria for group formation were the following: no distant metastases; satisfactory hematological and biochemical measurements; no specific treatment for this disease. All patients suffered from chronic smokers cough. 100% survey subjects were smokers at the moment of examination (Table 1). Thus, all patients could be referred to a homogeneous group by clinical and morphological characteristics of the disease and by social adaptive criteria. A follow-up period varied from 12 to 45 months, an average period — 21 months. Overall survival was chosen as the basic criterion of treatment efficiency.

Control group included 10 males from different districts of Nizhny Novgorod region, with no previous oncology history (mean age — 56.21±0.93 years) suffering from smokers cough, who sought medical advice in outpatient department of N.A. Semashko Nizhny Novgorod Regional Clinical Hospital (Russia) for chronic inflammatory diseases of upper respiratory tract. The volunteers were comparable with oncology patients by social adaptive criteria (See Table 1).

The study complies with the declaration of Helsinki (adopted in June, 1964 (Helsinki, Finland) and revised in October, 2000 (Edinburg, Scotland)) and was performed following approval by the ethic committee of Nizhny Novgorod State Medical Academy (Russia). Written informed consent was obtained from every patient.

Patients' ecological conditions were assessed by anthropogenic load index (Jan) developed in 2003 by Prof. D.B. Gelashvili et al. [12]. The index takes into consideration the main factors deforming the environment and characterizes the condition of socio-eco-economic systems. By this index the districts of Nizhny Novgorod region were divided into four clusters: A cluster — relatively satisfactory situation (Jan<0.6); B cluster — moderately tight ecological situation (0.6<Jan<1.1); C cluster — tense ecological situation (1.1<Jan<1.7); D cluster — critical ecological situation (Jan>1.7).

The districts the patients lived in we compared with clusters by anthropogenic load index. On the basis of ecological zoning the patients were divided into four groups: group 1 — relatively satisfactory environment (n=20), group 2 — moderately satisfactory environment (n=19), group 3 — tense (n=21), group 4 — critical ecological situation (n=20).

Table 1

Characteristics of the experimental subjects

Parameters	Oncology patients	Volunteers
Age, years	58.63±0.68	56.21±0.93
Smoking experience, years	41.15±3.10	37.00±1.82
Number of cigarettes per day, pcs	20–40	
Comorbidity	Smokers cough, inflammatory processes of upper respiratory tract	

Using chemiluminescence induced by hydrogen dioxide and iron sulfate in blood plasma we assessed integral indices of free-radical activity: Imax (мВ) — maximum chemiluminescence intensity of the study samples and 1/S index (relative units) inversely proportional to chemiluminescence light sum over 30 s of measurement — total antioxidant activity (TAA) [13]. The measurements were made on biochemiluminometer BCL 06-M (Russia) [14]. In blood plasma we determined the content of lipid peroxidation (LP) molecular products: diene conjugates (DC), triene conjugates (TC), MDA. Their concentration was expressed in optical density units in relation to common lipid amount. Common lipids were determined using a standard reagent kit Lachema (Czech Republic). In blood plasma we measured protein oxidative modification degree by the level of carbonyl derivatives based on the reaction between oxygenated aldehyde and ketonic amino acid protein residues and 2,4-dinitrophenylhydrazine forming aldehyde- and ketone-dinitrophenylhydrazones (at spontaneous and metal-induced oxidation, respectively: ADNPHsp, ADNPHind and KDNPHsp, KDNPHind) [15, 16]. Optic density of the formed compounds was recorded at wavelength of 270 and 363 nm, expressed in optic density units per 1 g protein. Total protein was determined using a reagent set by Vital diagnostic (Russia) spectrophotometrically (Genesis-10UV; Thermo Scientific, USA). SOD activity was determined by the reaction with nitroblue tetrazolium, catalase — by H_2O_2 degradation rate in neutral medium in red blood cells. The findings were presented as activity units per 1 mg of hemoglobin per min (activity units/mg Hb per minute) [17]. Human tumor necrosis factor α (TNF-α) in patients' blood plasma was determined using a reagent set alpha-TNF-IFA-BEST (Russia); the findings were recorded using a spectrophotometer TECAN (Austria). The results were expressed in pg/ml.

The results were statistically processed using ME and Statistica 8.0. programs. The data obtained were found to have normal distribution, therefore, the results were assessed using parametric statistical techniques. We used Newman–Keuls test to determine the differences between the groups. The results were presented in a form of diagrams showing mean values (M) of the indices under study and errors of mean (m), as well as standard deviation. The patients' survival was analyzed using Kaplan–Meier method. Regression analysis was used to search the combinations of independent characteristics affecting the disease prognosis. Consistency degree of the changes of the parameters under study was determined by Pearson correlation coefficient.

Results and Discussion. The patients composing a homogeneous group by clinical, morphological, anatomical, as well as social and adaptive criteria were found to have different degrees of oxidative stress intensity.

Taking into consideration a regulatory effect of redox-

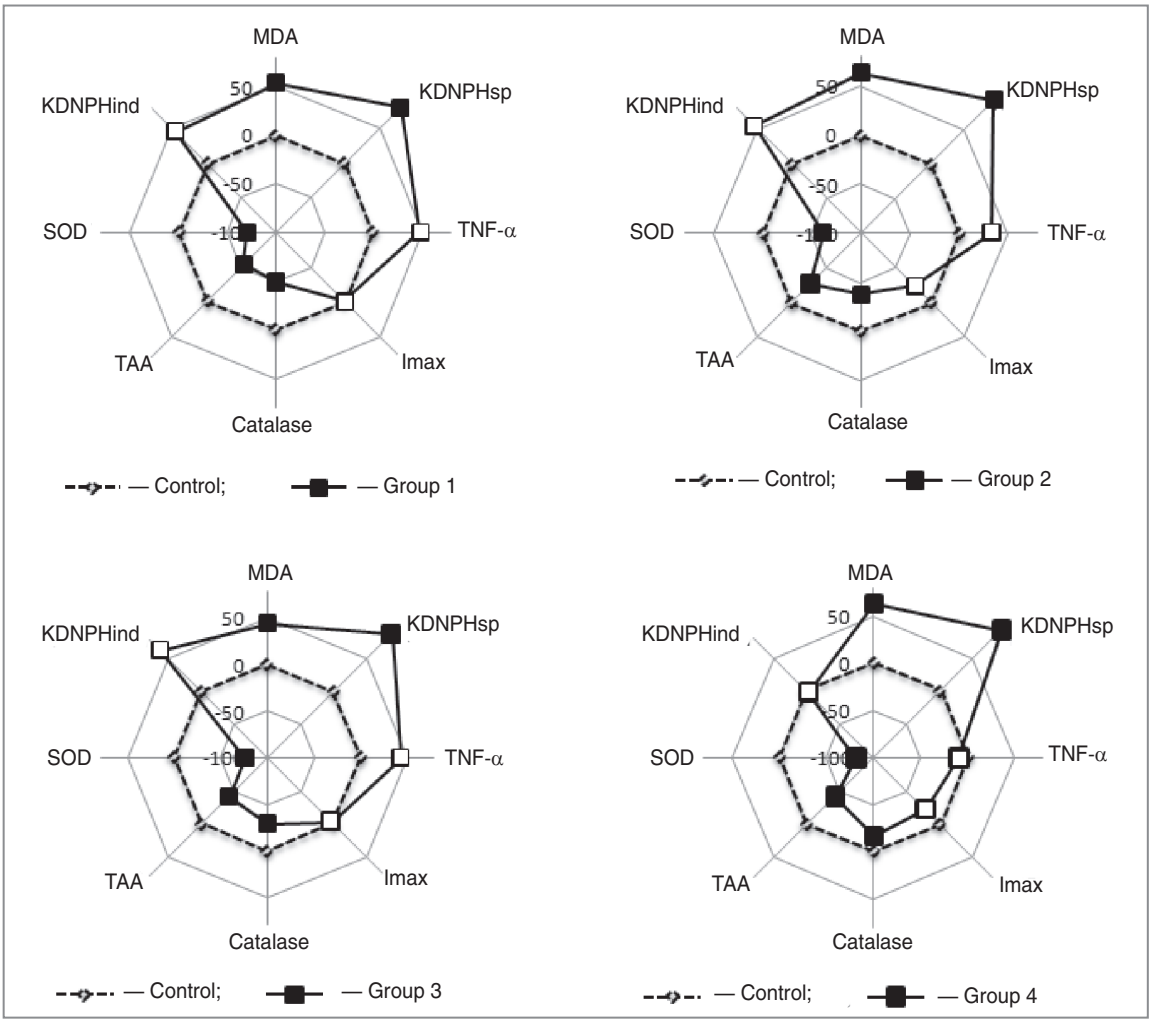

Fig. 1. Multiple-vector diagrams showing the parameters of pro-, antioxidant system imbalance in 4 patient groups

component in adaptation [3], we believe pro-, *antioxidant balance can be considered as one of the key elements joining such phenomena as stress*, the change of functional status of nervous and immune systems.

Figure 1 demonstrates multi-vector diagrams showing the disorders in pro-, antioxidant system, and specific parameters most clearly reflecting its imbalance in oncology patients living in districts with different anthropogenic load level. Vector ray length corresponds to control group variance percentage shown in the diagram as zero reference point.

The diagrams clearly demonstrate the shift of pro-, antioxidant equilibrium towards free-radical oxidation intensification. From the indices under study we distinguished those contributing to the most imbalances in pro-antioxidant profile of patients against the background of a heavy oxidative stress in oncology patients. These indices are Imax, TNF-α, MDA, KDNPH, SOD and catalase activity. The consistency between these parameters and anthropogenic load index is expressed in moderate correlations (Fig. 2).

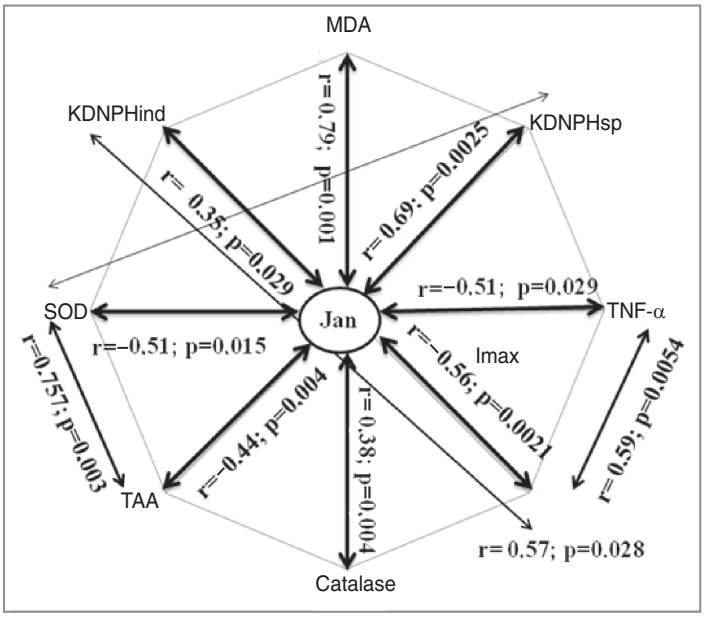

Fig. 2. Correlation relationship between the parameters of pro-, antioxidant homeostasis, TNF-α in patients and anthropogenic load index values in the districts of patients' living

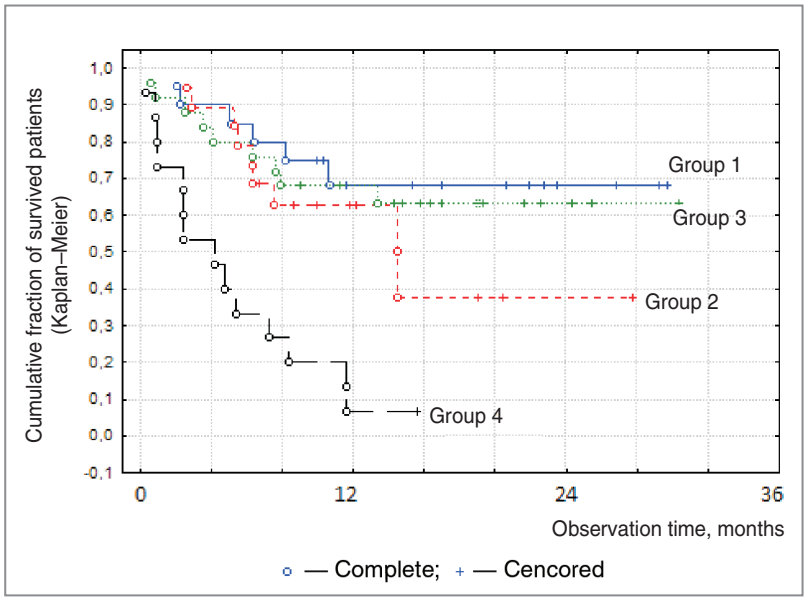

Fig. 3. Life expectancy of oncology patients living in districts with different anthropogenic load

Malignant neoplasm development results in marked disorders in patient's homeostasis systems. Prolonged living under environmental factors typical of a certain region is likely to form various disturbances of regulatory mechanisms. Chemoradiotherapy promotes exacerbation of these disturbances that can affect antitumor therapy efficiency and tolerance, as well as patients' life expectancy.

By applying a Kaplan–Meier method we plotted three-year survival curves depending an anthropogenic load level in different districts (Fig. 3), where Complete — complete observation with lethal outcome, and Censored — censored or incomplete observation. In case of a censored observation a patient had no lethal outcome and for three years was followed up for past malignancy at place of residence. Life expectancy comparison of patients' group showed statistically significant difference of group 4 from groups 1, 2 and 3, $p \leqslant 0.05$.

Survival analysis of patients showed the lethality rate for three years after chemoradiotherapy in group 1 was 30%, in group 2 — 48%, in group 3 — 36%, and in group 4 — 93%. Anthropogenic load index value of the patients' districts correlates with patients' life expectancy after chemoradiotherapy ($r = -0.89$; $p = 0.0021$).

Despite certain success of radiotherapy and chemotherapy, the management of oncology patients is empirical. The rise of new antitumor drugs in the past decade has not result in survival rate increase of oncology patients. S.A. Protsenko, MD, the head of the department of chemotherapy and innovative technologies, N.N. Petrov Research Institute of Oncology, says that currently there are no save prognostic factors of management efficiency and toxicity. Patients are treated according to standard treatment schedules based on group prognosis (age, stage, tumor histology). And survival rate of III–IV stage tumor patients is 60%. Therefore, the problem of individual management and search of prognostic factors of its efficiency is urgent [2].

A prolonged oxidative stress reduces spare and adaptive capacities. Chemoradiotherapy by inducing increased radical formation increases oxidative load due to a tumor process.

We used multi-factorial regression analysis to determine the dependence of life expectancy of oncology patients on oxidative homeostasis state. The method application conditions were observed: all test parameters were quantitative and normally distributed, no strong linear correlations being between them.

The calculations showed that anthropogenic load index, maximum chemiluminescence intensity, MDA concentration, KDNPHind, TNF-α level in blood plasma and SOD activity in red blood cells of patients are significant independent unfavorable prognostic factors in oropharyngeal tumors. We calculated Beta coefficients showing weight value of the factors affecting life expectancy in patients under study (Table 2).

Standardized rates of multiple regression show how mean dependent variable (life expectancy) would change if the value of the corresponding independent variable increased by a standard deviation, while other variable remained unchanged [18].

The regression analysis resulted in establishing the following equation of the relationship between life expectancy (LE) of oncology patients and prognostic parameters under study: LE=1.420–0.090 Jan– 0.004 SOD– 4.058 KDNPHind +0.328 Imax –0.056 MDA+0.081 TNF-α.

All beta coefficients are significant at 5% level ($p<0.05$). Zero hypothesis probability (p) is significantly lower than 0.05 indicting total significance of regression equation. This equation explains 77.2% (RI=0.772) of regressand variation.

Thus, Beta coefficient values enable to compare a relative contribution of each factor to life expectancy prognosis.

Determination coefficient value (RI=0.772) indicates a good approximation of a regression line to observable data and prognosis possibility.

Anthropogenic load index studied in this work and showing ecological problems of the patients' districts [12]

Table 2

Weight value of factors affecting the disease prognosis

Factors (n=80)	Beta	p
Test reliability	—	0.04864
Jan	–0.3238	0.00205
KDNPHind	–0.1816	0.00165
MDA	–0.2318	0.00005
SOD	0.1767	0.00020
Imax	0.2215	0.00015
TNF-α	0.2124	0.00000
R=0.85677 — multiple correlation coefficient RI=0.772704 — determination coefficient		

is a calculated parameter for 47 administrative districts of Nizhny Novgorod region within a narrow temporal course (1997–2000). Such narrow specificity of a parameter for different regions does not enable to use Jan for other territories and temporal courses and requires individual calculation in each case. Therefore, we carried out regression analysis using the data on air condition and utility and drinking water supply sources in districts of patients' living consistent with the year of patients' admission as a quantitative alternative to anthropogenic load index (2005–2009) (Table 3). Such data are presented in a state report "On sanitary and epidemiologic situation in Nizhny Novgorod region in 2010" [19].

Table 3

Weight value of factors affecting the disease prognosis using the data on air condition and utility and drinking water

Factors (n=80)	Beta	p
Test accuracy	—	0.04877
Community air (% of samples with increased MAC)	–0.2921	0.00245
Utility and drinking water supply sources (% of samples with increased MAC)	–0.2433	0.00125
KDNPHind	–0.1715	0.00114
MDA	–0.2231	0.00004
SOD	0.1867	0.00016
Imax	0.2312	0.00022
ΦНО-α	0.2212	0.00023
R=0.86233 — multiple correlation coefficient RI=0.78776 — determination coefficient		

Here: MAC — maximum allowable concentration.

Regression analysis results showed that the substitution of anthropogenic load index for water and air pollution index in the districts of patients' living has no effect on significance level of Beta coefficients for both the parameters characterizing ecological problems and pro-, antioxidant system activity indices. The values of determination and multiple correlation coefficients do not change within the range of tenths either (Table 3). It proves the relationship of life expectancy of patients with oropharyngeal tumors and the extent of ecological problem in districts these patients live.

Regression analysis, one of statistical modeling method, enabled to calculate prognostic model based on reliable factors revealed.

The results of comparison between actual life expectancy and estimated calculated by weight coefficients of prognosis factors under study is of interest (Fig. 4).

So, if estimated coefficient was over 1.5 units — all patients survived three-year follow-up period; coefficient decrease under 1.5 units before treatment was associated with survival rate under 12 months.

In case anthropogenic load index is excluded from the analysis, an error increases up to 15.6%, and if MDA, SOD, ADNPHind are ignored, an error varies from 6.1 to 7.3%. Thus, removal of these factors from regression analysis results in significant predictive error increase. However, Imax and TNF-α are interchangeable parameters, and in case one of them is excluded, the model remains functioning.

Thus, only integrated use of pro-, antioxidant homeostasis parameters with due account for anthropogenic load index of districts where oncology patients with oropharyngeal tumors live enable to prognosticate individual treatment results (Fig. 5) and, thereby, optimize the management.

Conclusion. Pro-antioxidant system components were found to be the markers of latent inhomogeneity in a group of patient with III–IV stage oropharyngeal tumors. Oxidative stress level in blood of patients with similar clinicopathologic and anatomical characteristics appeared to be different and

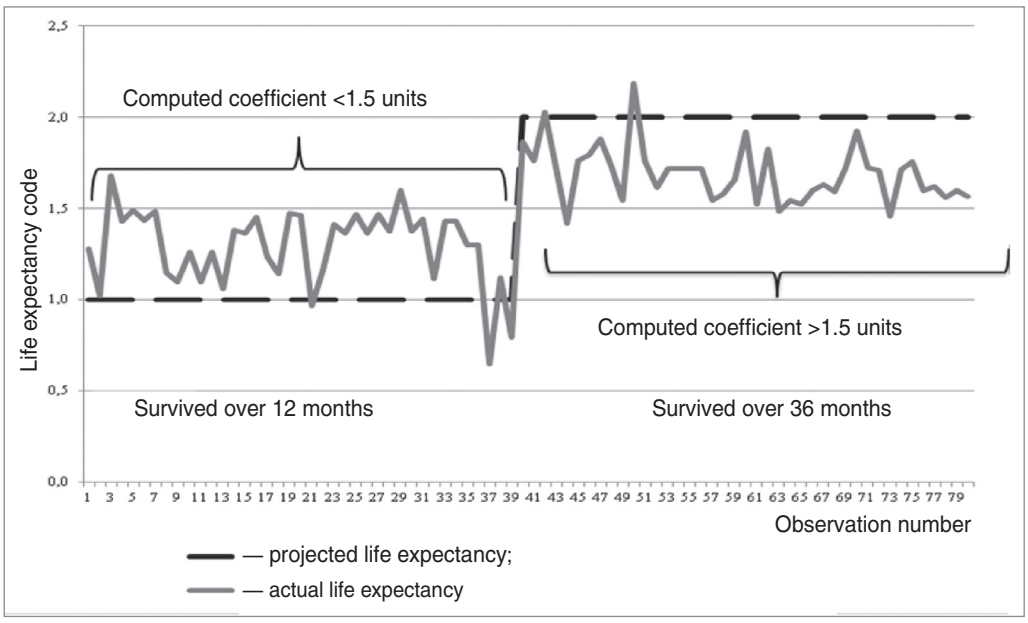

Fig. 4. Actual life expectancy and probability of survival of oncology patients after treatment

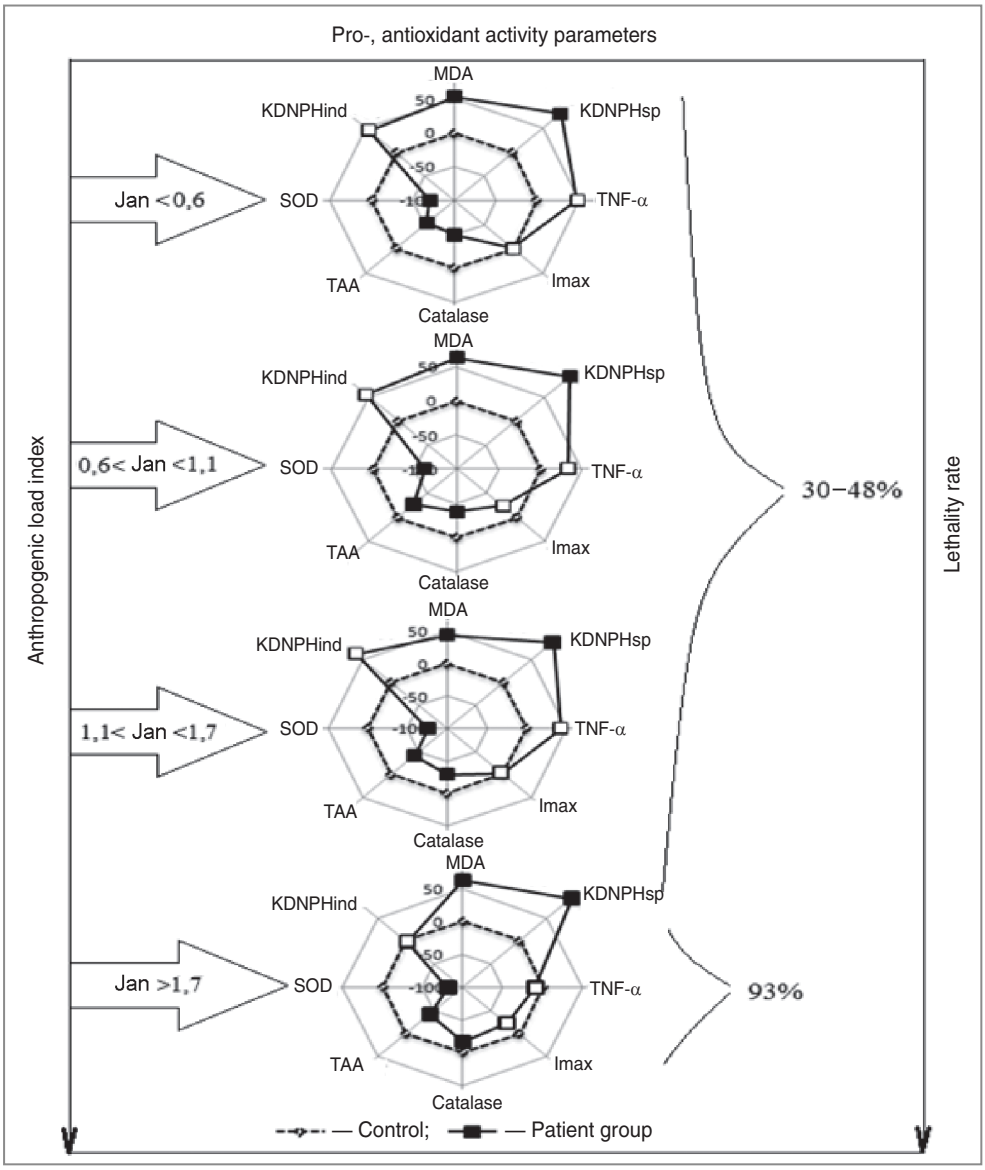

Fig. 5. The dependence of lethality of oncology patients on anthropogenic load and oxidative stress degree

associated with oxidative homeostasis under conditions of districts with different anthropogenic load. The study has demonstrated for the first time the relationship of environmental factor and oxidative homeostasis parameters, and their integrated effect on life expectancy of oncology patients.

Parameters of pro-, antioxidant balance, as well as anthropogenic load index of districts are the markers of individual life expectancy prognosis of patients with oropharyngeal tumors. Therefore, the developed prognostic model enables to improve the survival assessment quality after standard chemoradiotherapy and can be used to optimize management in clinical oncology (e.g., termination of known inefficient standard scheme of chemoradiotherapy, and standard scheme correction).

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Conflict of Interests. The authors have no conflict of interest to disclose.

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