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ORIGINAL RESEARCH

A.S. Goncharova, G.Yu. Egorov, E.N. Kolesnikov, A.Yu. Maksimov, M.V. Romanova, A.B. Sagakyants, E.S. Bondarenko

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УДК 57.084.1

STUDY OF XAV-939 EFFECT ON CANCER STEM CELLS OF HUMAN COLORECTAL CANCER IN HETEROTOPIC MODELS IN VIVOABSTRACT

The purpose of this study was to investigate the numbers of cells with CSC markers in samples of human colorectal cancer (CRC) xenografts isolated in animals with tumors after exposure to the small molecule XAV-939, 5-fluorouracil, and to the combination of XAV-939 with 5-fluorouracil. The model was created in Balb/c Nude mice. Tumor fragments of human CRC were transplanted into adipose tissue of animals under the skin of the right thigh. The fourth xenograft generation was used for the study. Animals were divided into groups receiving XAV-939, 5-fluorouricil, their combination, and the control group. Tumor nodes were measured twice a week; at the end of the experiment, the number of cells with the CSC phenotype was determined in the tumor material of the animals. By the end of the experiment, tumor nodules in the group of animals receiving the combination of drugs were significantly smaller than in the control group. The highest levels of cells with phenotypic signs of CSCs were characteristic of samples from the control group, and for samples obtained from the group receiving 5-fluorouracil as monotherapy. Decreased numbers of these cells were observed in groups receiving XAV-939 and the combination of XAV-939 and 5-fluorouracil.

Keywords: colorectal cancer, CRC, cancer stem cells, CD44, CD133.

Background. Today, colorectal cancer (CRC) is among the most common malignancies and one of the most frequent causes of cancer-related deaths. Poor treatment outcomes are believed to be associated with a population of cancer stem cells (CSCs) characterized by a low proliferation rate and the ability for multipotent differentiation, as well as resistance to various antitumor effects [1]. This cell population is characterized by various specific markers with biological functions contributing to the formation of features characteristic of CSCs [2]. Proteins such as CD44 and CD133 are often used for CSC identification, and as prognostic markers of the CRC course [6].

The resistance of long-lived, low-proliferative CSCs to cytotoxic effects is also important, which indicates the need for the development and use of targeted drugs aimed at CSCs and disruption of their signaling, for example, the Wnt pathway, which is important in maintaining the CSC pool. In addition, a number of studies showed an association between its activation and the progression of some malignant tumors, including CRC; so, the inhibition of the Wnt signaling pathway is considered as a promising therapeutic direction in CRC [5; 11].

In recent years, researchers have actively discussed specific therapy aimed at CSC-associated signaling in combination with traditional chemotherapeutic regimens to eliminate both differentiated cancer cells and the CSC population in order to prevent possible recurrence [8]. At the moment, several dozens of different compounds, including small molecules, are at various stages of preclinical and clinical studies [2]. The small molecule XAV-939 is considered as one of Wnt signaling inhibitors [7]. Studies on its possible mechanisms of action reported that SW480 CRC cells treated with XAV-939 demonstrated stabilization of the protein complex, including the proteins Axin, APC, GSK-3b, Ck1a, and consequently blockade of abnormally activated Wnt signaling pathway [3]. In addition, studies on the H446 cell culture showed that, in comparison with other inhibitors of the Wnt pathway, XAV-939 exhibits strong specificity for this signaling without affecting other molecular pathways [4]. However, the mentioned studies were performed on cell cultures, and therefore it is a necessary task to study the effect of the Wnt signaling pathway inhibitor XAV-939 on CSCs in *in vivo* models obtained by xenotransplantation of clinical specimens, which are largely able to reflect the complex biology and behavior of human tumors in response to a therapeutic strategy.

In this regard, the aim of this study was to investigate the numbers of cells with CSC markers in samples of human CRC xenografts isolated in animals with tumors after exposure to the small molecule XAV-939, 5-fluorouracil, and to the combination of XAV-939 with 5-fluorouracil.

Material and methods. *Tumor material.* Subcutaneous xenografts were created using a transplantable strain of human CRC obtained at the National Medical Research Centre for Oncology. The primary tumor material was obtained from a patient diagnosed with T4N1M0 splenic flexure cancer during surgical treatment (resection of the transverse colon). The patient provided written consent to the transfer of biological material.

Recipient animals. The experiment was performed in 20 immunodeficient Balb/c Nude mice (females). The animals were obtained from the SPF-vivarium of the Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences (Novosibirsk). The mice were 5–6 weeks old, with an average weight of 22 g. The animals were kept in the SPF vivarium of the Experimental Laboratory Center at the National Medical Research Centre for Oncology. All manipulations during the experiment were in compliance with the ethical principles

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established by the European Convention for the Protection of Vertebrate Animals used for experiments or for other scientific purposes (ETSN 123, Strasbourg, March 18, 1986). The study protocol was approved by the local ethics committee of the National Medical Research Centre for Oncology.

Creation of a tumor model. The fourth generation of subcutaneous xenografts was used for this study: fragments of the third generation tumor xenograft were implanted under the skin of the right thigh in recipient mice. Animals were anesthetized using injection anesthesia with the veterinary drugs Xila 20 mg/kg and Zoletil-100 50 mg/kg.

Investigated drugs, doses, methods and modes of their administration, distribution of animals into groups. The studied drug XAV-939 (Sigma-Aldrich) – orally at a dose of 25 mg/kg, 5-fluorouracil – intraperitoneally at a dose of 25 mg/kg, 0.9% NaCl - intraperitoneally, sterile water – orally; administered twice a week. The control group received carrier substances in the same mode: 0.9% NaCl solution, sterile water.

Tumor-bearing animals were divided into 4 groups, each of which included 5 animals: group 1 – 5-fluorouracil (5-Fu); group 2 – XAV-939; group 3 – XAV-939+5-fluorouracil (XAV-939+5-Fu); group 4 – control.

Animals were divided into groups depending on the sizes of tumor nodes at the time of the beginning of the drug administration - 50±20% mm^3. When randomizing animals, we were guided by the minimum scatter of the average values of the volume of tumor nodes in groups.

Analysis of the growth of human CRC xenografts. Tumor nodules were measured twice a week, their sizes were calculated by the formula V=a×b×c×π/6, where V is the tumor volume (mm^3), and a, b, c are linear measurements of the ellipsoid in three planes (mm).

CSC determination by flow cytometry. The numbers of cells with CSC markers CD45-44+, CD45-133+, CD45-44+133+ were determined in samples of human CRC xenografts isolated from tumor-bearing animals using the FacsCanto II flow cytometer (Becton Dickinson, USA) with two lasers with fluorophore excitation wavelengths of 488 nm and 633 nm, with the ability to use up to 6 monoclonal antibodies in one tube simultaneously. A set of monoclonal antibodies was used for this study: CD44 FITC (BD Pharmingen, USA)/CD133 APC (BD, USA)/CD45 APC-Cy7 (BD, USA).

Statistical analysis. The data were analyzed using the Statistica and Excel program package. The Shapiro–Wilk test was used to verify the hypothesis of the normal distribution, and the differences between the groups were evaluated by the Mann-Whitney non-parametric-criterion.

Results and discussion. The experiment revealed the dynamics of growth of xenotransplants in three experimental groups and the controls. The volumes of the tumors in the group receiving 5-fluoruracyl and in the group with XAV-939 did not significantly differ from the control group. The volumes of tumor nodes in the experimental group with XAV-939+5-Fu were 335.2 mm^3, which was statistically significantly lower than the volumes of tumors in the control group – 609.3 mm^3 ($p<0.05$). The data demonstrating the dynamics of the growth of tumor nodes are presented in Figure 1.

Note: * - differences are statistically significant by the Mann-Whitney test ($p<0.05$).

The level of cells with the CD45-44+ phenotype in the control group was 7.9% [7.7%; 8.1%], and 8.1% [7.9%; 8.5%] in the group with 5-Fu. The percentage of cells carrying the CD44 molecule in the group with XAV-939 amounted to 3.7% [3.4%; 3.9%], which was statistically lower than the number of cells of this phenotype in the samples of the control group ($p <0.05$). The content of cells with the CD45-44+phenotype in the group with XAV-939+5-Fu amounted to 1.9% [1.6%; 2.1%], and it was statistically lower, both as in the group with XAV-939 monotherapy, than the number of CD45-44+ cells in the samples of the control group ($p<0.05$).

Experimental data on the numbers of cells with the CD45-44+ phenotype are presented in Figure 2.

Note: * - differences are statistically significant by the Mann-Whitney test ($p<0.05$).

An analysis of cells with the CD45-133+ phenotype demonstrated that their content in the control group was 8.1% [7.9%; 8.4%]; in the group with 5-Fu – 6.4% [6.3%; 7.7%]; in the group with

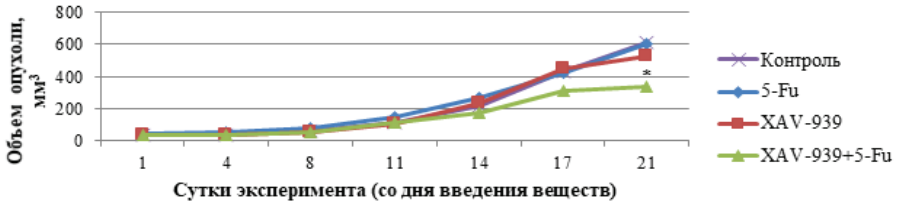

Fig. 1. The dynamics of tumor growth in experimental and control groups.

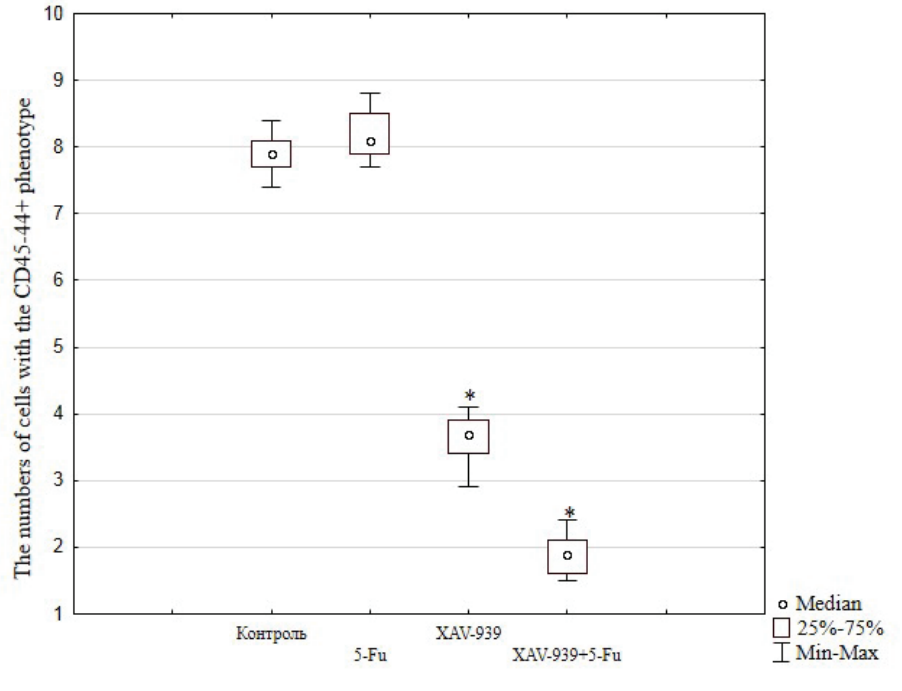

Fig. 2. The numbers of cells with the CD45-44+ phenotype in experimental and control groups.

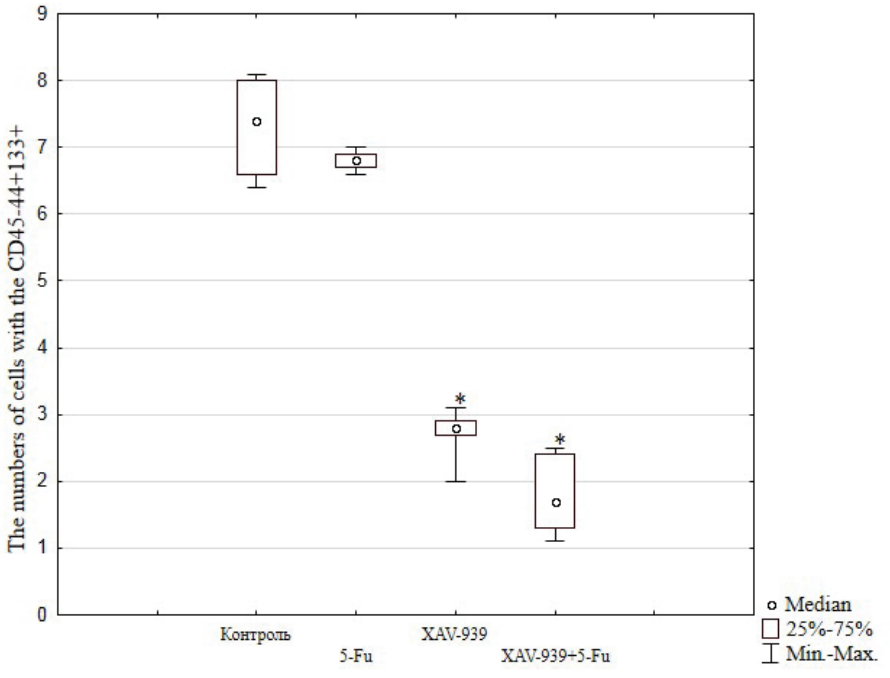

Fig. 3. The numbers of cells with the CD45-133+ phenotype in experimental and control groups.

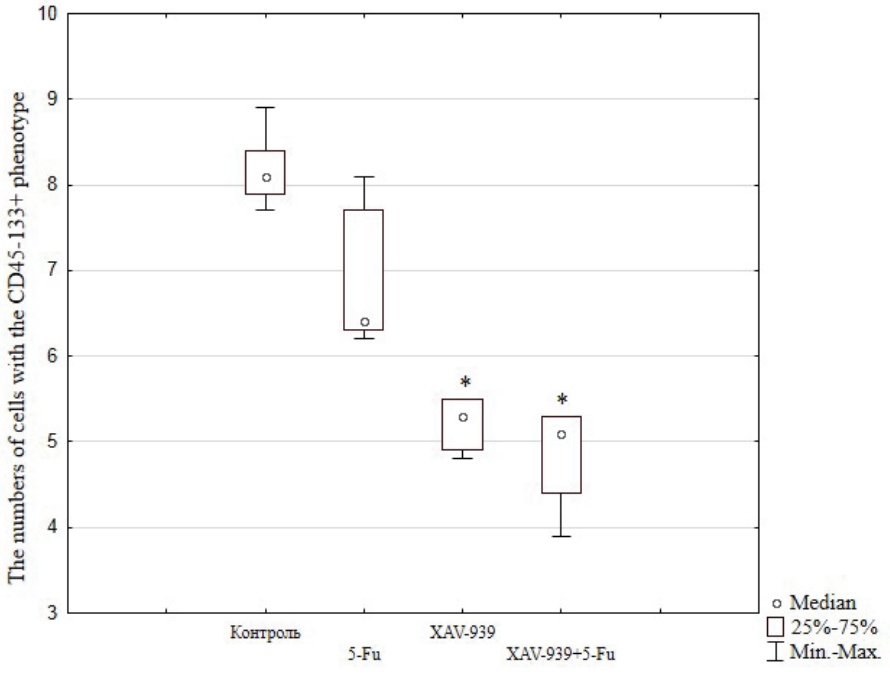

Fig. 4. The numbers of cells with the CD45-44+133+ phenotype in experimental and control groups.

XAV-939 – 5.3% [4.9%; 5.5%]; in the group with XAV-939+5-Fu – 5.1% [4.4%; 5.3%]. Both in the group with XAV-939 monotherapy and in the group with XAV-939+5-Fu, the number of CD45-133+ cells was statistically significantly lower than in the control group (p=0.0122).

The numbers of cells with the CD45-133+ phenotype in experimental groups are presented in Figure 3.

Note: * - differences are statistically significant by the Mann-Whitney test (p<0.05).

The number of cells with the CD45-44+133+ phenotype in the control group was 7.4% [6.6%; 8%]; in the group with 5-Fu – 6.8% [6.7%; 6.9%]; in the group with XAV-939 – 2.8% [2.7%; 2.9%], and in the group with XAV-939+5-Fu – 1.7% [1.3%; 2.4%], which was was statistically significantly lower than in the control group (p<0.05).

The data on the numbers of cells with the CD45-44+133+ phenotype are presented in Figure 4.

Note: * - differences are statistically significant by the Mann-Whitney test (p<0.05).

An analysis of the scientific literature of recent years demonstrates a significantly increasing interest in CSCs, their biological functions and the role in carcinogenesis [7, 10, 11]. A number of studies on the prognostic potential of the CD44 and CD133 CSC markers in CRR reported that higher expression of these proteins is associated with a high risk of tumor recurrence and metastasis [9, 10]. According to Spelt et al. (2018), patients with tumors characterized by a high content of cells with the CD45-133+ phenotype had poor survival, compared to patients without this marker [9]. On the contrary, the CD44 knockdown suppressed invasion, proliferation and migration of tumor cells. In addition, the study on SW620 CRR cell culture showed that the expression of the CD44+ and CD133+ markers was associated with the phenomenon of drug resistance to cytostatics [3].

Our study revealed that the highest levels of cells with phenotypic signs of CSCs were characteristic of samples from the control group and from the group receiving 5-fluorouracil as monotherapy, which, together with the absence of a significant decrease in the volume of tumor nodes in this group, can be considered as a sign of resistance to this drug, which is consistent with the trends described in the literature that characterize CSCs and their dynamics. Decreased numbers of cells expressing CSC markers in groups receiving XAV-939 and the combination of XAV-939 and 5-fluorouracil can be considered as confirmation of the Wnt signal path inhibition, which might help overcome the resistance of tumor cells to standard chemotherapy, and this was confirmed by a decrease in the volume of tumor nodes in the group of animals receiving the combination of drugs.

Conclusions. This study demonstrated the reduction in the number of cells with the CSC phenotypes CD45-44+, CD45-133+, CD45-44+133+ in the samples of xenotransplants in animal groups receiving the XAV-939 tested substance, as well as a combination of XAV-939 and 5-fluorurationils, which can be considered a sign of the Wnt signal path inhibition. The data characterize XAV-939 as a promising substance for further studies on its effectiveness in combination with standard therapy against CSCs in CRR.

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O.O. Alyoshina, I.V. Averyanova

PHYSICAL DEVELOPMENT OF WOMEN IN MAGADAN REGION IN AGE AND ETHNIC ASPECTS

Anthropometry is known to be a qualitative measure that reflects the level of health state of a population. For the first time, an assessment study on physical development of women in age and ethnic aspects was conducted in the territory of Magadan Region. This research aimed at studying age dynamics in basic anthropometric indicators among women of different ethnicity from different regions of residence, at mature and old ages.

Materials and methods. In the year of 2022 we analyzed data of medical records of one thousand and sixty-four women from Severo-Evensk District and the city of Magadan. The following anthropometric indicators were included in the general database: Body Length, Body Mass, and Waist Circumference, with further calculating the Body Mass Index. The subjective sample was divided into six groups according to the ethnicity, as well as based on the age. The main indicators of physical development were evaluated by standard research methods.

Results. From the obtained data we could see a reduction in subjective Body Length variables with those of Body Mass, Body Mass Index, and Waist Circumference growing with increasing age, from the middle age (maturity) to the elderly period of ontogenesis. In the settlement of Evensk, the percentage of middle-aged women diagnosed with obesity was 40% among Aboriginals and 24% among Caucasians growing up to 68% and 47%, respectively, in old women. The obesity incidence among women of Magadan made up 25% and 45%, respectively.

Conclusion. The observed characteristics indicate unhealthy tendencies, which the increased Body Mass Index suggests owing to shorter Body Length and significantly bigger Body Mass indices progressing from mature to old ages. To a greater extent, obesity is experienced by women of the Aboriginal population of Magadan Region. All the examinees show Waist Circumference measurements that excess the normal standards, which indicate the development of such an unfavorable factor as abdominal obesity.

The age- and ethnicity-based somatometric status was also specified for Magadan Region women, which should be considered when forming the region-related standards of physical development.

Keywords: anthropometric indicators, ethnicity, human population, women of the Magadan region, obesity.

Introduction. According to the WHO, obesity is recognized as the epidemic of the XXI century due to the constant spread of this disease [27]. For some experts' estimates, the incidence of obesity among the female population is expected to 50% increase by the year of 2025 [24]. To reveal abnormalities in physical development, anthropometric indicators have been used in clinical healthcare practice for many decades as a way to identify some diseases, classify impairments and evaluate further treatment [29]. They are also integrative characteristics that ascertain the quality of life, respond to environmental, social, and hygienic changes [18]. The anthropometric method is inexpensive, non-invasive and universally applicable for assessing the size, proportions and composition of the human body – all these vary throughout human life [31, 5].

The growth of the elderly population is an inevitable consequence of social and economic development and improvement of medical technologies. [22]. This group is characterized by a higher risk of many diseases including cardiometabolic ones [14, 33]. Being a way to detect overweight and obesity, the careful monitoring of physical development and BMI indicators throughout life also appears to be a predictor of a number of diseases including cardiovascular and metabolic diseases in old age [28].

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The conditions of the North bring unfavorable climatic factors as an additional negative impact on the human body [3]. Because of the worse functional abilities as well as limited adaptive capabilities, the elderly people are the most vulnerable to the climatic and geographical factors of the North [2]. At the same time, the study of somatometric picture needs to be conducted among both newcoming and indigenous populations, despite the fact that the latter perceive the sub-extreme and extreme conditions of the northern regions as quite adequate [6]. Monitoring of physical development of the Aboriginal population of Russia's north is still relevant since during the first decade of the XXI century they experienced the growth in diseases of civilization including cardiovascular and endocrine pathologies [4].

Thus, the purpose of this research was to analyze somatometric pictures of mature and elderly women residing in Magadan Region who differ by their ethnicity.

Materials and Methods. Outpatient records and the results of medical examination of residents of Severo-Evensk District were studied in the survey. Similar work was carried out in the city of Magadan. The data were taken from the Severo-Evensk District Hospital and Department No. 1 of Magadan Regional State Healthcare Institution of the City Polyclinic.

In the course of the work, we collected basic somatometric data: BL (cm), BM (kg), and WC (cm). Then, the Body Mass Index (BMI) was calculated using the formula BMI = BM/BL^2, where BL is the length of the body in meters. The interpretation of the Caucasian women's BMI was carried out in accordance with the recommendations of the World Health Organization (WHO) [34]. The Aboriginal women's variables were processed using the interpretation proposed for the Asian population [21].

In total, 1064 mature and elderly women's medical records were analyzed. The age classification of women was carried out according to the periodization adopted at the VII All-Union Conference on Problems of Age Morphology, Physiology and Biochemistry in 1965 [8]. The data obtained were divided into six groups according to ethnic and age criteria: Groups 1 and 2 were made up of the female Aboriginal population of mature and elderly ages from the settlement of Evensk. Groups 3 and 4 were represented by the female Caucasian population of mature and elderly ages from the settlement of Evensk. Groups 5 and 6 were the Caucasian females of mature and elderly ages from the city of Magadan. The Aboriginal sample was represented by two peoples: Evens and Koryaks who belong to the Mongoloid anthropological type [15]. The Caucasian sample was represented by those born in Magadan Region in the 1st-2nd generations, mainly the Slavs.

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The results were processed using the Statistica 7.0 application software package. Verification of the normality of the distribution of the measured variables was carried out based on the Shapiro-Wilk test. Statistical analysis of the data obtained was made using the parametric Student t-test for independent samples. The results presented were an average value and its error (M±m). In the work, the critical significance level (p) was assumed to be 0.05.

Results and Discussion. Table 1 shows basic physical indicators of the females of Aboriginal and Caucasian populations of Magadan Region. Table 2 demonstrates statistically significant differences among the studied characteristics. It should be noted that Body Length is the main indicator for assessing physical development which is genetically deter-

Table 1

Anthropometric variables in women of different age and ethnicity, (M±m)

Indicator	Aboriginal Population, Evensk		Caucasian Population, Evensk		Caucasian Population, Magadan	
	Mature Age (1)	Old Age (2)	Mature Age (3)	Old Age (4)	Mature Age (5)	Old Age (6)
Mature age, yrs	41.3±1.3	62.3±1.0	39.5±1.1	62.8±1.2	43.4±0.4	67.3±0.4
Body Length, cm	157.8±1.1	154.5±1.2	164.3±1.1	160.3±1.3	163.3±0.3	160.3±0.4
Body Mass, kg	62.1±1.2	65.8±1.4	71.7±1.2	74.9±1.1	71.0±0.6	74.5±0.9
Waist Circumference, cm	81.4±1.6	87.5±2.6	89.3±1.8	89.7±2.5	82.6±1.7	87.9±0.8
BMI, kg/m^2	25.0±0.6	27.6±1.0	26.±1.0	29.7±1.1	27.3±0.5	29.4±0.4
N	52	22	34	23	702	226

Table 2

Statistically significant differences between the study groups

Indicator	Groups								
	1-2	3-4	5-6	1-3	1-5	3-5	2-4	2-6	4-6
Body Length. cm	p<0.05	p<0.05	p<0.001	p<0.001	p<0.001	0.38	p<0.01	p<0.001	p=1
Body Mass. kg	p<0.05	p<0.05	p<0.001	p<0.001	p<0.001	0.60	p<0.01	p<0.001	p=0.78
Waist Circumference. cm	p<0.05	p=0.89	p<0.001	p<0.01	p=0.61	p<0.01	p=0.54	p=0.88	p=0.49
BMI. kg/m^2	p<0.05	p<0.05	p<0.001	p=0.39	p=0.99	p=0.24	p=0.16	p=0.09	p=0.79

mined [16] and tends to change with age [30]. The study revealed a pronounced and statistically significant shortening in the Body Length with age among the examined females of both Aboriginal and Caucasian populations from both Evensk and Magadan. Statistically significantly lowest Body Length variables in mature and elderly ages were recorded in the Aboriginal women of Evensk in comparison with the Caucasian subjects as characteristic of the indigenous small-numbered peoples of the North [1, 12]. At the same time, the Caucasian samples did not differ from each other in this indicator.

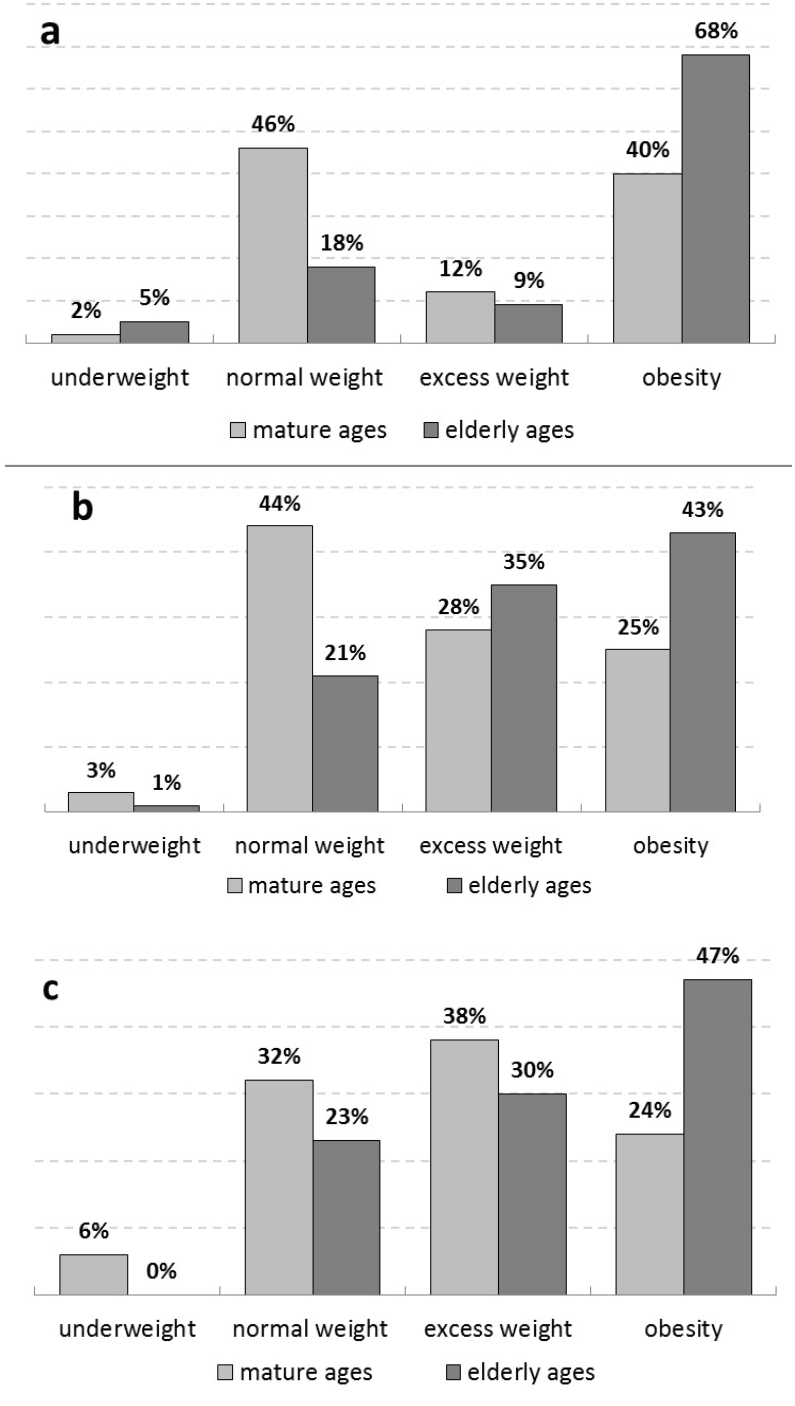
Body Mass as an indicator of the morphological state of the body [16] reflects a person's health state and their lifestyle [25]. In the course of the work, we could see a statistically significant increase in this indicator with age in each group. The Aboriginal subjects exhibited significantly lower values of both Body Mass and Body Length indicators in comparison with those of the two Caucasian groups. It is worth noting that none of the Caucasian women of Evensk and Magadan showed any differences in this indicator in mature or elderly age.

Of greatest interest is the BMI indicator since its growth accelerates the risk of chronic non-communicable diseases and health deterioration [17] and it is considered as a predictor of cardiovascular and metabolic diseases [19, 32]. This index is used to identify excess weight and obesity which denote the body overweight resulting from an imbalance in the three energy components: food consumption, energy expenditure and energy storage [23]. During the analysis of the average BMI values, the Aboriginal women of the settlement of Evensk, both mature and old examinees, were diagnosed with obesity. The Caucasian women of Magadan Region showed excess weight. We found statistically significant growth in this indicator in each group with age owing to the pronounced increase in the Body Mass with the simultaneous shortening in the Body Length.

For a more detailed study of the Body Mass Index, we differentiate the analyzed groups by this indicator and presented the results in Figure 1. It can be seen that 2% of mature and 5% of elderly representatives of women of the Aboriginal population were underweight (Fig. 1a). Normal or excess weight was characteristic of 46% and 12% of mature women, and 18% and 9% of elderly women, respectively. Serious obesity was observed in 40% of mature women growing to 68% with increasing age.

Figure 1b shows the BMI variables ranging through the subjects of the Caucasian population in Evensk. From the above data, it can be seen that 6% of mature women are underweight, with none of the elderly applying to this category. Normal weight is observed in 32% of mature women and 23% of the elderly. Excess weight was exhibited by 38% of mature examinees, and 30% by the elderly. Among the Caucasian women of the settlement of Evensk, 24% of middle-aged women are obese, and 47% of the elderly can be diagnosed with serious overweight.

Among the mature females of Caucasian ethnicity in Magadan (Fig. 1c), we detected underweight in 3% of the subjects, 44% of normal weight cases, with excess weight and obesity being characteristic of 28% and 25% of the examined women, respectively. In the elderly, only 1% of the subjects proved to be underweight, 21% of the surveyed women showed normal weight, excess weight was found in 35%,



Body Mass Index variables in mature and elderly women: (a) Aboriginal population, Evensk, (b) Caucasian population, Evensk, (c) Caucasian population of Magadan

and 45% of female residents of Magadan were obese.

Thus, the female population of the settlement of Evensk, both Aboriginal and Caucasian, showed a sharp growth in obesity with increasing age due to a smaller number of those with normal or excess weight: the proportion of obese people from middle-aged to the elderly increased by 28% and 23% in Aborigines and Caucasians, respectively. A distinctive characteristic of the Caucasian women of Magadan was less common incidence of normal weight due to a bigger number of obese people and those with excess weight. However, the proportion of obese women in Magadan, as they age from maturity to the elderly, proved to increase by 18% which was rather smaller than in Evensk. All these variables could be compared with the average values of this indicator through the groups.

Despite being a common indicator of detecting obesity, BMI does not reflect the localization of adipose tissue or the ratio of fat and muscle components in the body [13, 20]. A more accurate indicator that shows the accumulation of the abdominal fat appears to be Waist Circumference. It is also strongly correlated with risks of cardiovascular and metabolic diseases and can serve as an identifier of these diseases [28].

It is believed that the BMI value of ≥25.0 kg/m^2 in Caucasian women and ≥23.0 kg/m^2 in Mongoloid women with the WC value of ≥80 cm testify to abdominal obesity [9] and therefore identify increased risks of concomitant diseases. In this Magadan Region survey, Aboriginal and Caucasian women of all ages have their Waist Circumference values exceeding 80 cm which, together with excess weight and obesity, suggests visceral obesity and increases the risk of metabolic syndrome [20], cardiovascular diseases [9], disorders in carbohydrate and lipid metabolism [10], and reproductive impairments [11]. Visceral obesity in women is associated with a higher risk of heart failure than in men [26]. We could see a significant growth of this indicator with increasing age in Aboriginal women of Evensk and Caucasian women of the city of Magadan, however the Caucasians of Evensk did not show any big changes in this regard, which suggests an accelerated risk of the above mentioned diseases that mature women can develop owing to abdominal obesity. Thus females of the Caucasian ethnicity of the settlement of Evensk appear to be the most vulnerable when it comes to the risk of a metabolic syndrome which may already have its origin at the age of maturity owing to rather high values of the WC indicator. Our Waist Circumference measuring examination among the mature female populations, Aborigines in Evensk and Caucasians in Magadan, showed the average statistical values of the indicators being practically compared with the normal standards except for the Caucasian women in Evensk who demonstrated the values significantly exceeding the upper limit of the range.

Conclusion. Our comparison study on women's physical development depending on their ethnicity, age (mature and old ages), and the region of residence has shown that women of Magadan Region, as they age from mature to old age, develop such unfavorable age-related changes as shortening Body Length with increasing Body Mass Index and bigger Waist Circumference which is the risk of cardiovascular diseases. The female population of the settlement of Evensk, both Aboriginal and Caucasian, exhibited a sharp growth in obesity with increasing age due to a smaller number of people with normal or excess weight. As for the Caucasian women of Magadan, a less common incidence of normal weight could be seen due to a bigger number of obese people and those with excess weight. In this survey, women of all ages were diagnosed with abdominal obesity. The Caucasian women in the settlement of Evensk who were rather high in their WC values at the age of maturity could therefore be referred to having a risk of developing a metabolic syndrome.

In this research, we have identified age- and ethnicity-related features of somatometric status of women in Magadan Region which need to be seen in a regional context when forming physical development standards.

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NAD-DEPENDENT DEACETYLASE GENES OF SIRTUIN FAMILY AND RISK OF DEVELOPING VARIOUS PHENOTYPES OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Chronic obstructive pulmonary disease (COPD) is one of the most common chronic respiratory diseases with high morbidity and mortality. The pathogenesis of COPD is closely related to oxidative stress, that is the main mechanism causes accelerated cell senescence. Published data suggest that the COPD pathogenesis may involve stress responses dysregulation that inhibit cellular senescence. We aimed to assess the contribution of sirtuin genes (*SIRT2, SIRT1, SIRT3, SIRT6*) to the various COPD phenotypes risk.

SNPs of *SIRT2* (rs10410544), *SIRT1* (rs3758391, rs3818292), *SIRT3* (rs3782116, rs536715), *SIRT6* (rs107251) genes were genotyped by the real-time polymerase chain reaction (PCR) among 1245 samples (severe COPD with frequent exacerbations (N=331), stable COPD with rare exacerbations (N=290) and control (N=624)). Logistic regression was used to detect the association of studied SNPs in different models.

Significant associations with severe COPD phenotype were identified for *SIRT1* (rs3818292) (P=0.0097, OR = 1.49 for AG genotype), *SIRT3* (rs3782116) (P = 0.0034, OR =0.63) and *SIRT3* (rs536715) (P = 0.00001, OR =0.53) under dominant model, and *SIRT6* (rs107251) (P = 0.00001, OR =0.55 for CT genotype). Stable COPD phenotype with rare exacerbations was associated with *SIRT1* (rs3818292) (P = 0.0055, OR =1.54 for AG genotype), *SIRT3* (rs536715) (P = 0.00001, OR =0.48 under dominant model), and *SIRT6* (rs107251) (P = 0.0002, OR =0.54 for CT genotype).

The obtained results indicate the contribution of NAD-dependent deacetylase genes of sirtuin family and cellular senescence mechanisms to COPD development. The *SIRT3* (rs3782116) identified as a specific marker for severe COPD phenotype with frequent exacerbations.

Keywords: Chronic obstructive pulmonary disease; sirtuins; oxidative stress

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Introduction. Chronic obstructive pulmonary disease (COPD) is a multifactorial chronic heterogeneous inflammatory disease of the respiratory system, with a predominant injury of the distal respiratory tract and lung parenchyma [6]. COPD is characterized by the development of systemic effects that cause the severe complications further aggravating the course of the disease in patients. COPD is one of the most common chronic respiratory diseases with high morbidity and mortality rates [6].

Tobacco smoking is the principal risk factors of COPD; however, COPD develops as result of complex interaction between genetic and environmental factors [4; 6]. Further research is needed to understanding the pathobiology of COPD. At the moment, another aspect of COPD pathogenesis is being discussed, that the COPD pathogenesis may involve stress responses dysregulation that inhibit cellular senescence [4]. Oxidative stress is a key factor in accelerated cellular senescence [8]. Many endogenous molecules counteract to cellular senescence and NAD-dependent protein deacetylases from the sirtuins family are considered as potential factors that decrease senescence [15]. Sirtuin deficiency is considered as one of the mechanisms of accelerated lung aging in COPD [4; 10]. Sirtuins are involved in the formation and functioning of mitochondria, mtDNA protection, and thus may play a key role in the pathogenesis of age-associated diseases [5].

We aimed to assess the contribution of NAD-dependent protein deacetylases from the sirtuins family genes (*SIRT2, SIRT1, SIRT3, SIRT6*) to the various COPD phenotypes risk.

Materials and methods. DNA samples were collected from unrelated subjects who were Tatars in ethnicity and resided in the Republic of Bashkortostan. The study was approved by the Ethics Committee at the Institute of Biochemistry and Genetics (Protocol No 17, December 7, 2010, №19, November 11, 2022). All participants of this study provided written

Table 1

Genotypes and alleles distribution of sirtuins genes loci in studied groups

Gene. SNP	Genotypes and alleles	COPD абс. (%)	Control абс. (%) (N=624)	P
Severe COPD with frequent exacerbations (N=331)				
SIRT2 rs10410544 T>C	CC/CT/TT	144/134/53 (43.50/40.48/16.01)	254/271/99 (40.71/43.43/15.87)	0.653
	C/T	422/240 (63.75/36.25)	779/469 (62.42/37.58)	0.602
SIRT1 rs3758391 T>C	TT/TC/CC	104/131/96 (31.42/39.58/29.00)	168/294/162 (26.92/47.12/25.96)	0.081
	T/C	339/323 (51.21/48.79)	630/618 (50.48/49.52)	0.799
SIRT1 rs3818292 A>G	AA/AG/GG	175/144/12 (52.87/43.50/3.63)	375/213/36 (60.10/34.13/5.77)	0.011
	A/G	494/168 (74.62/25.38)	963/285 (77.16/22.84)	0.236
SIRT3 rs3782116 G>A	GG/GA/AA	165/118/48 (49.85/35.65/14.50)	239/287/98 (38.30/45.99/15.71)	0.002
	G/A	448/214 (67.67/32.33)	765/483 (61.30/38.70)	0.007
SIRT3 rs536715 G>A	GG/GA/AA	222/73/36 (67.07/22.05/10.88)	324/249/51 (51.92/39.90/8.17)	0.00001
	G/A	517/145 (78.10/21.90)	897/351 (71.88/28.13)	0.004
SIRT6 rs107251 C>T	CC/CT/TT	212/86/33 (64.05/25.98/9.97)	333/243/48 (53.37/38.94/7.69)	0.00001
	C/T	510/152 (77.04/22.96)	909/339 (72.84/27.16)	0.052
Stable COPD with rare exacerbations (N=290)				
SIRT2 rs10410544 T>C	CC/CT/TT	128/116/46 (44.14/40.00/15.86)	254/271/99 (40.71/43.43/15.87)	0.575
	C/T	372/208 (64.14/35.86)	779/469 (62.42/37.58)	0.512
SIRT1 rs3758391 T>C	TT/TC/CC	81/133/76 (27.93/45.86/26.21)	168/294/162 (26.92/47.12/25.96)	0.930
	T/C	295/285 (50.86/49.14)	630/618 (50.48/49.52)	0.919
SIRT1 rs3818292 A>G	AA/AG/GG	148/129/13 (51.03/44.48/4.48)	375/213/36 (60.10/34.13/5.77)	0.01
	A/G	425/155 (73.28/26.72)	963/285 (77.16/22.84)	0.08
SIRT3 rs3782116 G>A	GG/GA/AA	129/111/50 (44.48/38.28/17.24)	239/287/98 (38.30/45.99/15.71)	0.097
	G/A	369/211 (63.62/36.38)	765/483 (61.30/38.70)	0.368
SIRT3 rs536715 G>A	GG/GA/AA	201/76/13 (69.31/26.21/4.48)	324/249/51 (51.92/39.90/8.17)	0.00001
	G/A	478/102 (82.41/17.59)	897/351 (71.88/28.13)	0.00001
SIRT6 rs107251 C>T	CC/CT/TT	184/75/31 (63.45/25.86/10.69)	333/243/48 (53.37/38.94/7.69)	0.00001
	C/T	443/137 (76.38/23.62)	909/339 (72.84/27.16)	0.121

Notes: P-value for Chi-square test

informed consent. The COPD group included 621 patients (539 (86.79%) males and 82 (13.21%) females) with a mean age of 64.42±10.71 years. There were 510 (82.13%) smokers and former smokers, the smoking index was 45.34±23.84 pack years; and 111 (17.87%) nonsmokers. In order to identify genetic markers associated with COPD phenotypes, the control group and patients were compared; COPD patients differentiated according to the modern classification which included an integral assessment of the COPD phenotype, taking into account the number of exacerbations per year, the results of specialized questionnaires: the COPD assessment test (CAT - COPD Assessment Test), the Medical Research Council Dyspnea Scale (MRC - Medical Research Council Dyspnea Scale) and indicators of the study of lung function [7]. Two phenotypes were identified: group 1 – severe COPD with frequent exacerbations (**N=331**), with a mean age of 65.39±10.01 years; group 2 - COPD with rare exacerbations (**N=290**), with a mean age of 65.03±8.17 years. The control group included 624 subjects (555 (88.94%) males and 69 (11.06%) females) with a mean age of 59.67±12.31. There were 526 (84.29%) smokers and former smokers and 98 (15.71%) nonsmokers in the group; the smoking index was 38.75±24.87 pack years in the smokers. Inclusion and exclusion criteria for the COPD and control have been previously described [2].

Genotyping. DNA was isolated from peripheral blood leukocytes by phenol–chloroform extraction. The set included SNPs of the following genes: *SIRT1* (rs3758391, rs3818292), *SIRT2* (rs10410544), *SIRT3* (rs3782116, rs536715), *SIRT6* (rs107251). SNP genotyping was performed by real-time polymerase chain reaction (PCR) using commercial kits for fluorescence detection (DNK-Sintez, Russia; https://www.oligos.ru) and a BioRad CFX96™ instrument (Bio-Rad Laboratories, United States). The methods of analysis were described in detail previously [2]. *Statistical Analyses.* Statistical analyses of the results were performed using the software packages IBM SPSS 22.0. The methods were described in detail previously [2].

Results and discussion. Data on the distribution of genotypes and alleles frequencies of the studied loci, and the significance of differences between groups in the frequencies of genotypes and alleles of *SIRT1* (rs3758391, rs3818292), *SIRT2* (rs10410544), *SIRT3* (rs3782116, rs536715), *SIRT6* (rs107251) genes

Table 2

Significant association of studied sirtuins genes loci with COPD stratified by phenotypes

Gene, SNP	Rare allele	N	Genotype / model	OR_{adj} (CI95%)	P_{adj}	$P_{cor-FDR}$
Severe COPD with frequent exacerbations (N=331)						
SIRT1 rs3818292 A>G	G	955	AA AG+GG dominant	1.00 1.35 (1.01-1.81)	0.046	0.046
			AA+GG AG	1.00 1.49 (1.10-2.00)	0.0097	0.0125
SIRT3 rs3782116 G>A	A	955	GG GA+AA dominant	1.00 0.63 (0.46-0.86)	0.0034	0.0068
			AA+GG AG	1.00 0.66 (0.48-0.90)	0.0088	0.0125
			Log-additive	0.77 (0.62-0.97)	0.022	0.0244
SIRT3 rs536715 G>A	A	955	GG GA+AA dominant	1.00 0.53 (0.39-0.72)	0.00001	0.000033
			AA+GG AG	1.00 0.43 (0.30-0.59)	0.00001	0.000033
			Log-additive	0.74 (0.59-0.94)	0.01	0.0125
SIRT6 rs107251 C>T	T	955	CC CT+TT dominant	1.00 0.65 (0.48-0.86)	0.003	0.0068
			CC+TT CT	1.00 0.55 (0.40-0.75)	0.00001	0.000033
Stable COPD with rare exacerbations (N=290)						
SIRT1 rs3818292 A>G	G	914	AA AG+GG доминантная	1.00 1.45 (1.08-1.96)	0.015	0.015
			AA+GG AG	1.00 1.54 (1.14-2.08)	0.0055	0.007
SIRT3 rs536715 G>A	A	914	GG GA+AA доминантная	1.00 0.48 (0.35-0.66)	0.00001	0.000035
			AA+GG AG	1.00 0.53 (0.38-0.75)	0.0002	0.00035
			лог-аддитивная	0.55 (0.42-0.72)	0.00001	0.000035
SIRT6 rs107251 C>T	T	914	CC CT+TT доминантная	1.00 0.66 (0.48-0.89)	0.0061	0.0071
			CC+TT CT	1.00 0.54 (0.39-0.75)	0.0002	0.00035

Note: N - is the number of individuals included in the analysis; Padj, significance in the likelihood ratio test for the regression model adjusted for age, sex, smoking status and pack-years; ORadj, adjusted odds ratio and CI, 95% confidence interval; Pcor-FDR, significance after the FDR correction; in the log-additive model per rare allele dosage, the rare allele dosage increases in the following order: homozygote for the common allele (0)–heterozygote (1)–homozygote for the rare allele (2).

are presented in Table 1. The groups of patients with severe COPD and healthy controls differed significantly in the genotypes and / or alleles frequency distributions of *SIRT1* (rs3818292), *SIRT3* (rs3782116, rs536715), *SIRT6* (rs107251) genes. Statistically significant results of association analysis of studied gene loci and severe COPD are shown in Table 2.

An association of *SIRT1* (rs3818292) with severe COPD phenotype was established in the dominant model (P_{adj} = 0.046, OR = 1.35); the risk of COPD was increased in heterozygous individuals (P_{adj} = 0.0097, OR = 1.49). The rs3782116 and rs536715 of *SIRT3* gene were also associated with severe COPD; thus for rs3782116, the association was statistically significant in the dominant (P_{adj} = 0.0034, OR =0.63) and additive (P_{adj} = 0.022, OR =0.77) models, and with the heterozygous genotype (P_{adj} = 0.0088, OR = 0.66). The most significant associations were established for the *SIRT3* (rs536715) in the dominant model (P_{adj} = 0.00001, OR = 0.53) and the heterozygous genotype (P_{adj} = 0.00001, OR = 0.48) and the *SIRT6* (rs107251) with the heterozygous CT genotype (P_{adj} = 0.00001, OR=0.55).

Association with stable COPD phenotype was detected to *SIRT1* (rs3818292) in dominant model (P_{adj} = 0.015, OR =1.45) and heterozygous genotype (P_{adj} = 0.0055, OR =1.54); *SIRT3* (rs536715) in dominant (P_{adj} = 0.00001, OR =0.48) and additive (P_{adj} = 0.00001, OR =0.55) models and heterozygous genotype (P_{adj} = 0.0002, OR =0.53). *SIRT6* (rs107251) associated with stable COPD phenotype in the dominant model (P_{adj} = 0.0061, OR =0.66), significant association was established for heterozygous CT genotype of *SIRT6* (rs107251 (P_{adj} = 0.0002, OR =0.54) (Table 2).

SIRT1 is the most studied member of the mammalian sirtuin family. It has been shown that *SIRT1* plays an important role in signaling pathways involved in cellular senescence and cell death [5]. *SIRT1* deacetylates many key regulatory proteins and transcription factors involved in DNA repair, inflammation, expression of antioxidant genes, and cellular senescence [14]. Previously, it was shown that SIRT1 levels are reduced in peripheral pulmonary and peripheral blood mononuclear cells of patients with COPD [12]. We found that the risk of developing both COPD phenotypes is higher in heterozygous carriers of the *SIRT1* (rs3818292).

SIRT3 is the main mitochondrial deacetylase regulating many enzymes involved in energy metabolism, respiratory chain components, the tricarboxylic acid cycle, ketogenesis, and fatty acid beta-oxidation [15]. *SIRT3* can directly control the production of reactive oxygen species (ROS) by deacetylating manganese-superoxide dismutase (SOD2), the main mitochondrial antioxidant enzyme [13]. SIRT3 plays a pro- and anti-apoptotic role in various pathological conditions [15]. We have studied the association of two functional polymorphisms (rs3782116 and rs536715) of the *SIRT3* gene with different COPD phenotypes. The association with the development of severe COPD with frequent exacerbations was established for both polymor-

phic loci, while in stable COPD association was shown only with the rs536715 locus. Our data indicated that *SIRT3* (rs3782116) gene locus is the specific marker of frequent exacerbations COPD phenotype.

The associations of *SIRT3* gene loci with age-associated diseases in which oxidative stress and cellular senescence play a key role were extensively investigated [11].

The *SIRT6* (rs107251) is associated with the development of both COPD phenotypes. *SIRT6* exhibits ADP-ribosyltransferase and histone deacetylase activity, and plays role in DNA repair [9]. In the study [3], a decrease of *SIRT6* levels was shown in respiratory epithelial cells of COPD patients due to cigarette smoke exposure. An association of *SIRT6* gene loci with cardiovascular diseases has been shown; cardiovascular diseases are often a comorbid pathology in COPD and have similar pathogenetic mechanisms associated with oxidative stress and cellular senescence [1].

Conclusion. As a result of the study, we have identified significant associations with the development of various COPD phenotypes with polymorphic variants of the *SIRT1* (rs3818292), *SIRT3* (rs536715) and *SIRT6* (rs107251) genes. A specific marker for COPD phenotype with frequent exacerbations is the *SIRT3* (rs3782116) gene locus. The data obtained confirm the hypothesis of a significant role of NAD-dependent protein deacetylases from the sirtuin family and the mechanisms of cellular senescence in the hereditary predisposition to COPD development.

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MATURATION OF MONOCYTES INTO DENDRITIC CELLS BY MORPHOLOGICAL SIGNS IN BREAST CANCER

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Taking into account the complex maturation process of dendritic cells under culturing conditions, as well as the available data of morphological characteristics at various pathological conditions, we find it interesting to assess the features of cell morphology in oncological patients. The purpose of this study was to assess the morphological characteristics of the processes of maturation of dendritic cells in breast cancer. In cancer patients, the potential for cellular viability and maturation compared to healthy individuals is reduced in the early days of cultivation, probably due to the cytotoxicity of chemotherapy and radiation therapy at the time of the study. Cell analysis in the last days of cultivation indicates that the processes of activation of monocyte maturation in dendritic cells in in vitro were significantly higher in oncobols than in healthy individuals.

Keywords: cultivation, monocytes, dendritic cells, morphology, breast cancer.

Introduction. Anticancer vaccines are designed to induce an immune response against tumor antigens. Despite decades of research and development, only a few anti-cancer vaccines have been approved for human use. The success of

these anti-cancer vaccines depends on several factors, including the type of antigens used, the microenvironment of the tumor, the immune landscape of the tumor and various vaccine formulations [5]. Dendritic cells (DCs) are the main antigens that process and represent antigens through the molecules of the main histosis complex MHC I and II to innate and adaptive immune systems [11, 8, 4, 6].

DCs can be grown (differentiated) in large quantities under special laboratory conditions and have become widely studied on experimental models. DCs are rare among peripheral blood leukocytes (less than 1%) and typically exhibit complex phenotypic and functional heterogeneity of the population. Several subtypes of DC with unique and specific functions, morphology and localization: 1) Langerhans cells are described; 2) Myeloid dendritic cells; 3) Dendritic cells derived from monocytes; 4) Lymphoid dendritic cells; 5) Plasmocyte dendritic cells.

Immature DCs have a rounded and smooth surface, while mature DCs have a rough surface with multiple pseudotypes. In the immature state of DC, lower levels of bone-inducing molecules such as CD80, CD86, CD83, and MHC II are expressed and secrete lower levels of immunostimulating cytokines such as IL-12, IL-10, and TNF. In contrast, mature DCs express high levels of bone-inducing molecules and immunostimulating cytokines, indicating that DCs are in phenotypically and functionally mature state [3]. Immature DCs with low levels of activation and high phagocytic ability absorb antigens and mature, acquiring a more active phenotype. After maturation, DCs form dendrites and rapidly migrate to lymph nodes to enhance immune response [7].

An important difference between the properties of newborn babies and adult DCs is the more pronounced suppression of mitogenic activity in cultures with a competitive influence of conventional and modulated DCs [2]. Under the influence of Kureha polysaccharide, immature dendritic cells develop into mature dendritic cells with reduced antigen capture, but also high expression of key surface molecules of MHC-II class, CD40, CD80, CD86 and CD83, as well as larger IL-12p70 and TNF-α [9].

Immature dendritic cells were observed to have large rounded cells, almost without processes. Towards maturation, cells acquire dendritic process elongated forms. These changes occur before and after activation on dendritic cells of patients and on dendritic cells of conditionally healthy persons. The study results are based on the fact that the maturation capacity of dendritic cells in osteomyelitis disease is not impaired. The ability of dendritic cells isolated from peripheral blood monocytes, patients with chronic osteomyelitis caused by Staphylococcus aureus to maturation under the influence of in vitro activators is not impaired [1].

In the study [10] a comparison was made of maturation of DCs from monocytes of patients with cirrhosis of liver and healthy persons. Higher DC output from monocytes was found in patients with cirrhosis of the liver. However, with phenotyping of CD14+ cells, there was no significant difference between cirrhosis of the liver and healthy donors in terms of DC output from CD14+ precursors. The study concluded that when using adequate conditions for DC maturation and T-cell DC absorption from cirrhosis patients, they retain the same ability to activate, mature and present the antigen as healthy donors.

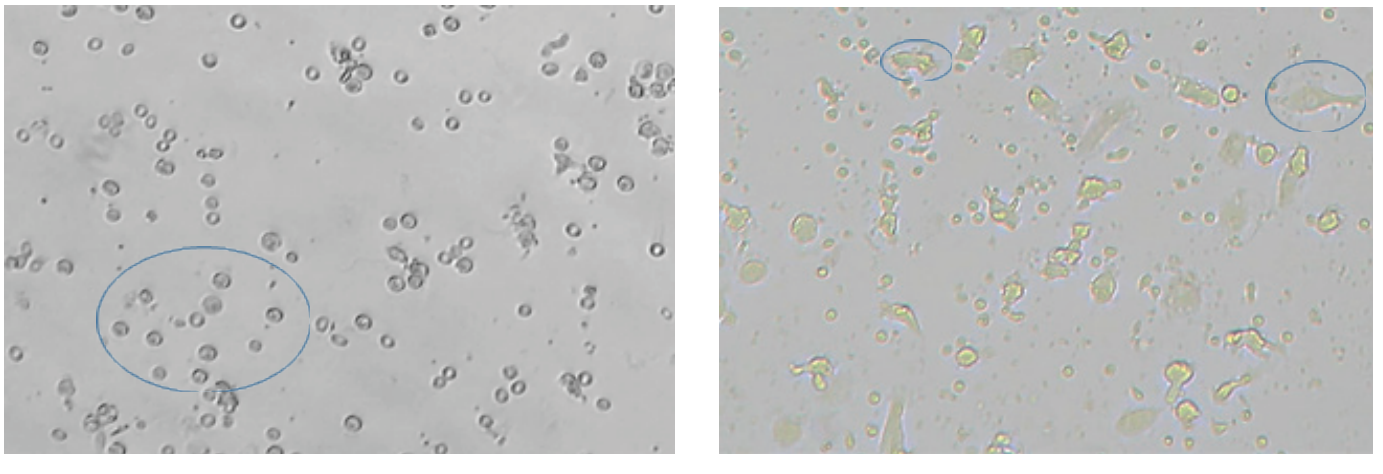
Dendritic cell maturation is a complex heterogeneous process that can give different distinctive functional properties. In studies by Chinese scientists [12] tested the influence of surractin on the maturation of dendritic cells. The results showed that mature DCs form longer processes than immature DCs. Surfactin can induce morphological, phenotypic, and functional maturation of dendritic cells and in this process NF-κB has been involved in signaling.

Thus, given the complex process of maturation of dendritic cells under cultivation, as well as the available data of morphological characteristics at various pathological conditions, it seems interesting to us to assess the features of cell morphology in oncological patients.

The purpose of this study was to assess the morphological characteristics of the processes of maturation of dendritic cells in breast cancer.

Material and methods of research. The research was carried out on the basis of the laboratory of medical biotechnologies of the Medical institute NEFU. Venous blood of 19 patients with a verified diagnosis of breast cancer with informed voluntary consent was given to SBU RS(Y) «Yakut Republican Oncological Dispensary». Dendritic cells were isolated from adult venous blood monocytes. The control group consisted of 4 practically healthy volunteers without inflammatory, autoimmune and oncological diseases.

All studies were carried out in sterile conditions and laminar box. Venous blood is taken sterily into vacueters containing EDTA (Lab-Vac), the volume of venous blood was about 18 ml (2 vials of 9 ml) and diluted with an equal volume of the nutrient medium RPMI-1640 («Biolot», Russia). Mononucleic cells were isolated by the standard method, by



Drawing. Images of cells under an inverted microscope (lens 40): 2 day of cultivation (left) prevails round cells, 7 day (right) - elongated cells (examples of cells are highlighted by an oval)

The ratio of the number of counted cells in the dynamics, $\left(\begin{smallmatrix} Me \\ Q1;Q3 \end{smallmatrix}\right)$

Cultivation days	Cages	Control group (16 fields of vision)	Cancer group (76 fields of vision)	p…
2-й	«Round»/ Immature	113.00 (74.75; 138.00)	89.00 (42.00; 126.00)	0.184
	«Elongated»/ Ripening	27.00 (18.25; 46.75)	18.00 (13.00; 27.00)	0.024
7-й	«Round»/ Immature	69.00 (47.50; 87.5)	12.00 (6.00; 25.75)	0.000
	«Elongated»/ Ripening	27.50 (22.25; 35.00)	18.00 (9.75; 25.00)	0.001

centrifugation in the gradient density Ficoll (LLC «PanEco») 1500 rev/min for 40 min. and centrifuged twice at 1000 rev/min by 10 min. For the adhesion of monocytes to the bottom of the cultural vials of 75 cm2preliminary incubated at 1.5 h at 5% CO2 at 37oC. The non-attached cells (lymphocytes) were washed with incomplete RPMI medium (OOO «BioloT», Russia). 10 ml of RPMI (OOO «BioLOT», Russia) with 20% of FBS500SA (LLC «Diaem») was added to the attached monocytes as well as growth factors and differentiation factors - GM-CSF (40 microns) and IL-4 (40 microns), which were applied to 1-5 days.

Photographs were taken of field of view with native (unpainted) cells in the cultivated vials under the inverted microscope (JSC «LOMO», Russia) with an increase of x10 and x40 on the 2nd and 7th days of cultivation. Clear images with uniform cell distribution throughout the microscope's field of vision were selected for counting. The initial concentration of cells in the examined slurry on the day of planting in vials was within 1-1.5 million cells per ml. A total of 16 fields of vision of healthy persons and 76 areas of vision of oncobols were selected for analysis. The differentiation of the analyzed cells has been made according to morphological form into two types: 1 - «round cells», i.e. rounded cell shape without cell processes (immature) and 2 - «elongated cells», i.e. cells with elongated shapes and cells with processes (ripening).

Statistical analysis was carried out using IBM SPSS Statistics 19.0. The Kolmogorov-Smirnov test was used to determine whether the data corresponded to normal distribution law. The equality of the sample averages was tested using the Student parametric t-criterion (in the case of a normal distribution) and the Mann-Whitney non-parametric U-criterion for independent samples (in the case of deviation from the normal distribution). The criterion χ2was used to assess whether or not the two categorical variables were connected. The data are presented in the table as Me (median), Q1 and Q3 (quartiles 25 and 75%). The results were considered statistically significant at the achieved level of significance p <0.05. The study was conducted in full compliance with the ethical recommendations of the Helsinki Declaration of the World Medical Association and the Basics of the Russian Federation Law on Health Protection of Citizens (1993).

Results and discussion. A comparative analysis of the data showed that the number of round cells on the second day of cultivation did not reveal statistically significant differences, although the median of round cells in the control group was 1.31 times higher than that of oncobols (tables). However, we found a significant difference in the number of elongated cells on the second day of cultivation, so in the group of oncobols their number was significantly lower (p=0.024) than in the control group. The percentage of elongated cells in the healthy group was 19.28%, and in the cancer group it was 16.82%. On the second day of cultivation, the morphological characteristic of cells according to the degree of change in the form of monocytes into an elongated group in oncobols tends to decrease (χ2 = 51.56; p=0.056) compared to control group. Thus, in the early days of cultivation, the maturation process is somewhat delayed in cancer patients.

Analysis of the obtained cell count data for the seventh day of cultivation indicates that the total number of cells in oncobols decreased from 2 days of cultivation by 3.56 times, when as in the control group - 1.45 times. This significant percentage of loss, i.e. the low viability of oncobols cells to culturing conditions, may be due to the cytotoxic effect of chemotherapy and radiation therapy received at the time of the patient's study with breast cancer.

Comparative analysis of the data on the 7th day of cultivation showed that the number of round and elongated cells was statistically significantly lower in oncobols (p=0,000 and p=0,001, respectively). Nevertheless, it should be noted that the percentage of elongated cells in oncobols was 45.85%, which is 1.56 times higher than in the control group (29.32%), with the ratio of round and elongated cells having statistically significant difference (χ2 = 82.14; p=0.010) and the share of elongated cells is significantly higher in oncobles. Thus, in the last days of cultivation, despite the significantly low content of cells, the processes of activating the maturation of monocytes into dendritic cells under in vitro conditions were higher in oncobles than in healthy individuals.

Conclusion. Thus, the morphological analysis we have obtained indicates that the degree of maturation of dendritic cells from peripheral blood monocytes under culturing conditions has a significant difference between breast cancer patients and healthy individuals. In cancer patients, the potential for cellular viability and maturation compared to healthy individuals is reduced in the early days of cultivation, probably due to the cytotoxicity of chemotherapy and radiation therapy at the time of the study. Cell analysis in the last days of cultivation indicates that the processes of activation of monocyte maturation in dendritic cells in in vitro were significantly higher in oncobols than in healthy individuals. The data we have obtained require further in-depth study in order to find ways to improve the effectiveness of the use of autologous dendritic cell oncoduction in breast cancer patients.

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GENERATION LENGTH IN THE YAKUT POPULATION IN 18th-19th CENTURIES

The intergenerational time interval in the Sakha people (Yakuts) was determined by an analysis of genealogical data of 712 families from Namsky, Verkhnekolymsky, Srednekolymsky, Nizhnekolymsky and Elgetsky districts recorded in the 18th – 19th centuries. The male generation interval in the Yakuts averaged 35.7 years, the female generation interval was 30.5 years, which is much higher than the mean general intervals used earlier in population-genetic studies for calculating the time of genetic divergence by the Y chromosome (31-32 years) and mtDNA (25-28 years).

Keywords: generation length, Yakuts, population.

Introduction. Generation interval (sometimes referred to as the generation length) is the most important parameter for calculating the mutation rate at microsatellite repeats in the Y chromosome and autosomal loci, in mitochondrial DNA, as well as for calculating the time of genetic divergence. In early genetic studies, the mean generation interval of 25 and 30 years was used for the paternally inherited Y chromosome; 20 years – for the maternally inherited mtDNA; and 20 and 25 years – for autosomes [15]. In particular, in the Zhivotovsky's fundamental work on assessing the mutations rate at the Y chromosome STR loci in populations with short-term documented histories – in Polynesians of New Zealand and Gypsies of Bulgaria, the male generation interval was estimated as ~25 years [19]. This value has been used by many authors to calculate the time of the most recent common ancestor in the reconstruction of the genetic history of various ethnic groups [1,10-12,14]. Later, an estimate of about ~30 years was considered more reasonable for male generation interval, and ~25–28 years – for female generation interval [1,15]. Only in one study of the genetic history of the Iberian population by the Y-chromosome, the male generation length was assumed to be 35 years [16].

However, one should take into account differences in marriage traditions and demographic characteristics in various populations (age of first marriage, adult mortality rate), which can greatly affect the intergenerational time interval. In order to clarify this indicator for the Yakut population, we determined the male and female generation intervals according to the genealogical data of the Yakuts of the 18^{th} – 19^{th} centuries.

Materials and Methods. The generation intervals were calculated by a direct count in deep-rooted pedigrees of Central and Northern Yakuts, restored according to the census records of 1768, 1795, 1816, and 1858; church registers for the period from 1768 to 1918; and the materials of the 1917 census [6-9]. The sample included genealogical data of 120 families of the Modut *nasleg* (community) [8, Tables 1-14] and 64 families of the Khatyryk nasleg [9, Tables 1-2] of Namsky district; 58 families of the I Baidun nasleg [6, Tables 88-93] and 96 families IV Myatyuzhsky nasleg [6, Tables 94-101] of Verkhnekolymsky district; 187 families of the II Baidunsky nasleg [6, Tables 1-12] and 90 families of the I Kangalassky nasleg [6, Tables 14-22] of Srednekolymsky district; 55 families of the I Myatyuzhsky nasleg of Nizhnekolymsky district [6, Tables 102-104); and 42 families of the Indigirka Yakuts of Elgetsky* disctrict [7, tables 2, 4]. **Note*. Elgetsky district was founded after 1770; it occupied a vast territory in the basin of the upper, middle and lower reaches of the Indigirka River. Since the 1930s, most of the territory of Elgetsky district has been part of modern Abysky and Momsky districts, and the smaller part belongs to Allaikhovsky district. The name of Elgetsky district is almost forgotten today.

The male generation interval I_m (father-child), which is of interest for the study of the Y-chromosome, is calculated as the mean age of the father at birth of all his children. The female generation interval I_f (mother-child), which is used in the study of mtDNA, is defined as the mean age of the mother at birth of all her children. For the population as a whole, the generation interval in a given time period is equal to the weighted average of the total generation intervals for the families included in it. The overall human generation interval required for studies using autosomal loci is calculated by the formula $I_o = (I_f + I_m)/2$ [15]. These generation intervals, I_m, I_f, and I_o, depend only on the reproductive adults; people who do not have children cannot influence these indicators. Thus, childhood mortality and infertility do not affect the values of the generation interval of a population.

In addition to the intergenerational

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Table 1

Generation lengths in 8 naslegs of Yakutia in 18th-19th centuries

District: Nasleg	Number of families	Father-child	Father-son	Mother-child	Mother-daughter	Overal generation interval
Namsky:						
Modut	120	36.0	34.7	30.3	29.9	33.2
Khatyryk	64	33.4	34.6	29.2	29.4	31.3
Verkhnekolymsky:						
I Baidunsky	58	36.0	34.6	29.8	31.3	32.9
IV Myatyuzhsky	96	35.5	36.1	31.3	31.1	33.4
Srednekolymsky:						
II Baidunsky	187	36.1	37.2	31.5	31.3	33.8
I Kangalassky	90	37.2	36.7	30.7	30.1	34.0
Nizhnekolymsky:						
I Myatyuzhsky	55	33.0	33.0	28.7	28.9	30.9
Elgetsky	42	38.9	37.6	30.9	32.8	34.9
Total	712	35.7	35.6	30.5	30.4	33.1

time intervals of father-child and mother-child used in the most genealogical studies, we also calculated the average father-son and mother-daughter intervals as more adequate indicators for calculating the rate of mutation of STR loci in the Y chromosome and in mtDNA.

Results and Discussion. Table 1 presents the mean male and female generation intervals in residents of 8 naslegs of Namsky, Verkhnekolymsky, Srednekolymsky, Nizhnekolymsky and Elgetsky distircts of Yakutia. The mean male generation intervals ranged from 33.0 to 38.9 years for various naslegs, female intervals – from 28.9 to 32.8 years. There were no significant differences in the generation lengths between the indigenous inhabitants of Namsky district and the northern Kolyma Yakuts. The maximum values of male generation interval (38.9 years) were recorded in families of the Indigirka Yakuts of Elgetsky district, which is apparently due to the fact that the sample included the genealogies of the two most noble families of Efimovs and Sleptsovs, which are distinguished by numerous offspring and currently constitute the main part of the Yakut population of Abyisky district [7]. When comparing the values between the generation lengths of father-child and father-son, mother-child and mother-daughter, no significant differences were found. For the entire studied sample of Yakuts, the male generation interval averaged 35.7 years, the female one – 30.5 years, with the overall generation length at 33.1 years.

The generation lengths calculated by us for the Yakut population significantly exceed the mean global values proposed earlier by Fenner, which were calculated on the basis of the United Nations (2000)

Table 2

Mean age of parents at first and last birth in Yakutia in 18th-19th centuries

District: Nasleg	Fathers		Mothers	
	1	2	1	2
Namsky:				
Modut	29.8	43.0	28.4	32.2
Khatyryk	30.5	40.5	23.7	33.9
Verkhnekolymsky:				
I Baidunsky	30.0	40.7	24.5	35.1
IV Myatyuzhsky	31.2	40.0	26.0	34.4
Srednekolymsky:				
II Baidunsky	30.5	42.5	27.6	35.1
I Kangalassky	30.2	42.1	25.3	34.9
Nizhnekolymsky:				
I Myatyuzhsky	27.4	39.2	23.1	33.7
Elgetsky	31.0	44.7	25.0	38.0
Total	29.9	41.4	26.2	34.2

Note: 1 is the average age at birth of the first child, 2 is the average age at birth of the last child

analysis of the mean-age-at-first-marriage data for 191 countries (84% of all countries in the world) and data from the Council of Europe (2002) [15]. Additional sources of information for this work were the results of national censuses, special surveys conducted in 1970 – 1998, and ethnographic data collected in the 19th and 20th centuries from the study of 157 hunter-gatherer societies from Africa, Eurasia, Australia, Northern, and South America. In developed countries, the author estimated the male generation length at 30.8 years, the female one – 27.3 years; in less developed countries – 31.8 and 28.3 years; and in the populations of hunter-gatherers – 31.5 and 25.6 years, respectively. Fenner proposed to use the value of generation length equal to 31-32 years for studying the divergence of populations by the Y-chromosomes, 25-28 years – for mtDNA, and 28-30 years – for autosomes [15]. The differences in the data for the Yakut population could be explained by the fact that in the Fenner's study, the generation intervals were estimated by indirect methods based on such parameters as age at first and last births, rate of mortality, and the average difference between the ages of men and women at first marriage. At the same time, it should be noted that direct methods for estimating generation lengths from genealogical data of several population are more accurate than indirect ones.

The values obtained by us for the Yakut population are comparable with the data of Tremblay for the French-Canadian population of Quebec, where the

mean values of the generation lengths for the Y-chromosome were estimated at 35 years, for mitochondrial DNA – 29 years, and for autosomal loci – 32 years [20]. Tremblay and Vézina analyzed the demographic parameters of the Quebec population from the 17th to the 20th centuries, just as we did, by direct methods of analyzing genealogical data and came to the conclusion that the Catholic population of Quebec then lived in conditions characteristic of many ancient populations: the demographic context included natural birth rates without contraceptive use, high mortality, and a relatively young age at marriage. Both populations, Yakut and Canadian, are characterized by developing in relatively isolated conditions, high birth rates, and the accumulation of rare hereditary diseases [2-4, 17].

It is known that the main factor affecting the intergenerational time interval is the average age of first marriage: the higher the age at which people get married, the longer the intervals between generations [20]. In tribes where traditions do not restrict early sexual intercourse outside marriage, the age of menarche is taken as the starting point of the reproductive period. However, it has been shown that in the first years after menarche there is a low birth rate and children born to women under 20 years of age account for a small proportion of all children [18]. In the sample of Yakuts of the 18th – 19th centuries studied by us, the proportion of children born to women under 20 was only 12%. The generation interval is also affected by the adult mortality rate in different age groups: people who die earlier leave fewer children and the intergenerational time interval in their lineages is shorter. On contrary, long-lived people have more children, greater genetic contributions to subsequent generations, and longer generations on average.

The mean age of a Yakut mother at birth of her first child in the 18th – 19th centuries was 26.2 years, and for a Yakut father – 29.9 years, while for the Canadians of Quebec it was 22.8 and 26.2 years, respectively [20]. Based on these data, one would expect that the generation length of the Yakuts should be on average ~3.5 years longer than that of the Canadians, but since this was not observed, it is sensible to assume that the mortality among Yakuts at a young age was higher than in the Canadian population, which led to smoothed differences and approximately equal values in the generation lengths for the both populations.

It should be noted that the mean age of the mother at birth of her first child among the Yakuts (26.2 years) turned out to be much higher than those in 11 hunter-gatherer tribes of Africa, Australia, Asia and America (on average 19.4 years) and in 40 less developed countries (average 20.5 years) [15]. The value obtained by us, apparently, cannot be explained by the late entry of women into marriage, since in the 19th - early 20th centuries, the mean marriage age for Yakut women ranged from 16 to 21 years; for Yakut men – from 17 to 25 years [5]. The discrepancy with data for other populations rather indicates a high infant mortality rate among young Yakut mothers and the fact that these early-dead children may not have been included in the censuses and church registers. Therefore, our calculated age of 26.2 years should be attributed to the mean age of the mother at birth of her first *surviving* child. At the same time, the mean age of the mother at birth of her last child among Yakuts (34.2 years) turned out to be comparable with the same indicator in hunter-gatherer populations – an average of 34.6 years, and in less developed countries – an average of 36.1 years. [15].

In Helgason et al. (2003), generation intervals were calculated for 131,060 modern Icelanders and their ancestors born between 1698 and 1742 and between 1848 and 1892 [13]. The authors noted a trend towards a decrease in the generation lengths over the past 300 years. For the female and male lineages extending up to 1848 – 1892, the generation lengths were 28.12 and 31.13, respectively. In the female and male lineages connecting modern Icelanders with their ancestors born in 1698 – 1742, the corresponding intervals were 28.72 and 31.93. To determine whether the same trend is observed in the Yakut population, we calculated the generation lengths for people born in the 18th century, and in the first and second halves of the 19th century (Table 3). It was found that the female generation interval among the Yakuts gradually decreases from 32.33 in the 18th century to 28.50 in the second half of the 19th century; the male generation interval remained high in the 18th century (36.79) and the first half of the 19th century (37.01), but significantly reduced in the second half of the 19th century (32.3). We assume that marriage traditions and the age of first marriage could hardly have changed over such a short period of evolution of the Yakut ethnos; therefore, the gradual decrease in mortality at a young age could have influenced the decrease in the generation length to a greater extent. From historical data it is known that this period from the mid-18th century until the late 19th century in Yakutia was characterized by the eradication of slavery, a stronger policy of Christianization, and improving of living conditions of the population [3].

Table 3

The generation intervals in Yakut population by time periods in 18th-19th centuries

Period	Generation interval	
	Father-child	Mother-child
18th century	36.79	32.33
1st half of 19th century	37.01	30.54
2nd half of 19th century	33.51	28.50

Conclusion. Thus, the results obtained indicate higher intergenerational intervals for the Yakuts in comparison with other populations: an average of 35.7 years for male generations and 30.5 years for female generations. There is a tendency towards a gradual decrease in the generation lengths in the Yakut population in the period of the 18th – 19th centuries.

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GENETIC AND CLINICAL MARKERS OF LACTASE DEFICIENCY IN ADOLESCENTS OF THE CENTRAL AND SOUTHERN REGIONS OF EASTERN SIBERIA

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Research objective: to establish the genotype frequency of single nucleotide polymorphisms rs4988235 and rs182549 of the MCM6 gene depending on the ethnicity of adolescents in Eastern Siberia (Russians, Khakasses, Tuvans) and to identify the relationship between lactase deficiency (LD) and the clinical characteristics of recurrent abdominal pain (RAP).

Materials and Methods: 449 adolescents aged 11-18 years old were examined at schools in three cities of Siberia (Krasnoyarsk, Abakan, Kyzyl) and in-patient hospital in Krasnoyarsk. Lactase deficiency (LD) was diagnosed by the hydrogen breath test (HBT) after oral lactose load using the Gastrolyzer apparatus (Bedfont, UK). In schoolchildren, genomic DNA was isolated from saliva samples by the sorption method using the DIAtom DNA Prep kits (IsoGen, Russia). In inpatient children, DNA was isolated from whole blood by the sorption method from 0.1 ml of a suspension of leukocytes using the DNA-Sorb-B kit (103-20, AmpliPrime, Russia). Genotyping for the carriage of allelic variants rs4988235 and rs182549 of the MCM6 gene was performed on the basis of TaqMan allelic discrimination technology using real-time polymerase chain reaction (RT-PCR) on a detecting thermal cycler «Rotor-Gene 6000» (Corbett Life Science, Australia).

Results: The CC genotype of the rs4988235 polymorphism of the MCM6 gene occurs almost 5 times more often (93%) with a positive HBT than with a negative HBT (22%), p<0.001. Moreover, carriage of the rs4988235*CC genotype has a high sensitivity for LD diagnostics, i.e. 93 (81-99) %, with a relatively low specificity of 77 (69-85) %, which is likely to be due to the presence of secondary LD. A significantly higher prevalence of CC genotypes of both polymorphisms associated with LD has been observed in Mongoloid adolescents (Khakas - 82% and Tuvans - 91%), compared with Russian adolescents - 49%, p<0.001. There was no relationship between genetic markers of LD and RAP, verified according to the J. Apley and N. Naish criteria.

Conclusion: A high diagnostic significance of the rs4988235*CC genotype for LD diagnostics in Siberian adolescents was established. The CC genotype prevalence of both polymorphisms, associated with LD, in Russian adolescents (49%) does not differ from European data, whereas these genotypes were found in the great majority of Mongoloids examined (82-91%), which can be considered to be "paradoxical", given that the southern regions of Central Siberia are characterized by a historically high level of dairy farming development.

Keywords: lactase deficiency, adolescents, hydrogen breath test (HBT), genetic polymorphisms, recurrent abdominal pain.

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Introduction: Lactase deficiency (LD) is the most common variant of disaccharidase deficiency. LD is realized to be the reduced intestinal lactase activity, i.e. the parietal digestion enzyme that breaks down lactose, composed of glucose and galactose. There are primary (infant), primary constitutional (adult, late), and secondary hypolactasia.

Lactose, the main carbohydrate in

milk, is one of the main energy source during breastfeeding. To implement the energy function, the disaccharide has to be hydrolyzed to glucose and galactose, effected using β-galactosidase (lactase-phlorizin hydrolase, LPH), which is encoded by the lactase (*LCT)* gene localized at chromosome 2q21 [5]. The LPH enzyme is expressed only in the apical region of the villi of the small intestine, with the highest activity appearing to be at an early age. The enzyme activity decreases rapidly in most people in older age, which can cause full dairy intolerance with specific clinical consequences. However, the enzyme activity is preserved for about a third of the human population, i.e., lactase persistence (LP) seems to take place by adult age. There is currently strong evidence for the genetic basis of LP, encoded by at least five single nucleotide polymorphisms in a dominant inheritance pattern. These polymorphisms are localized upstream of the *LCT* gene in the regulatory region that regulates the *LCT* gene expression, i.e. *MCM6* (minichromosome maintenance complex component 6) [7].

The population LP frequency, being the highest in northern European populations, decreases towards the south of Europe and the Middle East, with it being minimal in non-livestock regions of Africa, Asia and the Far North [1]. Currently, population differences in the LP prevalence are considered by most authors to be a consequence of positive natural selection after the domestication of cattle in the Middle East and North Africa 7500-9000 BC. The four most likely hypotheses of the positive selection advantage for individuals with LP-determining mutations in populations with developed dairy farming have recently been provided [3, 8, 9]:

• the ability to take a highly nutritional food product (milk) without restrictions for life, and not only in childhood;

• the possibility of using milk as a liquid energy drink during heat and drought;

• the ability for lactose to enhance calcium absorption, with that being especially important in areas with vitamin D deficiency;

• the ability of milk to positively affect growth and fertility by stimulating insulin-like growth factor-1.

Currently, there have been known five single nucleotide polymorphisms associated with LP, evidenced by transfection and clinical studies, i.e. −13910:C>T (rs4988235), −13907:C>G (rs41525747), −13915:T>G (rs41380347), −14009:T>G (rs869051967), −14010:G>C (rs145946881). The most ancient and studied polymorphism is rs4988235, which almost completely determines LP in European populations. Other polymorphisms are likely to determine LP in populations of the Middle East and Africa [1]. In recent years, 18 more rare polymorphisms of the MCM6 gene, associated with LP in various relatively small populations have been described [1]. Among them is the mutation −22.018:G>A (rs182549) described, showing full linkage with the rs4988235 polymorphic region.

In the given study, we aimed to establish the genotype frequencies of single nucleotide polymorphisms rs4988235 and rs182549 of the MCM6 gene depending on the ethnicity of adolescents in Eastern Siberia (Russians, Khakasses, Tuvans) and to identify the relationship between LD and the clinical RAP characteristics.

Materials and methods: Lactase deficiency was diagnosed by determining the concentration of hydrogen in the exhaled air after an oral lactose load using the clinically approved apparatus the Gastrolyzer (Bedfont, UK). The basal hydrogen concentration in the exhaled air was measured, then a child drank a specially prepared lactose solution, followed by measurement series of hydrogen in the exhaled air carried out every 30 min. within 2 hours. Gastrointestinal symp-

Table 1

Clinical characteristics and genotype distribution of single nucleotide polymorphisms rs4988235 and rs182549 of the MCM6 gene depending on the results of the hydrogen breath test (HBT) with a lactose load

Clinical characteristics	negative HBT (n=115)	positive HBT (n=43)	p
Age (years)	12.6±0.29	12.7±0.43	0.956
Boys/girls	50/65	25/18	0.110
Weight (kg)	50.8±2.8	49.8±2.5	0.599
Height (cm)	156.1±1.9	159±2.6	0.304
Dyspeptic symptoms after a lactose load	29 (25)	26 (61)	<0.001
MCM6 (rs4988235) genotypes			
TT	18 (16)	2 (5)	0.067
CT	70 (61)	1 (2)	<0.001
TT+CT	88 (77)	3 (7)	<0.001
CC	27 (23)	40 (93)	<0.001
MCM6 (rs182549) genotypes			
TT	18 (16)	2 (5)	0.067
CT	71 (62)	1 (2)	<0.001
TT+CT	89 (78)	3 (7)	<0.001
CC	26 (22)	40 (93)	<0.001

Table 2

Indicators of diagnostic significance for the carriage of the rs4988235*CC genotype for diagnosing lactase deficiency compared with the hydrogen breath test ("gold standard") in adolescents of Eastern Siberia

Indicator	Value	95% confidence interval
Sensitivity	93	81-99
Specificity	77	69-85
Positive Likelihood Ratio	4.11	2.91-5.83
Negative Likelihood Ratio	0.09	0.03-0.27
Positive Predictive Value	61	52-69
Negative Predictive Value	97	91-99
Accuracy	82	74-87

toms were assessed during and after the test. The test was considered to be positive when the concentration of hydrogen in the exhaled air was more than 10 ppm from the basal one.

Genomic DNA was isolated from saliva samples collected using special containers “Saliva DNA Collection and Preservation Devices” (Norgen Biotek Corp., Thorold, ON, Canada) by the sorption method using the Diatom DNA Prep kits (IsoGene, Russia) according to the manufacturer’s instructions.

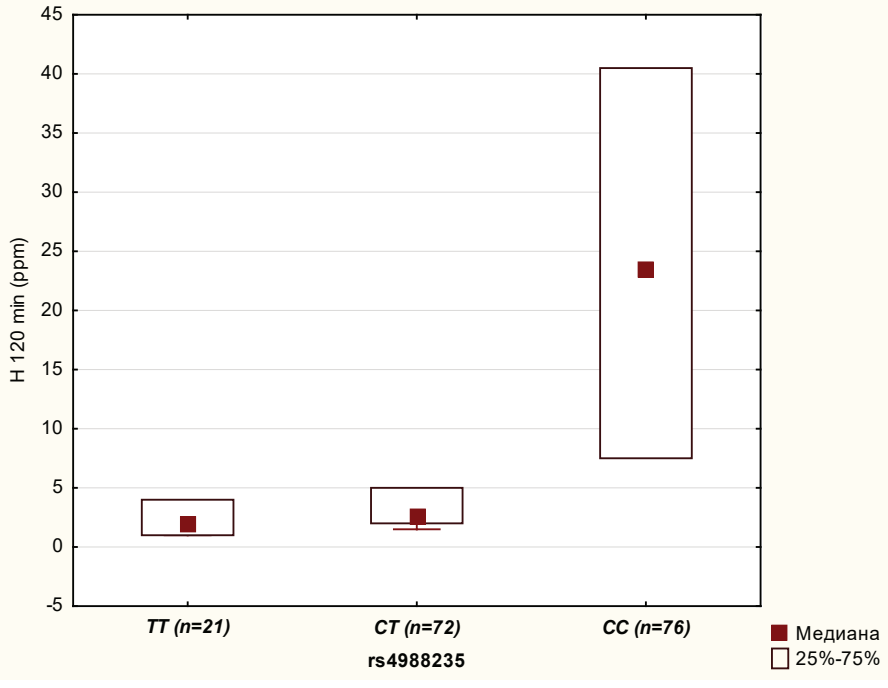
Whole blood was used as a source of genomic DNA in a separate group of experimental subjects (n = 170). Blood sampling in a volume of 3-5 ml was taken aseptically in vacuum tubes "IMPROVACUTER" (Guangzhou Improve Medical Instruments Co., Ltd, Китай) containing 0.5 M EDTA solution. Isolation of genomic DNA was carried out by the sorption method from 0.1 ml of a suspension of leukocytes using the DNA-Sorb-B kit (103-20, AmpliPrime, Russia) according to the manufacturer's instructions.

Genotyping for the carriage of allelic variants rs4988235 and rs182549 of the MCM6 gene was carried out on the basis of TaqMan allelic discrimination technology using real-time polymerase chain reaction (RT-PCR) on a detecting thermal cycler «Rotor-Gene 6000» (Corbett Life Science, Australia). The master mix included a 2.5-fold reaction mixture containing 2.5-fold buffer B (KCl, TrisHCl (pH8.8), 6.25 mM MgCl2), SynTaq DNA polymerase, deoxy-Nucleoside triphosphates, glycerol, Tween 20 (M-428, Synthol, Russia), ddH_2O (M-428, Synthol, Russia), primers and fluorescent probes (DNA-Synthesis, Russia). Amplification was performed in 25 µl of reaction mixtures containing approximately 30 ng of DNA, according to the following protocol: 95°C - 3 min; 95°C - 15 s, 55°C - 30 s, 72°C - 30 s (50 cycles). Each experiment included a negative control, where the DNA template was replaced with distilled water.

Results and discussion: To identify the relationship between genetic markers of LD and the HBT results, 158 adolescents were tested. The results are presented in Table 1.

As follows from the data presented in Table 2, with a positive HBT result, the presence of the CC genotype of the rs4988235 polymorphism of the MCM6 gene occurs almost 5 times more often than with a negative HBT, i.e. in 93% and 22%, respectively (p<0.001).

Additionally, indicators of diagnostic significance for the carriage of the rs4988235*CC genotype for diagnosing lactase deficiency compared with the hydrogen breath test (“gold standard”) in adolescents of Eastern Siberia were calculated. The results are presented in Table 2.



Hydrogen concentration in exhaled air 120 - minutes after experiment started, depending on MCM6 (rs4988235) genotype (K-W test, p<0.001).

The data given in Table 2 have shown the high diagnostic significance for the carriage of the rs4988235*CC genotype for diagnosing lactase deficiency in adolescents of Eastern Siberia.

However, a relatively low carrier specificity of the rs4988235*CC genotype for LD to be diagnosed may be due to the presence of secondary LD caused, for example, by intestinal infections or celiac disease, as well as other genetic loci and/or epigenetic mechanisms of primary LD.

Additional evidence of diagnostic significance may be the estimation of hydrogen concentration in the exhaled air 120 - minutes after experiment started, depending on the MCM6 (rs4988235) genotype. As illustrated in Figure 1, the rs4988235*CC genotype is associated with significantly higher exhaled hydrogen concentrations after lactose load when compared to TT and CT genotypes.

To assess the population frequencies of the genetic marker distribution of LD, there were tested 449 adolescents aged 12-18 years of three nationalities - Russians, Khakasses and Tuvans, living in the central (Krasnoyarsk) and southern (Abakan and Kyzyl) regions of Eastern Siberia.

Data on genotype distribution of single nucleotide polymorphisms rs4988235 and rs182549 of the MCM6 gene, depending on the ethnicity of adolescents, are presented in Table 3. The results obtained have shown a significantly higher prevalence of CC genotypes for both polymorphisms associated with LD in Mongoloid adolescents, i.e. both Khakasses and Tuvans, compared with Russian ones (p<0.001). Therefore, the

Table 3

Genotype distribution of single nucleotide polymorphisms rs4988235 and rs182549 of the MCM6 gene depending on the ethnicity of adolescents (Russians, Khakasses, Tuvans)

Genotypes	Russians (n=231)	Khakasses (n=66)	Tuvans (n=152)
MCM6 (rs4988235) genotypes			
TT	17 (7)	2 (3)	0 (0)
CT	101 (44)	10 (15)*	13 (9)*
TT+CT	118 (51)	12 (18)*	13 (9)*
CC	113 (49)	54 (82)*	132 (91)*
MCM6 (rs182549) genotypes			
TT	17 (7)	2 (3)	0 (0)
CT	105 (46)	11 (17)*	13 (9)*
TT+CT	122 (53)	13 (20)*	13 (9)*
CC	109 (47)	53 (80)*	132 (91)*

Note: * - statistical significance of differences when compared with the ethnic group “Russians” < 0.001.

rs4988235*CC genotype was found in about half (49%) of Russian adolescents, whereas this genotype appeared to take place in the great majority of Khakasses and Tuvans examined (82% and 91%, respectively). Thus, genetic markers for lactase persistence (LP) seems to take place by adult age only in 20% of Khakass adolescents and 9% of Tuvans, with that being 53% in Russian adolescents of Eastern Siberia (determined by the frequencies for rs4988235 TT + CT, Table 1), corresponds to frequencies found in Caucasoids of Central Europe [1]. These data can be considered to be "paradoxical", taking into account that the southern regions of Eastern Siberia (Khakassia and Tyva) are characterized by a historically high level of dairy farming development and high consumption of dairy products.

Extremely low prevalence rates of LP genetic markers have previously been given for other populations, including those in regions with historically high consumption of dairy products [1]. It is not surprising that the extremely low LP prevalence among the Nenets from the Russian Far North (10%), who did not eat milk until the beginning of the 20th century [7]. The same low LP prevalence (14%) was also recorded, e.g., in northern Yakuts, characterized by low dairy farming [9]. However, similar low LP prevalence was also registered in the southern regions with a high consumption of dairy products, namely, among Kazakhs (21%), Kyrgyzes (12%), Buryats (18%), Mongols (13%) [5].

The low LP prevalence we have found for the Khakassia and Tyva populations, as well as for the other populations of the South Asian regions mentioned above, can be represented to be a combination of several cultural, nutritional, and environmental factors. Features of keeping animals (farming or predominant grazing in meadows, seasonal factors), food traditions in some populations leading to the need for fermentation of dairy products (for example, as cheese, koumiss) or mixing milk with other products (for example, drinking milk with tea and salt, characteristic of Tuvans), reducing the influence of evolution factors on lactase-producing objects [8]. There may also be a modifying effect of gut microbiota, characteristic of certain populations, on the intra-intestinal fermentation of lactose. [2, 4, 6].

Finally, migration processes could result in populations mixing and positive selection leveling of lactase-producers [1]. We have not found a relationship between genetic markers of LD and recurrent abdominal pain (RAP), verified according to the criteria of J.Apley and N.Naish (three or more episodes of abdominal pain in the last three months, disrupting the child's daily activities). Therefore, in Russian adolescents, the frequency of carriage of the CC variant of polymorphism (rs4988235) in children without RAP was 51%, and in children with RAP, that was 43% (p=0.515); in Khakass adolescents that was 78% and 93%, respectively (p= 0.446); among Tuvan adolescents - 93% and 85%, respectively (p=0.221). Thus, the etiopathogenetic relationship between LD and RAP in the total unbiased sample of adolescents seems to be unlikely.

Conclusion: The high diagnostic significance of the rs4988235*CC genotype for LD diagnostics in Siberian adolescents was established. The CC genotype prevalence of both polymorphisms genotypes associated with LD in Russian adolescents (49%) does not differ from European data, whereas these genotypes are found in the great majority of Mongoloids examined (82-91%), which can be considered to be "paradoxical", given that the southern regions of Central Siberia are characterized by a historically high level of dairy farming development.

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DIAGNOSTIC AND TREATMENT METHODS

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STABILOMETRY IN THE COMPLEX REHABILITATION OF PATIENTS AFTER CEREBRAL STROKE

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Postural disturbance are detected in about 80% of people after a stroke and significantly limit the patient's household and social activity, increase the risk of falls. Stabilometry is a highly informative method for studying the equilibrium function based on the analysis of the parameters of the center of pressure. The purpose of the study: clinical and stabilometric analysis of postural disorders in the complex rehabilitation of patients after a stroke. Materials and methods. The study involved 60 patients (40 men and 20 women, median age 61.0 [56.25; 65.75] years, median rehabilitation start time 30.0 [25.0; 40.75] days). All patients were divided into 2 groups: the first group consisted of 30 people with hemiataxy; the second group - 30 people with hemiparesis. All patients underwent complex rehabilitation and stabilotraining sessions. The dynamics of stabilometric parameters and clinical scales (NIHSS, Rivermead index, Rankin scale, Barthel scale) were assessed. Results. Both groups received statistically significant improvements in the area of the statokinesiogram, velocity of the center of pressure, energy index in both phases of the study, as well as in all clinical scales ($p < 0.001$). We did not reveal differences in the degree of changes in stabilometric parameters in patients with different stroke syndromes (hemiataxia or hemiparesis) before and after complex rehabilitation ($p > 0.05$). No correlation was found between stabilometric parameters and clinical scales. Conclusion. Stabilometry is a highly informative method for studying the balance function in patients after a stroke in complex rehabilitation, however, one should take into account the lack of correlation of its parameters with clinical scales.

Keywords: stroke, rehabilitation, stabilometry, stabilotraining, postural disorders, biofeedback.

Introduction. Stroke remains an urgent medical and social problem of modern society in the world and in Russia. According to the study of the global burden of stroke, from 1990 to 2010 there was an absolute increase in the number of patients with ischemic and hemorrhagic stroke in the world (by 37% and 47%, respectively). However, in high-income countries, the incidence and mortality from stroke have decreased significantly (ischemic - by 13% and 37%, hemorrhagic - by 19% and 38%, respectively) [14]. In 2015, the incidence and mortality from stroke in Yakutsk (Sakha Republic (Yakutia), Russia) were 3.64 and 0.83 cases per 1000 population per year, respectively [8].

Walking disorders are detected in about 80% of stroke survivors and significantly limit the patient's household and social activity, increase the risk of falls [5, 16]. Immediately after a stroke, 50% of patients cannot move independently, 12% of patients can only move with assistance, and after rehabilitation measures, the proportion of people able to walk independently increases to 50%, but 18% still have severe postural disorders. disorders [10]. Thus, motor rehabilitation of stroke patients is a key task.

The postural balance of a person is maintained by integrating incoming visual, vestibular, somatosensory, and proprioceptive information [9]. In addition, the cognitive sphere plays an important role in the control of movements [12]. In cerebral stroke, the breakdown of the above systems leads to the development of postural disorders [11]. A highly informative method for studying the balance function, based on the analysis of the parameters of the center of pressure (displacement along the sagittal and frontal axes, movement speed, statokinesiogram area, energy consumption), is stabilometry [4, 6]. In addition, using a stabilometric platform, it is possible to carry out dosed stabilotraining aimed at improving the patient's motor functions using biofeedback (BFB) [3, 13].

The aim of the study: clinical and stabilometric assessment of posture disorders in the complex rehabilitation of patients after a stroke.

Materials and research methods. This prospective study was conducted on the basis of the Department of Medical Rehabilitation of Patients with Functional CNS Disorders, Treatment and Rehabilitation Center of the Republican Clinical Hospital No. 3 (Yakutsk). All patients participating in the study signed an informed consent.

Criteria for inclusion: 1) the ability to maintain balance without support at the time of study and training; 2) no cognitive impairment (MMSE > 24 points); 3) the absence of severe somatic diseases that prevent the study.

Non-inclusion criteria: 1) inability to maintain balance without support; 2) cognitive impairments that impede the quality of understanding tasks and following instructions; 3) presence of neurodegenerative diseases of the nervous system, history of epilepsy; 4) cardiac and respiratory insufficiency, preventing the implementation of stabilometry and stabilotraining; 5) severe orthopedic disorders; 6) patients with only sensory disorders or without neurological symptoms; 7) patients with polyneuropathies of any etiology.

The study included 60 patients (40 men and 20 women, median age - 61.0 [56.25; 65.75] years, median period for the start of rehabilitation - 30.0 [25.0; 40.75] days). Depending on the neurological deficit, the patients were divided into 2 groups. The first group consisted of 30 people with hemiataxy; the second group - 30 people with hemiparesis. In each group, patients with ischemic and hemorrhagic stroke were equally divided.

All patients received treatment in accordance with current clinical guidelines and medical standards. The following scales and indices were used: NIHSS, Rivermead Mobility Index, Rankin Disability Scale, Barthel Daily Activity Index.

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Patients of both groups were comparable in terms of gender and age characteristics ($p > 0.05$). In patients of the 2nd group (with hemiparesis), the motor deficit was initially more pronounced according to the NIHSS scale (Table 1).

The stabilometric study was performed on the ST-150 platform (Mera, Russia) in accordance with standard recommendations [4]. Patients were examined before the first and after the last stabilotraining in the morning until 11 am, while any studies and procedures that could affect the result (for example, intravenous injections, fibrogastroscopy, etc.) were excluded. Stabilometry was carried out according to the European version of the installation of the feet (heels together, toes apart by 30°) in two phases (with open (EO) and closed (EC) eyes), each lasting 30 seconds. The following indicators of stabilometry were analyzed: the average position of the center of pressure (CP) along the sagittal (Y) and frontal (X) axes; area of the statokinesiogram (S); velocity CP (V), energic index (Ei).

Stability training with the use of BFB was carried out using the static test "Target" and the dynamic test "Hares" (Fig. 1). Each patient included in the study completed 10 workouts over 2 weeks (5 times a week) for 20 minutes (10 minutes for the Target test and 10 minutes for the Hares test). Training were carried out in the first half of the day, while the interval from the previous procedure (for example, massage, physiotherapy) was at least 30 minutes.

Statistical research methods. Statistical analysis was performed using the SPSS Statistics 22.0. Quantitative data are presented as median and 25th and 75th quartiles (Me [Q25; Q75]). For comparison of paired quantitative data, the Wilcoxon test was used; the Kruskal-Wallis test was used to compare four independent data. To level the lateralization of the process, the data module was analyzed by axial parameters (X, Y). To compare the dynamics before and after rehabilitation measures, the difference in indicators was calculated as a percentage. Spearman's test was used for correlation analysis. Spearman's test was used for correlation analysis. Differences were considered statistically significant at $p \leq 0.05$.

Results. When evaluating stabilometry parameters in patients with both hemiataxy and hemiparesis, statistically significant improvements were obtained in the parameters of the statokinesiogram area, velocity CP, and energic index in both phases of the study. With hemiparesis, an improvement was also revealed in the

Table 1

Patient characteristics

Parameter	Group 1 (patients with hemiataxy) n = 30	Group 2 (patients with hemiparesis) n = 15	p-value
Median of age, years	62.0 [58.75; 68.0]	61.0 [53.0; 64.25]	0.182
Medain time to start rehabilitation after a stroke, days	27.5 [25.0; 39.25]	30.0 [25.0; 50.0]	0.219
Male / female, abs.	21 / 9	19 / 11	0.584
NIHSS, score	3.5 [2.0; 5.75]	6.0 [4.0; 8.0]	0.005*
Rivermead Mobility Index, score	11.0 [8.0; 13.0]	11.0 [7.0; 12.0]	0.549
Barthel index, score	85.0 [80.0; 95.0]	85.0 [71.25; 95.0]	0.403
Rankin scale, score	3.0 [2.0; 3.0]	3.0 [3.0; 4.0]	0.67

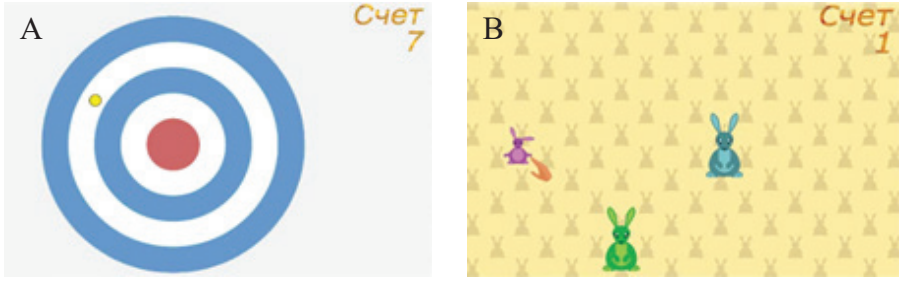

Fig. 1. Stability training: A - static test "Target", the patient, by shifting the center of the pressure, needs to move the yellow dot to the center of the target and hold it in this position for the maximum amount of time (until the end of the test series); B - dynamic test "Hares", the patient needs to move his hand by shifting the center of the pressure to capture the hares

Table 2

Summary results of stabilometry before and after complex rehabilitation

Sign	Significance before training	Significance after training	P-level
Group 1 (patients with hemiataxy)			
X (EO), mm	9.45 [2.35; 13.45]	9.35 [3.95; 16.73]	0.443
X (EC), mm	8.65 [2.58; 15.45]	8.2 [6.1; 18.9]	0.53
Y (EO), mm	10.05 [6.4; 20.75]	12.35 [5.45; 18.6]	0.853
Y (EC), mm	12.65 [3.95; 19.6]	15.3 [6.9; 23.63]	0.504
S (EO), mm²	594.95 [315.6; 941.03]	262.8 [224.0; 421.7]	< 0.001*
S (EC), mm²	1431.85 [694.48; 2393.1]	600.15 [295.95; 895.23]	< 0.001*
V (EO), mm/s	16.35 [15.1; 21.4]	14.15 [11.75; 16.25]	0.001
V (EC), mm/s	26.35 [21.71; 42.35]	20.15 [15.63; 24.85]	< 0.001
Av (EO), мJ/s	299.04 [173.44; 622.3]	167.12 [137.47; 372.63]	0.001
Av (EC), мJ/s	830.16 [475.31; 2283.0]	396.67 [210.53; 1191.36]	< 0.001*
Group 2 (patients with hemiparesis)			
X (EO), mm	6.95 [4.23; 13.95]	8.2 [4.65; 11.23]	0.984
X (EC), mm	7.0 [3.38; 12.75]	8.1 [5.42; 12.6]	0.472
Y (EO), mm	14.2 [8.73; 24.63]	9.3 [5.0; 19.8]	0.041*
Y (EC), mm	14.45 [8.8; 24.53]	12.25 [4.13; 22.23]	0.175
S (EO), mm²	465.1 [360.4; 657.65]	267.85 [186.95; 421.7]	0.003*
S (EC), mm²	766.8 [552.48; 1149.25]	413.55 [211.78; 740.73]	< 0.001*
V (EO), mm/s	10.8 [9.92; 17.2]	10.65 [8.55; 13.4]	< 0.001*
V (EC), mm/s	21.1 [16.56; 26.9]	14.53 [12.83; 21.0]	< 0.001*
Av (EO), мJ/s	175.47 [84.94; 323.62]	108.62 [72.82; 207.23]	0.004*
Av (EC), мJ/s	457.07 [243.9; 928.93]	253.93 [138.04; 573.8]	< 0.001*

* - statistically significant differences.

shift of the CP in the sagittal axis in EO phase, however, the p-value approaches 0.05 (p=0.041). Summary results of stabilometry before and after complex rehabilitation are presented in Table. 2.

We did not reveal differences in the degree of changes in stabilometric parameters in patients with different stroke syndromes (hemiataxia or hemiparesis) before and after complex rehabilitation (p > 0.05) (Fig. 2). However, patients with ataxia initially had statistically significantly larger statokinesiogram areas in the EC phase (p=0.005).

Left boxes - patients of the 1st group (with hemiataxy), right boxes - patients of the 2nd group (with hemiparesis). Abbreviations: CP, center of pressure; phase EO - phase with open eyes; phase EC - phase with closed eyes.

For all clinical scales and indices in all groups, statistically significant differences were obtained before and after complex rehabilitation (Table 3).

Correlation analysis did not reveal the dependence of the dynamics of changes in stabilometric parameters before and after complex rehabilitation on the age of patients, as well as on the time of the start of rehabilitation after a stroke (the maximum period is 129 days). There was no correlation between the dynamics of stabilometric parameters and the dynamics of assessments according to clinical scales (NIHSS, Rivermead mobility index, Barthel and Rankine scales) before and after complex rehabilitation. Before the start of complex rehabilitation, there was also no correlation between stabilometric parameters and clinical scales (p > 0.05), except for a moderate negative correlation between the area of the statokinesiogram in the EC phase and the total score on the Barthel scale in patients with ataxia (r = –0.468, p =0.01).

Discussion. We have shown the use of stabilometry to assess the effectiveness of complex medical rehabilitation, incl. with the use of stability training, in patients with stroke and various neurological deficits (hemiparesis or hemiataxia).

Stabilometry is a highly informative method of objectifying imbalances; in addition, it can be used as biofeedback training in patients with movement disorders [2, 6]. Bronnikov V.A. and co-authors, on the basis of a stabilometric study of patients with stroke before and after rehabilitation, identified 4 variants of parameter changes that reflect different stages of the formation of a pathological stato-locomotor stereotype [7].

We did not set ourselves the task of determining the effectiveness of stability training in the complex rehabilitation of patients with cerebral stroke, since a number of studies have already been devoted to this. A study by Walker C. and colleagues in the early 2000s compared physiotherapy and physiotherapy in combination with biofeedback training in patients with hemiparesis after a stroke. 46 patients who had a stroke within 80 days were included. BFB trainings were held daily for 30 minutes a day until discharge from the hospital. As a result of the study, scientists did not find significant differences in the improvement of motor functions in the two groups and concluded that biofeedback training does not provide benefits in the early rehabilitation of patients with stroke [18].

Barkala L. and colleagues studied the effect of biofeedback balance training in patients with hemiplegia after a stroke. The main group received training using the Nintendo Wii Fit biofeedback system in a playful way along with traditional physical therapy, while the control group received only physical therapy. Training was carried out for 5 weeks, 2 sessions per week for 30 minutes. Scientists did not find a statistically significant difference in the two groups in terms of body symmetry (baropodometry), static balance (stabilometry) and functional independence and concluded that the improvement was achieved through physiotherapy [17].

The effect of biofeedback training on stroke recovery in a later period (more than 3 months) was shown by Srivastava A. and colleagues. A study that included 45 post-stroke patients (mean 16.51± 15.14 months) and 20 sessions of biofeedback training (20 min per day, 5 days per week, for 4 weeks) found statistically significant differences according to the Berg balance scale, functional status and Barthel index [15]. The successful use of stabilometric training using BFB in combination with functional electrical stimulation in the late recovery period of

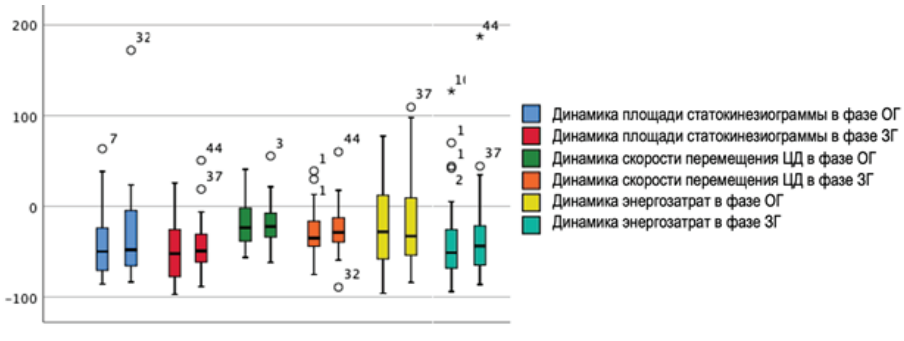

Fig. 2. Changes in stabilometric parameters before and after complex rehabilitation (in % of the initial level).

Table 3

Results of clinical scales and indices in patients of various groups before and after complex rehabilitation

Scales		Group 1	Group 2
NIHSS, scales	Before	3.5 [2.0; 5.75]	6.0 [4.0; 8.0]
	After	1.5 [0.0; 2.0]	3.0 [3.0; 4.0]
	p-value	< 0.001*	< 0.001*
Mobility index Rivermead	Before	11.0 [8.0; 13.0]	11.0 [7.0; 12.0]
	After	13.0 [12.0; 14.0]	14.0 [12.0; 14.0]
	p- value	< 0.001*	< 0.001*
Barthel scale	Before	85.0 [80.0; 95.0]	85.0 [71.25; 95.0]
	After	95.0 [90.0; 100.0]	100.0 [95.0; 100.0]
	p- value	< 0.001*	< 0.001*
Rankin scale	Before	3.0 [2.0; 3.0]	3.0 [3.0; 4.0]
	After	2.0 [2.0; 2.5]	2.0 [2.0; 3.0]
	p-value	< 0.001*	< 0.001*

ischemic stroke was also demonstrated by domestic scientists who conducted training every other day for 5 weeks (15 procedures in total) [1].

Virtual reality technologies have advantages over traditional rehabilitation measures in patients with cognitive decline and motor deficits. This was shown in individuals with cerebral palsy, who showed greater interest, initiative, learning ability and higher motivation when using virtual reality technologies in rehabilitation [19].

The difference of our study is the assessment of stabilometric parameters in patients with various post-stroke syndromes (hemiataxia or hemiparesis). In both groups, we obtained statistically significant improvements in all stabilometric parameters, except for the parameters of the support symmetry (displacement of the CP in the frontal and sagittal axes).

Although improvements were obtained in the process of complex rehabilitation in all clinical scales and indicators, we did not find a significant relationship with stabilometric indicators both before and after rehabilitation. In our opinion, this is due to the different sensitivity of clinical scales and stabilometry to assess the balance function.

The limitations of our study are the lack of consideration of cognitive functions for balance and the outcome of rehabilitation (patients with moderate and severe cognitive impairment were not included in the study). We also did not take into account neuroimaging data (volume, location of ischemia or intracerebral hematoma).

Thus, stabilometry is a highly informative method for studying the balance function in patients after a stroke in complex rehabilitation, however, the lack of correlation of parameters with clinical scales should be taken into account. Further studies may be aimed at identifying the role of cognitive impairment in the process of complex rehabilitation of patients after a stroke using stabilometry.

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RELATIONSHIP OF THE SYSTEMIC LEVEL OF CHEMERIN AND CLINICAL AND LABORATORY PARAMETERS IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Objective: to study the relationship between the level of chemerin in blood serum and clinical and laboratory parameters of patients with RA.

Materials and methods. We observed 88 women diagnosed with RA, the average age was 56.4 years [47.5-60.7]. Disease activity according to DAS 28 was 3.50±1.11 (3.27-3.74) points. Articular erosions were present in 69 (78.4%) patients. Serum chemerin levels were determined using the commercial HUMAN CHEMERIN ELISA kit (BioVendor). Statistical processing was performed using the Statistica 12.0 software package for Windows.

Results. The average level of chemerin in patients with RA was 463.5 ng/mL [366-576.5]. The level of chemerin in the blood serum has a direct correlation with the weight and BMI of patients, with the number of painful joints, as well as with the average annual dose of corticosteroids. The average concentration of chemerin in patients with an early stage of RA was significantly higher than in patients with an advanced stage (p=0.037). The difference between the level of chemerin in patients with early and late stages of RA is also close to statistically significant (p=0.066). The level of chemerin is significantly higher in RA patients with diabetes mellitus (p=0.007). The relationship was found chemerin with the level of CRP (Spearman - R=0.272758, p=0.010139). No correlations were found between the concentration of chemerin in the blood serum and the state of the bone tissue.

It can be assumed that chemerin is a typical pro-inflammatory adipokine that modulates inflammation in RA. In our study, in patients with RA, a positive correlation was found between the level of chemerin and weight, as well as BMI of patients. The level of chemerin is significantly higher in RA patients with diabetes mellitus.

Keywords: chemerin, rheumatoid arthritis, systemic inflammation, insulin resistance.

Introduction. In recent years, a class of biologically active substances called adipokines has been dynamically studied. It is well known that adipokines have a wide spectrum of activity, in particular, they play an important role in the development of immune responses and inflammation [4]. Chemerin is one of the least studied adipokines belonging to the cathelicidin/cystatin family of proteins, consisting of cathelicidin antibacterial proteins and cysteine protease inhibitors [5].

Unlike other adipokines, chemerin is present not only in adipose tissue, but is widely distributed in many organs. According to the Human Protein Atlas (HPA) database, chemerin mRNA is highly expressed in endocrine tissues (adrenals, parathyroid, etc.), liver, pancreas, female reproductive system (ovaries, cervix, endometrium, etc.), adipose tissue, lungs, kidneys and colon [6].

Recent studies have shown that chemerin plays an important role in modulating both physiological and pathophysiological processes. In animal experiments, Chemerin exhibits both pro-inflammatory and anti-inflammatory properties [9]. By reacting with the ChemR23 receptor, chemerin is involved in the early stage of acute inflammation. Presumably, chemerin synthesis is enhanced by pro-inflammatory cytokines, such as tumor necrosis factor alpha. Serum chemerin levels are significantly elevated in many inflammatory diseases [3]. Moreover, some authors believe that Chemerin is a potential marker of inflammation activity [1].

Many studies confirm that the level of circulating chemerin is closely and positively associated with body mass index (BMI), the amount of visceral fat and blood pressure [7]. To date, a relationship has been proven between serum levels of chemerin and conditions such as metabolic syndrome, obesity, and insulin resistance [10].

In our opinion, the elucidation of the pathological mechanisms of action of Chemerin can contribute to the development of new therapeutic methods for the treatment of various inflammatory diseases. However, at present, only a small number of studies have been conducted on the study of Chemerin in patients with a rheumatological profile. A number of studies have shown that the level of chemerin is associated with the activity of the RA disease [8]. However, in the literature available to us, we found single studies on the role of chemerin in the pathogenesis of rheumatoid arthritis (RA), and the relationship between the level of chemerin and comorbid pathology in this disease is also poorly covered.

Objective: to study the relationship between the level of chemerin in blood serum and clinical and laboratory parameters of patients with RA.

Materials and methods. The study was conducted on the basis of the Federal State Budgetary Scientific Institution "Research Institute of Clinical and Experimental Rheumatology named after N.N. A.B. Zborovsky, Volgograd. Inclusion criteria: age from 18 to 70 years, the presence of a diagnosis of RA. that RA is much more common in females, and that,

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according to the literature, the level of chemerin in identified individuals occurs in men and women, only females were identified in our study. Under our observation there were 88 women diagnosed with RA, the average age was 56.4 years [47.5-60.7], the duration of the disease was 10.0 years [4.5-17.0]. BMI was 27.5 kg/m2 [23.4-61.8]. According to the clinical stage, the patients were distributed as follows: early stage - 12 (13.6%) patients, advanced - 31 (35.2%), late - 45 (51.1%). Stage I radiological changes were in 8 (9.1%) patients, stage II - in 33 (37.5%), stage III - in 44 (50.0%), stage IV - in 4 (4.5%) patients. Disease activity according to DAS 28 was 3.50±1.11 (3.27-3.74) points. The number of painful joints (NPJ) and the number of swollen joints (NSJ) were 4.72+3.87 and 0.91+1.75, respectively. In the study group, patients positive for RF and ACCP prevailed (respectively, 68 (77.3%) and 59 (67%) patients). Articular erosions were present in 69 (78.4%) patients. The regimen of glucocorticosteroids (GCS) is shown in Table 1.

Table 1

The mode of taking GCS in patients of the study group

Cumulative dose of corticosteroids, g, Me (Q1-Q3)	5450 [363-13250]
Duration of GCS therapy, months, Me (Q1-Q3)	30 [10-72]
Number of patients currently taking corticosteroids, n (%)	57 (64,8)
The average dose of GCS at the moment, mg, Me (Q1-Q3)	5.0 [0-8]

Serum chemerin levels were determined using the commercial HUMAN CHEMERIN ELISA kit (BioVendor) according to the attached instructions. The reference values specified by the manufacturer in the instructions are given in table 2.

Table 2

Reference values of the level of chemerin in healthy females

Age, years	Chemerin level, ng/ml
3-19	207.2±28.3
20-39	199.3±29.6
40-59	206.4±42.2
60-79	247.5±63.1

Statistical processing was performed using the Statistica 12.0 software package for Windows. Most measures were non-parametric, so data are presented as median and first-third quartile (Me [Q1-Q3]). In the cases of a parametric measure, data are presented as the mean, standard deviation, and 95% confidence interval of the mean (M±Std. dev (95% CI). To calculate non-parametric indicators, Spearman's correlation (ρ) and Pearson's correlation (r –) were used. For intergroup analysis, an analogue of Student's t-test (t) was used, in the case of non-parametric data, Mann-Whitney analysis (Z). Comparison of several groups was carried out using the Kruskall-Wallis method (analogous to Anova for non-normally distributed data, presented in the tables as K-W χ2). For parametric indicators, post-hoc analysis of the ANOVA test was used.

Results. The average level of chemerin in patients with RA was 463.5 ng/mL [366-576.5]. We conducted a correlation analysis of the level of Chemerin in the blood serum with a number of clinical and laboratory characteristics of RA, as well as the dose of glucocorticosteroids (GCS) taken (see Table 3).

From the data obtained in Table 3, it follows that the level of chemerin in the blood serum has a direct correlation with the weight and BMI of patients, with the number of painful joints, as well as with the average annual dose of corticosteroids. Thus, we can assume the existence of a relationship between these indicators and the concentration of chemerin in the blood serum of patients with RA.

We also conducted an intergroup analysis of the level of Chemerin depending on the indicators of RA and comorbidity (Table 4).

According to the data presented, the average concentration of chemerin in patients with an early stage of RA was significantly higher than in patients with an advanced stage (p=0.037). The difference between the level of chemerin in patients with early and late stages of RA is also close to statistically significant (p=0.066). We also note that the level of chemerin is significantly higher in RA patients with diabetes mellitus (p=0.007). There were no correlations between BMI and the presence of diabetes mellitus with the GCS regimen.

When conducting a correlation analysis of chemerin with a number of general laboratory parameters, a relationship was found with the level of CRP (0,14±0,06, min 0,003, max 0,21 g/l Spearman - R=0.272758, p=0.010139), which is fully consistent with the literature data on the role of chemerin in the development of inflammatory reactions.

Considering the wide range of biological effects of adipokines, as well as the influence of many adipokines on bone metabolism, we investigated a possible relationship between the level of chemerin and indicators of bone metabolism (Table 5).

According to Table 5, no correlations were found between the concentration of chemerin in the blood serum and the state of the bone tissue.

Discussion of results and conclusions. Currently, the effect of Chemer-

Table 3

Chemerin level and correlation analysis with clinical and laboratory characteristics of patients

	Chemerin
Correlations with common factors:	
Age	0.232; p=0.030
Weight	0.254; p=0.017
Height	0.047; p=0.66
BMI	0.212; p=0.047
insulin resistance	-0.1; p=0.353
Correlations with RA:	
Duration	-0.079; p=0.462
RF	-0.075; p=0.488
ACCP	-0.144; p=0.18
DAS 28	-0.027; p=0.8
NPJ (number of painful joints)	0.213; p=0.046
NSJ (number of swollen joints)	0.166; p=0.123
Visual analog scale (VAS)	0.035; p=0.746
Dose of corticosteroids at present	ρ=0.025; p=0.825
Cumulative dose of corticosteroids	ρ=0.029; p=0.806
Duration of corticosteroids	ρ=-0.053; p=0.647
Average annual dose of corticosteroids	ρ=0.257; p=0.025

Note. ADCP - antibodies of cyclic citrulline peptide.

Table 4

Intergroup analysis of the level of chemerin depending on the parameters of RA and comorbidity of patients

	Чемерин
ACCP «+» (n=59) «-» (n=29)	Z=-0.435; p=0.664 464.0 [348.0-581.0] 437.0 [374.0-547.0]
RF «+» (n=68) «-» (n=20)	Z=-0.313; p=0.754 470.5 [363.0-576.5] 458.0 [381.5-577.0]
Stage of RA Early - 12 Deployed - 31 Late - 45	F=2.33; p=0.103 M=589.09±222.1 (448.0-730.2) M=469.29±148.8 (415.5-523.1) (p=0.037) M=488.8+161.8 (440.2-537.4)
Activity on DAS 28 1 - 19 2 – 11 3 - 51 4 - 7	K-W χ^2=0.31; p=0.959 462.0 [360.0-528.0] 497.0 [366.0-631.0] 463.0 [360.0-593.0] 499.0 [383.0-540.0]
X-ray stage 1 - 8 2 - 34 3 - 42 4 - 4	K-W χ^2=3.49; p=0.312 501.0 [379.5-581.0] 498.0 [374.0-591.0] 428.0 [360.0-540.0] 496.5 [452.5-574.0]
Presence of erosion Да 69 Нет 19	Z=-0.690; p=0.490 460.0 [366.0-572.0] 518.0 [360.0-603.0]
Function class 1 - 25 2 - 55 3 - 8 4 - 0	K-W χ^2=0.56; p=0.906 497.0 [360.0-603.0] 477.0 [371.0-581.0] 428.0 [324.5-484.5]
The presence of diabetes Yes (10) No (78)	Z=-2.69; p=0.007 598.0 [561.0-717.5] 479.5 [366.0-593.0]

in on systemic inflammation has been confirmed by experimental studies [2]. In our opinion, there are several possible explanations for the fact that higher concentrations of chemerin in patients with RA were detected in the early stages of the disease. On the one hand, it can be assumed that chemerin is a typical pro-inflammatory adipokine that modulates inflammation in RA, as in many other chronic diseases. In the future, in the later stages, against the background of a decrease in the level of systemic inflammation and disease activity, the serum level of Chemerin also tends to decrease. In this context, our data on the relationship between the level of chemerin and such a classical marker of inflammation as CRP also confirm this assumption. On the other hand, it is possible that Chemerin has an anti-inflammatory effect and is produced by the body as a response to the appearance of systemic inflammation. This point of view also has the right to exist, and final conclusions can only be drawn based on the results of subsequent large-scale studies.

Table 5

Correlation between the level of chemerin in blood serum and indicators of bone metabolism

Correlations with indicators of bone metabolism: МПКТ L1-L4 – 1.07 (0.95-1.15) МПКТ Neck – 0.89 (0.77-0.97) МПКТ Total – 0.94 (0.80-1.01) CTX-1 - 0.68+0.38 P1NP - 59.36+49.34	 ρ=0.005; p=0.960 ρ=0.011; p=0.921 ρ=-0.001; p=0.992 ρ=-0.046; p=0.668 ρ=0.025; p=0.814

Considering the important role of chemerin in the metabolism of adipocytes and adipogenesis, as well as the positive correlation of the level of chemerin in the blood serum with BMI, a significant role of this adipokine in metabolic diseases can be assumed. Since abdominal obesity is pathogenetically closely associated with insulin resistance and the development of type 2 diabetes mellitus directly depends on the presence of systemic inflammation, the data on a higher systemic level of Chemerin in patients with diabetes are logical and easily explained.

It is known that systemic inflammation is initially induced by excess visceral fat. In the future, as inflammation progresses, in different groups of patients, depending on the underlying disease and comorbidity, both a decrease in weight and BMI, and their increase can occur. In our study, in patients with RA, similarly to patients of other groups described in the literature, a positive correlation was found between the level of chemerin and weight, as well as BMI of patients. It can be assumed that systemic inflammation that develops against the background of RA, under the condition of an increased concentration of chemerin, does not lead to weight loss and a drop in BMI.

Thus, the study of the role of Chemerin in the development of systemic inflammation against the background of RA, as well as its relationship with clinical and laboratory parameters and comorbidity, is promising and practically significant.

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OSTEOBLASTIC METASTASES IN A PATIENT WITH POORLY COHESIVE GASTRIC CARCINOMA

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The article presents a clinical observation of osteoblastic metastases in a patient with poorly cohesive gastric carcinoma after several years of radical surgery. Multiple osteoblastic metastases sharply worsen the quality of life, adversely affect survival rate, and require timely comprehensive examination of patients with this pathology for early detection of "silent" bone metastases in order to improve long-term results of treatment.

Study objective: to describe a clinical case of osteoblastic metastases in poorly cohesive carcinoma.

Patient L., male, 48 years, was admitted to the RH#1 - NCM Therapy Department (Yakutsk, Russia) for examination with complaints of severe general weakness, severe pain in the hip joints and both lower extremities, sacrum, intensifying during walking and lying down, decreased appetite, dyssomnia due to pain syndrome.

In the Therapy Department the patient underwent CBC, biochemical analysis of blood, instrumental research. He received symptomatic treatment: table 5 diet, Nutriflex 40/80 intravenously. for parenteral nutrition, omeprazole with an antiulcer purpose, tramadol 2.0 ml and diclofenac 3.0 ml for pain control, vitamins B12 200 mcg per day, ketotifen 1 mg 1 time in the evening with a sedation. The condition is unchanged, the expressed pain syndrome in the hip joints and sacrum remains.

The patient was discharged with recommendations and referred to the Yakutsk Republican Oncological Dispensary to an oncologist and a chemotherapist for the selection of effective analgesic drug therapy and possible local radiation therapy.

Conclusion. Poorly cohesive gastric carcinoma is often diagnosed at late stages and has a poor prognosis. Our clinical case demonstrates that this form of cancer can be a source of multiple osteoblastic metastases, which drastically worsens the quality of life and adversely affects survival rate, and requires a timely comprehensive examination of patients with this pathology for early detection of "silent" bone metastases in order to improve long-term treatment results.

Keywords: poorly cohesive gastric carcinoma, osteoblastic metastases, computed tomography, bone scintigraphy.

Gastric cancer (GC) is one of the most common types of malignant tumors worldwide. In 2021, it caused 1 089 103 new cases and 768 793 deaths and ranked 6th in incidence and 3rd in mortality among all malignant neoplasms [1]. In 2021, in Russia, gastric cancer was newly diagnosed in 18.672 men and 13.359 women [4]. In the general structure (both sexes) of the incidence of malignant neoplasms in Russia, gastric cancer took the 6th place and in the structure of the mortality of the Russian population from malignant neoplasms - the 2nd place [3]. At the same time, gastric cancer is detected in Russia most often in the last stages, when there are already distant metastases (41.2% fall on the 4th stage). Most often, hematogenous metastasis of gastric cancer affects the liver and lungs. Bone metastases are detected in 0.9–2.8% of cases and rising to 13% at autopsies. The median survival time for detecting bone metastases is reduced to 100 days. Risk factors for the development of bone metastases in patients with gastric cancer include smoking, poorly differentiated adenocarcinoma, high levels of lactate dehydrogenase, alkaline phosphatase, and cancer embryonic antigen (CEA). The ability of malignant cells of malignant tumors to metastasize is determined by many factors: the weakening of intercellular contacts, the disruption of the connection of cells with the matrix, the acquisition of the ability of tumor cells to move. In addition, malignant cells produce a number of proteolytic enzymes that enable them to penetrate the vascular wall; they can maintain their viability in the liquid medium of the transport channel, and then again, having passed through the vascular wall, go beyond it and end up in organs far from the site of the primary localization of the tumor. Depending on the predominance of the type of bone tissue destruction in tumor lesions, 3 types of metastases are conditionally distinguished: *osteolytic, osteoblastic,* and *mixed*. *Osteolytic metastases* are characterized by the destruction of normal bone tissue. With this type, there is a high frequency of pathological fractures, which are most often observed in breast cancer, multiple myeloma, kidney cancer, non-small cell lung cancer. The destruction of bone tissue is mainly due to the activity of osteoclasts and is not a consequence of the direct impact of the tumor. *Osteoblastic (sclerotic) metastases* are characterized by pathological osteogenesis, in which the density of the resulting new bone tissue may be higher than normal values. They occur in prostate cancer [2], small cell lung cancer. The mechanism of formation of such metastases is still not well understood. *Mixed*

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metastases, characterized by the simultaneous presence of both lytic and blastic foci in the patient or the presence in the metastasis of both areas of bone tissue rarefaction and foci of osteosclerosis, are more common in breast cancer, stomach cancer, colorectal cancer. Analysis of the results of the biopsy material showed that there are no only osteolytic or only osteoblastic foci, since the processes of resorption and restoration of bone tissue proceed in parallel in both types of foci, but with different intensity [5]. Currently, methods such as computed tomography, magnetic resonance imaging, and bone scintigraphy are widely used to detect bone metastases [3].

Study objective: to describe a clinical case of osteoblastic metastases in poorly cohesive carcinoma.

Patient L., male, 48 yrs, was admitted to the RH#1 - NCM Therapy Department for examination with complaints of severe general weakness, severe pain in the hip joints and both lower extremities, sacrum, worsening during walking and lying down, decreased appetite, dyssomnia due to pain syndrome.

It is known from the anamnesis that in August 2020, in the Yakut Oncological Dispensary, the patient underwent a gastroenteroanastamosis with entero-enteroanastomosis for signet ring cell carcinoma, which, according to the new WHO classification of gastric epithelial tumors of 2019, belongs to one of the types of poorly cohesive carcinomas [6]. In August 2021 in Moscow, the patient underwent a radical operation - gastrectomy by abdominal access with removal of gastroenteroanastomosis and entero-enteroanastomosis and with planar resection of the pancreatic head (due to a carcinomatous lesion of the pancreatic capsule), D2 lymph node dissection (removal of lymph nodes of the 1st and 2nd order). The result of a histological study is a poorly cohesive carcinoma of the stomach of the diffuse type according to Lauren, with high mitotic activity and the presence of cancer emboli in the lumen of blood vessels, with perineural invasion and infiltration and ulceration of the mucous membrane, with germination of the submucosal layer and muscle membrane of the organ without germination of the serosa of the stomach, small omentum, with metastases in 2 (out of 16) lymph nodes along the lesser curvature of the stomach and 2 (out of 5) along the greater curvature of the stomach. Immunohistochemical study did not reveal the expression of the HER-2 oncoprotein in the tumor tissue. After the operation, he received courses of adjuvant polychemotherapy and felt satisfactory and continued to work as a mechanic. The patient's condition began to deteriorate since August 2022, when headaches appeared, in connection with which the patient was examined at the Yakutsk Republican Oncological Dispensary.

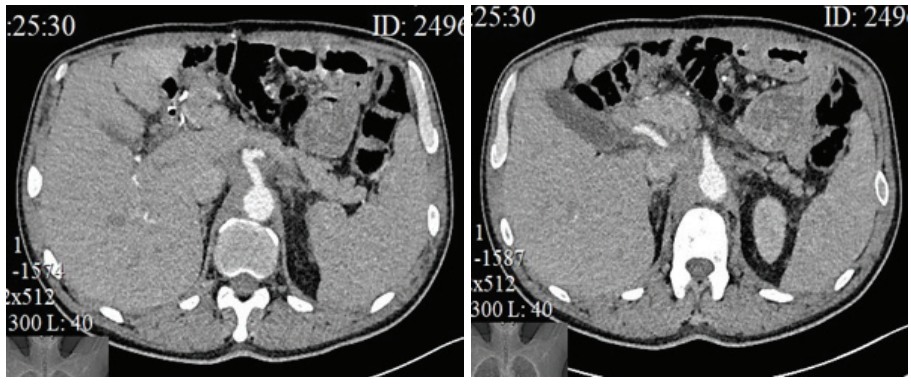

Fig. 1. Abdominal CT scan. CT signs of soft tissue infiltration around the abdominal aorta

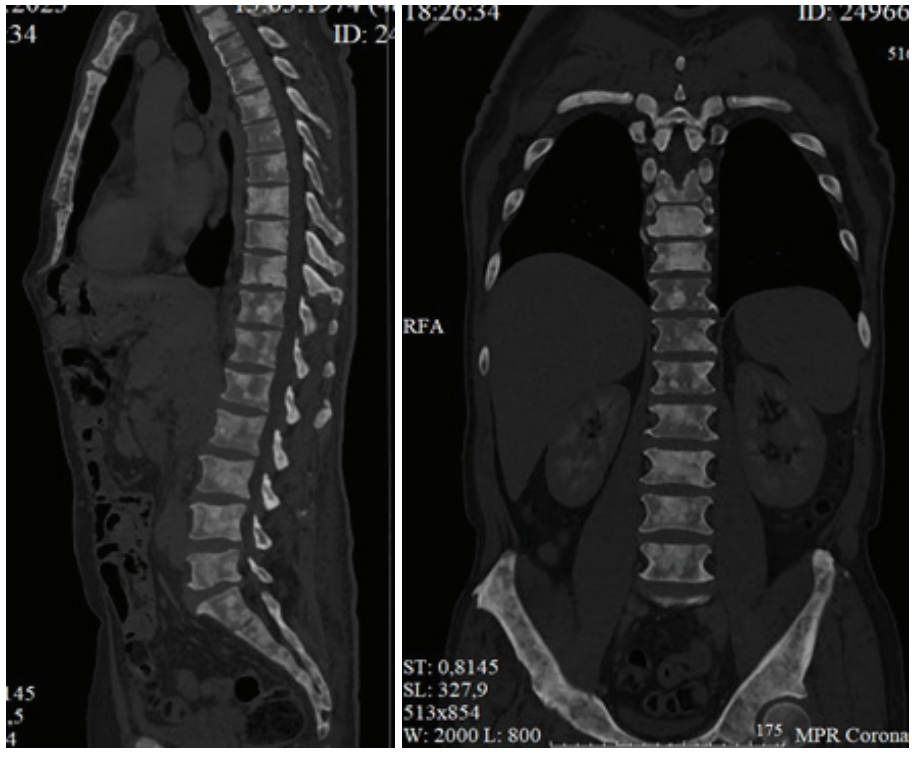

Fig. 2. CT of bones. Multiple foci of osteosclerosis in the bones

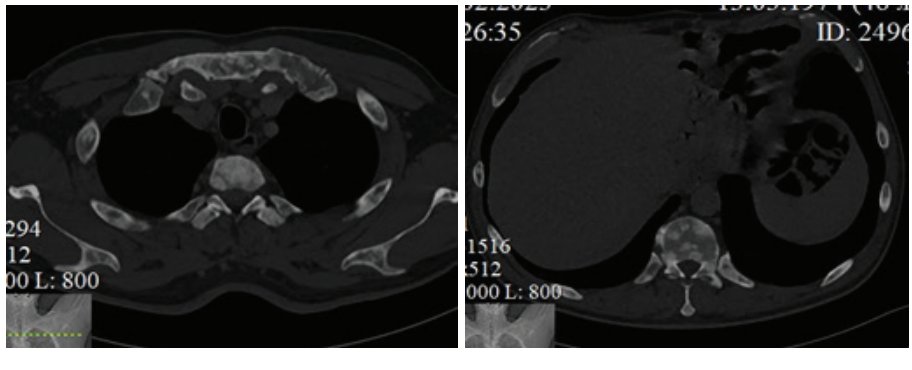

Fig. 3. Multiple foci of osteosclerosis in the bones in the chest, in the vertebrae

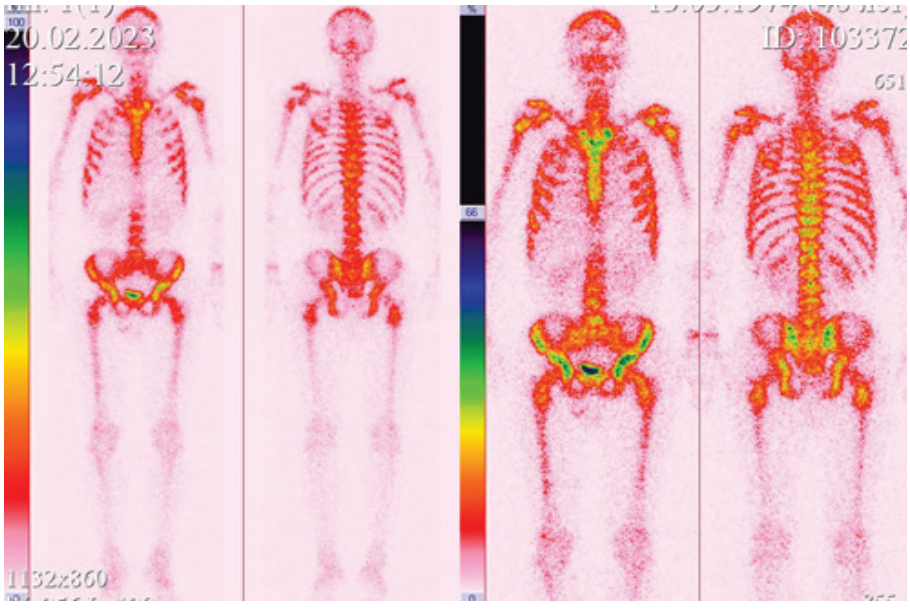

Fig. 4. Scintigraphic signs of multiple focal lesions of the hyperfixation of the radiopharmaceutical in the bones of skeleton

PET/ CT: data in favor of the presence of metabolically active tissue was not detected, hepatosplenomegaly. Ametabolic cystic cavity in the region of the iliopsoas muscle.

EGDS: condition after gastrectomy. Data for the recurrence of the disease was not revealed.

MRI of the brain: MRI picture of the cortical focus of gliosis in the occipital lobe on the right. There is no intracranial hypertension.

MRI of the abdominal cavity and small pelvis: there were no clear data for local recurrence and metastases in the studied areas. Single small cysts S 5-6 of the liver.

Colonoscopy: signs of colonoptosis and dolichosigma. No tumor pathology was detected in the examined departments.

In blood tests, tumor markers (total PSA, free PSA, AFP, CEA, CA 19-9) were within normal limits.

Since January 2023, severe pains in the pelvic bones and both lower extremities began to disturb. The patient was hospitalized in the Therapy Department of RHN№1-NCM to clarify the diagnosis.

On admission, the patient's general condition was severe. Patient changes his position slowly due to pain. Consciousness is clear. The skin and mucous membranes are clean, normal color, moderate humidity. There are no rashes. Peripheral lymph nodes are not enlarged, painless. Respiratory rate 16 per min. Vesicular breathing, no wheezing. Heart rate 67 bpm. SpO2 97%. Heart sounds are rhythmic, muffled. BP - 140/83 mm Hg. Tongue is wet, lined with white coating. Auscultation of the abdomen: intestinal peristalsis is auscultated. The abdomen is soft and painless. The liver is not enlarged. The spleen is not palpable. The chair is regular, decorated. The symptom of tapping is negative on both sides. Urination free, painless. There is no peripheral edema.

The biochemical analysis of blood revealed the following deviations from the norm: Ferritin ↑ 1428.00 mcg/l (20.00 - 250.00). Total cholesterol ↑ 7.05 mmol/l, total protein ↓ 59.30 g/l, LDH ↑ 530.90 u/l. C-reactive protein ↑ 49.92 mg/l (0.00 - 5.00).

In the coagulogram: prothrombin index ↓ 55.00%, prothrombin time ↑ 19.50 sec, INR ↑ 1.40, Fibrinogen ↑ 5.59 g/l. CBC: RBC ↓ $2.87*10^{12}$/l; WBC $6.08*10^{9}$ /l; Hgb ↓ 90.00 g/l, Hct ↓ 27.60%, PLT ↓ $127.00*10^{9}$/l, PCT ↓ 0.12%. In the leukocyte formula: stab neutrophils ↑ - 12.0%, ESR according to Panchenkov ↑ 61.00mm.

Instrumental Research data:

Abdominal CT scan: CT signs of soft tissue infiltration around the abdominal aorta (Fig. 1) and involving the body of the pancreas (relapse?). Multiple foci of osteosclerosis in the bones, possibly secondary (Fig. 2, 3).

Duplex scanning of vessels (arteries and veins) of the lower extremities showed no changes in the veins of the lower extremities.

Needle Electromyography (one muscle): according to EMG, a violation of the conduction along the peroneal nerve on the right of a rather pronounced degree, proximal sections.

MRI of the abdominal organs with contrast: the liver is not enlarged in size; its contours are even and clear. In the S4 segment of the right lobe, a small cyst up to 4 mm in size is determined, otherwise the structure of the parenchyma is homogeneous. The gallbladder is not enlarged, with clear, even contours, the bottom of the bladder is deformed like a "Phrygian cap". In the area of the body along the back wall, a local endophytic thickening of the bladder wall 3 mm in size is determined, adsorbing the paramagnetic preparation. MR-signal of the contents of the ducts is homogeneous. The intra- and extrahepatic bile ducts are not dilated, the common bile duct is 7 mm. Portal vein - 17mm. The spleen is enlarged, its dimensions are 141x53x-112mm (SI=836), it has a homogeneous structure. The pancreas is fully visualized, dimensions: head - 25mm, body - 24mm, tail - 18mm. The contours of the pancreas are clear, the parenchyma is homogeneous, without changes in the MR signal. Wirsung's duct is not dilated. Parapancreatic adipose tissue is not changed. The kidneys are usually located, with even and clear contours, not enlarged in size. The cortex and medulla are well differentiated, without changes in the MR signal. Pelvicalyceal system and ureters are not dilated. Perinephric tissue on the left is heavy and slightly edematous, against which the left adrenal gland is not clearly contoured. The right adrenal gland is not changed. No additional volumetric formations were found in the study area. Paraaortally, at the level of the supra-, juxta- and infrarenal sections of the abdominal aorta, a pathological infiltrate is determined, without clear boundaries, circularly surrounding the aorta itself and the proximal sections of the renal arteries, the celiac trunk, for about 109 mm, weakly and homogeneously absorbing the paramagnetic preparation. At the same time, the lumen of the abdominal aorta and its paired and unpaired branches are not narrowed. The inferior vena cava is not dilated. Enlarged lymph nodes of the abdominal cavity and retroperitoneal space were not revealed. DWI (b1000) revealed a pathological infiltrate with limited diffusion. At the study level, there is a diffuse decrease in the MR characteristics of the vertebral bone marrow signal on T2WI.

Conclusion. Pathological infiltrate at the level of the supra-, juxta- and infrarenal parts of the abdominal aorta, most likely, there is a secondary (metastatic) lesion of the para-aortic lymph nodes.

EGDS: esophageal mucosa is pale pink. The dentate line is clear. Cardia closes. The anastomosis is functioning. In the area of the adductor loop, a seal up to 0.5 cm (scar?) is determined, 2 biopsy fragments are taken. Hemostasis with cold NaCl solution 10% 10.0 ml. The mucosa of the efferent loop of the small intestine is pale pink. With biopsy: 2 fragments. ***Conclusion.*** Condition after gastrectomy, a biopsy was taken. The result of the biopsy: the tumor process was not detected.

Bone scintigraphy: planar scintigraphy of the skeleton, performed in the anterior and posterior projections, 3 hours after the introduction of the radiopharmaceutical, zones of hyperfixation of the radiopharmaceutical are determined in the proximal ends of the humerus and femurs, in the ribs, in the ilium and ischial bones of the pelvis on both sides.

Conclusion. Scintigraphic signs of multiple focal lesions of the shoulder and femur bones, ribs, sternum, pelvic bones on both sides (Fig. 4).

On the basis of complaints, anamnesis and studies, the final clinical diagnosis was made: Poorly cohesive carcinoma of the stomach, carcinomatosis of the pancreatic capsule. Condition after laparotomy, gastroenteroanastamosis from 08.2020. Condition after installation of the port system from 09.2020. Condition after 4 courses of polychemotherapy (PCT). Condition after 4 courses of PCT (second line). Progression of the disease (12.2020). Condition after 3 courses of PCT (second line). Condition after gastrectomy by abdominal access with removal of gastroenteroanastamosis and enteroenteroanastamosis and planar resection of the pancreatic head, lymph node dissection D2 (03.2021). pT3N1M1. Condition after 4 adjuvant courses of PCT. Progression of the disease: multiple metastases in the pelvis, femur and shoulder bones, ribs; limited soft tissue fibrosis around the upper half of the abdominal aorta and with probable involvement of the body of the pancreas. Severe pain syndrome, poorly controlled by non-steroidal anti-inflammatory drugs (NSAIDs).

The patient received symptomatic treatment: table 5 diet, Nutriflex 40/80 intravenously. for parenteral nutrition, omeprazole with an antiulcer purpose, tramadol 2.0 ml and diclofenac 3.0 ml for pain control, vitamins B12 200 mcg per day, ketotifen 1 mg 1 time in the evening with a sedation. The condition is unchanged, the expressed pain syndrome in the hip joints and sacrum remains. The patient was discharged with recommendations and referred to the Yakutsk Republican Oncological Dispensary to an oncologist and a chemotherapist for the selection of effective analgesic drug therapy and possible local radiation therapy.

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NEUROPSYCHOLOGICAL STUDY OF PATIENTS WITH SPINOCEREBELLAR ATAXIA TYPE 1

This article describes the neuropsychological study of patients with spinocerebellar ataxia type 1 according to the clinic register YSC CMP. The purpose of the study was to identify neuropsychological features of SCA1. This study involved 34 patients, of whom 9(26,5%) were men and 25(73,5%) women. The average age of the patients is 52. As a result of the study, neuropsychological features manifested in SCA type 1 were identified.

Keywords: neuropsychological research, spinocerebellar ataxia type 1, neurodegenerative disease.

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Spinocerebellar ataxia type 1 is an inherited disorder that is transmitted through an autosomal dominant inheritance type. Autosomal Dominant Type 1 SCA is a neurodegenerative disorder caused by trinucleotide re-expansion CAG within the coding region of the gene *Ataxin1* (*ATXN1*), which is characterized by a progressive type of course, clinical polymorphism. SCA1 are considered

a rare group of cerebellar ataxias, with an average prevalence of 2.7 cases per 100,000 population. Their most common forms are polyglutamine expansion diseases (ATXN1 / SCA1, ATXN2 / SCA2, ATXN3 / SCA 3, CACNA1A /SCA 6, ATXN7 /SCA 7, TBP /SCA 17, and ATN1 /DRPLA) [4]. Yakutia is a hotbed of accumulation of SCA1 type not only in Russia, but also in the world. In 1997, the prevalence of SCA1 among the Yakut's was 35 cases per 100,000 populations, and now this figure has increased to 77.6 cases [4]. This is the most common type of SCA. One of the features of the prevalence of this disease in Russia is the uneven distribution. According to WHO, the total incidence rate is 1-2 per 100 thousand populations, but in the eastern part of Russia, SCA1 is much more common - up to 48 people per 100 thousand populations, primarily due to Yakutia [3]. The significant increase in the prevalence of SCA1 can be explained by the introduction of the system of family case registration and modern molecular genetic methods of mutation diagnosis. On the other hand, monitoring results indicate the ongoing accumulation of mutation in the Yakut population. In addition, the boundaries of known geographic foci of mutation accumulation were expanded and the population heterogeneity of mutation carriers was assumed by the number of CAG repeats [4].

The clinical pattern is characterized by cerebellar-pyramidal syndrome, which is manifested by pronounced ataxia, impaired coordination of movements, paresis, speech impairment. As a result, as the disease progresses, patients cease to serve themselves, completely depend on loved ones in their environment, lead to social maladaptation [6]. Death occurs from intercurrent diseases, most often from respiratory failure, which is the result of aspiration and congestive pneumonia.

Thus, the spread of type SCA1 disease in Republic of Sakha (Yakutia) is a medical and social problem, since the working population suffers, the number of disabilities increases, the costs of long-term therapy and rehabilitation of patients increase. Treatment of SCA1 patients remains supportive, symptomatic, as there is currently no known therapy to delay or stabilize the neurodegenerative process in this disease. At the same time, the study of cognitive sphere in this category of patients could help in the development of a personalized approach in the provision of specialized medical care.

Purpose of the research - studying the cognitive sphere and features of its impairment during neuropsychological examination of patients with SCA type 1.

Materials and methods. In total, 199 patients with various neurodegenerative diseases were included in the hospital register of the YSC CMP clinic from January to May 2023 for neuropsychological research, of which 34 patients diagnosed with SCA type 1 (17.9%). According to the hospitalization log of the neurological department of the YSC CMP clinic, in 2020 the number of patients with SCA type 1 was 40, in 2021 - 57, in 2022 - 114. The increase in hospitalizations is associated with the limitation of hospitalizations in the context of the COVID-19 pandemic in 2020 and 2021.

Criteria for inclusion of patients in the study:

1. Patients with a confirmed diagnosis of SCA type 1 by molecular genetic testing.
2. Registration on the territory of the Republic of Sakha (Yakutia).
3. Availability of a signed informational consent form for patients or their legal representative to participate in the study.

Criteria for exclusion of patients from the study:

1. Absence of a verified diagnosis of SCA type 1.
2. No registration in the territory of the Republic of Sakha (Yakutia).
3. Unwillingness of the patient or his/her legal representative to comply with the protocol of this study.

Research Methods:

1. Modified version of A.R. Luriia's neuropsychological research scheme [2].
2. Schulte Table Test - the method was used to evaluate attention.
3. The assessment of task performance was carried out according to the modified scheme of quantitative assessment by Zm.M. Glozman [1].

Table 1

Analysis of the mutual distribution of frequencies by gender and signs of neuropsychological symptoms

	Gender		Pearson's chi-squared test (p)	
	man	woman		
Praxis				
norm	23.5% (n=8)	61.8% (n=21)	0.126	0.723
light disorders	2.9% (n=1)	11.8% (n=4)		
Speech				
accuracy of pronunciation	2.9% (n=1)	14.7% (n=5)	3.56	0.168
insufficiently clear pronunciation	0% (n=0)	17.6% (n=6)		
dysarthria	23.5% (n=8)	41.2% (n=14)		
Handwriting				
norm	2.9% (n=1)	14.7% (n=5)	0.36	0.55
light disorders	23.5% (n=8)	58.8% (n=20)		
Mindset				
norm	26.5% (n=9)	70.6% (n=24)	0.371	0.543
moderate intellectual-mnestic decline	0% (n=0)	2.9% (n=1)		
Memory				
norm	8.8% (n=3)	41.2% (n=14)	1.36	0.244
reduced memory capacity	17.6% (n=6)	32.4% (n=11)		
Calculate				
norm	26.5% (n=9)	58.8% (n=20)	2.11	0.55
counting errors	0% (n=0)	2.9% (n=1)		
forgetting the interim score	0% (n=0)	5.9% (n=2)		
reducing the speed of calculations	0% (n=0)	5.9% (n=2)		
Attention				
slight decrease	14.7% (n=5)	29.4% (n=10)	1.16	0.762
moderate decline	8.8% (n=3)	29.4% (n=10)		
pronounced decrease	2.9% (n=1)	8.8% (n=3)		
significant decrease	0% (n=0)	5.9% (n=2)		

Scoring of the performance of each sample on a 6-point scale. Error-free execution - evaluated at 0 points. Slow entry into the task, or 1 error with self-correction - 0.5 points. Slow but smooth execution or single impulsive errors with self-correction followed by correct execution - 1 point. Making at least three mistakes, corrected by teaching or speaking the program with subsequent correct execution - 1.5 points. Making errors in more than half of the performed sample, with partial correction - 2 points. Inability to perform a sample - 3 points [1]. In further work, the primary scores, after coding, were translated into a percentage coefficient.

In the study of gnosis we used tests for recognition of crossed out, superimposed and underdrawn images, for preservation of simultaneous perception - when looking at story pictures. The study of acoustic gnosis and auditory-motor coordination was conducted to analyze the properties of acoustic attention and to assess the preservation of phonemic hearing and auditory-motor coordination. Optic-spatial gnosis: recognition of time on a schematic clock without numbers, copying figures, tests for letter and color gnosis.

In the study of praxis, the following samples were used: reciprocal sample; fist-rib-palm test; Head's test, as well as an oral praxis study.

Study of speech functions: analysis of spontaneous speech (analysis of fluidity, correctness and unfolding of speech); a test for naming images of objects; test for understanding words (correct ratio of words with pictures); sample for associative series.

Study of handwriting: analysis of the correctness, nature and degree of automation of writing.

Reading study: correctness and smoothness were assessed.

The intellectual sphere was studied using the following tests: to understand the meaning of plot paintings; serial account "100-7" - to assess the safety of accounting transactions; definition of an unnecessary subject; level of generalization in sample "fourth extra". The study of abstraction was carried out by means of the proverbs and sayings meaning interpretation test.

The study of memory functions was carried out by means of auditory-verbal and visual memory tests - memorization of 10 unrelated words for 5 presentations, memorization of visual stimuli, and memorization of a story. In each test, immediate and delayed reproduction was analyzed.

Statistical processing of the data was performed using Microsoft Office Excel 2010 and Jamovi. Quantitative data were described using mean, standard deviation. Qualitative data were presented as frequencies and percentages. The Mann Whitney U test was used to compare the two independent groups. To assess the association of qualitative features, the criterion was used Pearson's chi-squared

Table 2

Analysis of the mutual distribution of frequencies by level of education and signs of neuropsychological symptoms

	Education level		Pearson's chi-squared test (p)	
	Higher education	Secondary education		
Praxis			5.86	0.015
norm	50% (n=17)	35.3% (n=12)		
light disorders	0% (n=0)	14.7% (n=5)		
Speech			0.848	0.654
accuracy of pronunciation	11.8% (n=4)	5.9% (n=2)		
insufficiently clear pronunciation	8.8% (n=3)	8.8% (n=3)		
dysarthria	29.4% (n=10)	35.3% (n=12)		
Handwriting			0.0	1.00
norm	8.8% (n=3)	8.8% (n=3)		
light disorders	41.2% (n=14)	41.2% (n=14)		
Mindset			1.03	0.31
norm	50% (n=17)	47.1% (n=16)		
moderate intellectual-mnestic decline	0% (n=0)	2.9% (n=1)		
Memory			0.118	0.732
norm	23.5% (n=8)	26.5% (n=9)		
reduced memory capacity	26.5% (n=9)	23.5% (n=8)		
Calculate			5.86	0.119
norm	50% (n=17)	35.3% (n=12)		
counting errors	0% (n=0)	2.9% (n=1)		
forgetting the interim score	0% (n=0)	5.9% (n=2)		
reducing the speed of calculations	0% (n=0)	5.9% (n=2)		
Attention			6.59	0.086
slight decrease	29.4% (n=10)	14.7% (n=5)		
moderate decline	11.8% (n=4)	26.5% (n=9)		
pronounced decrease	8.8% (n=3)	2.9% (n=1)		
significant decrease	0% (n=0)	5.9% (n=2)		

Table 3

Correlation matrix of «Calculate» and «Praxis» by variable «Education level»

		Education level
Calculate	Spearman correlation coefficient ρ(rho)	0.414*
	p- value	0.015
Praxis	Spearman correlation coefficient ρ(rho)	0.415*
	p- value	0.015

note. * p < 0.05

test. Correlation analysis was performed using Spearman's Rank Correlation Coefficient. Differences with bilateral p < 0.05 were considered statistically significant.

Results and discussion. The study included 34 patients aged 30 to 84 years (M age =52, SD = 10.6), of whom 9 (26.5%) were men and 25 (73.5%) were women. Patients were divided into 2 groups according to gender.

Frequency analysis of the obtained neuropsychological examination data showed that visual and visual-spatial gnosis in patients with SCA1 is generally preserved, patients have access to independent recognition of images without prompts, correct determination of images from neuropsychological samples (crossed out, overlaid and unordered objects), preservation of phonemic hearing and auditory motor coordination. No significant gnostic disorders were identified.

In women patients, in contrast to men patients, predominance of praxis disorders is observed. This is probably due to the predominance of women in the study group. At the same time, minor errors in the sequence and dynamics of movement execution were noted; the errors were quickly noticed by the patient and self-corrected. (Table 1).

With the preservation of the meaningful and communicative side of speech in patients of both groups, a predominance of the percentage of speech disorders such as dysarthria was observed. Patients with dysarthria are characterized by a normal beginning of speech expression with a quiet ending. Disorders of prosodics are expressed in the inability to subordinate the speech stream to intonational accents, speech becomes syllabic, "the chanted nature of the speech ". [5] (Table 1).

Most patients experienced fatigue of various intensities during the testing process, but didn't refuse to complete tasks.

When studying the neurodynamic parameters of mental activity in male patients, it was noted that the concentration and stability of attention in patients of this group were slightly reduced on average (M = 1,56; SD = 0,72). At the same time, in some patients there is a pronounced decrease in concentration of attention with a tendency to depletion (n=3). In the study of the cognitive sphere it is possible to note the preservation of flexibility of thinking, preservation of calculation operations. In the study of long-term memory using the method "Memorization of 10 words", fluctuations in the productivity of memorization are noted.

Table 2 shows the neuropsychological symptomatology data, according to the level of education. This table shows that patients who have secondary vocational education, compared to patients who have higher education, are dominated by statistically insignificant more pronounced impairments in counting operations when the neurodynamic component is examined *(p =0,019)*. Also, in patients with secondary education, there is a significant decrease in pace and forgetting the intermediate result at the score *(p=0,002)*. In the study of the cognitive sphere, this group has more pronounced difficulties of inclusion in the tasks, there is a pronounced cognitive-mnestic decline. At the same time, a more pronounced decrease in memorization productivity was revealed. The statistically insignificant study results obtained may probably be associated with a small sample of patients for statistical processing.

Spearman's correlation analysis also revealed no significant correlations between the signs of neuropsychological symptomatology according to the variable "Gender". A number of significant positive correlations are observed for the traits "Calculate" and "Praxis" on the variable "Education" (Table 3). Thus, the attribute "Calculate" is positively related to the variable "Education" (Ro=0.414, p <0.015), and the attribute "Praxis" is also positively related to the variable "Education" (Ro=0.415, p <0.015).

Conclusion. Thus, the neuropsychological study of patients with SCA type 1 allowed to reveal slowing of the rate of thinking in counting, decreased attention depending on the level of education, thus a correlation dependence of cognitive disorders was found: the higher the level of education, the less pronounced are these cognitive disorders. This suggests a slower destruction of neural connections in the field of higher nervous activity due to the neurodegenerative process in patients with higher professional education. The observed prosodic disorders or "the chanted nature of the speech" in most patients are the result of a neurodegenerative process in the cerebellar region. This study will continue to develop a further personalized approach in the provision of specialized medical care and the prevention of cognitive impairment in carriers of the mutant gene in the preclinical stage of SCA type 1.

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HEALTHY LIFESTYLE. PREVENTION

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THE ATTITUDE OF THE YOUTH OF YAKUTSK TO THE ISSUE OF ARTIFICIAL TERMINATION OF PREGNANCY

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A sociological study was conducted to study the attitude of young people to artificial termination of pregnancy. In our study, the majority of young people not only condemn abortions (31.3%), but also believe that it is possible to allow artificial termination of pregnancy only for strict medical reasons (26%), legislative prohibition is supported by 7.7% of respondents. About a third of the respondents (29.3%) adhere to liberal views on termination of pregnancy, they consider this procedure normal – "a woman has the right to decide for herself."A significant positive correlation was established between having children and the respondents' attitude to abortion ($\chi 2$= 20,815; p=0.000). In our study, having children determines the negative attitude towards artificial termination of pregnancy.

Keywords: youth, abortion, survey.

Introduction. The issue of artificial termination of pregnancy is an acute social, ethical and medical problem for the populations of all countries of the world, regardless of geographical location, socio-economic development and religiosity of members of society. There are varying opinions on this issue in different countries: from a woman's freedom of choice in the matter of termination of pregnancy, to carrying it out in exceptional cases and even a legislative ban. [2,3]. On the other hand, strict laws regarding termination of pregnancy lead to an increase in the number of unsafe manipulations, the result of which is a significant deterioration in women's reproductive health and infertility. Reducing maternal mortality by ensuring the safety of medical intervention is also an important part of the international obligations of states, and ensuring the safety of artificial termination of pregnancy is primarily the responsibility of governments in the field of public health [10]. The ways to solve this problem include mainly educational and awareness-raising activities. The availability and quality of medical care, the level of education of the population improve the indicators of reproductive health of young people and leads them to think about family planning and responsible attitude to childbearing.

In the Russian Federation, the number of abortions continues to remain at a fairly high level, it leads the world both in their number and in the negative dynamics of the rate of decline. The frivolous attitude of young people towards abortions may lead to significant demographic problems in the country in the future [2]. Sociological research on these complex issues is relevant. The purpose of this article is to analyze the attitude of young people of reproductive age in Yakutsk to some issues of artificial termination of pregnancy.

Materials and methods. The survey was conducted in Yakutsk by a standardized method of selective correspondence survey of respondents. The number of those who filled out the questionnaire was 300 people. The sociological study was conducted in order to study the attitude of the population of Yakutsk to DNA diagnosis of hereditary diseases as a new method used in practical medicine of the Republic of Sakha (Yakutia). The questionnaire consisted of 24 different types of questions: multiple-choice questions; dichotomous (yes, no); matrix questions (a question in the form of a table, where the desired value must be ticked).

The questionnaire data was processed in the IBM SPSS Statistics 22 software application. The confidence probability was 95%. Confidence interval (±%) 5.66.

For quantitative indicators, the normality of the distribution of data in groups was verified by the Shapiro-Wilk test. The distribution of indicators was different from normal and the samples were not large when distributed into groups, in this regard, we chose the Mann -Whitney U—test, when comparing groups according to the respondents' answers, the $\chi 2$ criterion was used.

The number of respondents who filled out the questionnaire was 300 people, including 154 men (51.3%), 146 women (48.7%). The average age of the respondents is 29.7 ± 0.7 years, a significant part (85.3% or 256 people) lives in Yakutsk.

The socio–demographic characteristics of the respondents are presented in Table 1.

Results and discussion. In our study, the majority of young people not only condemn abortions (31.3%), but also believe that it is possible to allow artificial termination of pregnancy only for strict medical reasons (26%), legislative prohibition of abortion is supported by 7.7% of respondents. About a third of the respondents (29.3%) adhere to liberal views on abortion, they consider this procedure normal – "a woman has the right to decide for herself" (Table 2).

In 2007, a survey was conducted in Yakutsk of 72 people aged 17-25 years on the attitude of young people to abortion. According to the authors' hypothesis, modern youth does not realize how serious the very fact of abortion is. As a result, it turned out that 23.6% of respondents define it as "forced action in a desperate situation" and only 18% consider abortion to be an "immoral act"[7].

In 2017, 60 people aged 17-40 years were interviewed in Voronezh on abortion issues. The results of the survey showed that 27.5% of women were in favor of banning abortions, while the majority (72.5%) were against banning abortions. In this study, the authors did not find an

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established connection between the opinion of women regarding abortion and having children or their marital status. The main factors of the ban on abortion were the murder of the child (100%) and the negative impact on the mother's body (83%) [5].

According to the Mann–Whitney test (U), when checking the dependence of respondents' responses on their age, we did not find significant differences (U= 4118; p= 0.959), nor did we find a link between the attitude to abortion and the marital status of respondents (χ2= 0.522; p=0.469)

We were interested to find out whether the respondents' attitude to abortion depends on having children. For a clearer interpretation, we divided all the answers to the question "on the attitude to abortion" into two types: negative - "I condemn abortions, but I consider it a forced measure", "allow only for strict medical reasons", "it should be prohibited by law" and positive - "I consider it normal, a woman has the right to decide for herself".

There is a weak reliable relationship between the presence of children and the respondents' attitude to abortion (r=0.1; χ2= 20,815; p=0.000). In our study, the presence of children determines the negative attitude towards artificial termination of pregnancy, which proves the exceptional value of children as a feature of the mentality of the Yakuts, since the majority (75%) of respondents were representatives of Sakha (Table 3).

The results of our early studies on the use of genetic testing and ethical problems of prenatal DNA testing of hereditary diseases revealed the predominant motives for DNA testing, among them the concern of young people for the health of their future children (50.3%). In addition, a significant number of respondents found it difficult to answer the ethically difficult question about termination of pregnancy with some given hypothetical fetal pathologies: Down syndrome - 40%, dwarfism – 45%, deafness – 42% [6,9].

Researchers of the Sakha mentality identified one of the qualities of the Yakut character – especially a reverent and careful attitude towards children [1,4,8]. According to Tyrilgin (2000), "... such a super-stable preservation by the Yakuts of their children, among whom many weakened ones were nursed ..." is one of the reasons for the "phenomenal vitality of the Sakha people"[8]. According to a questionnaire survey by Bravina (2005), the hierarchy of values of modern Yakuts is as follows: 1) "have a good family" (41.7%); 2) "well-being and success of children" (37.2%); 3) "live in prosperity" (30%); 4) "have respect for people" (19.4%); 5) "realization of their creative abilities" (15%) [1].

Interesting studies on abortion were conducted among 148 students of the University of Mostar. As a result, 81.1% of female students would have an abortion under certain circumstances. The majority of students answered that they would have an abortion if the fetus had malformations (χ2=3,892; P=0.49)

Table 1

Socio-demographic characteristics of respondents

Characteristics	Quantity (n=300)	%
Marital status:		
Unmarried	170	56.7
Married	84	28.0
Civil marriage	25	8.3
Divorced	12	4.0
Education:		
Higher education (unfinished)	214	71.3
Vocational education	44	14.7
Secondary general education	36	12.0
Basic general education	6	2.0
Number of children:		
No children	197	65.7
One child	46	15.3
Two children	33	11.0
Three or more	24	8.0
Occupation:		
Student	180	60.0
Employed	143	47.7
Nationality:		
Sakha	225	75.0
Other nationalities	75	25.0

Table 2

Respondents' attitude to artificial termination of pregnancy (abortion)

Survey question	N= 300	%
I think it's normal, a woman has the right to decide for herself	88	29,3
I condemn abortions, but I consider it a necessary measure	94	31,3
Allow only for strict medical reasons	78	26,0
It needs to be prohibited by law	23	7,7
Systemically missed	17	5,7

Table 3

Comparative analysis of the surveyed's attitude to abortion, depending on having children

What is your attitude on abortion?	Наличие детей	N	%	** r	
Negative	Нет	119	43.6	0.100	*χ^2 20.815 P 0.000
	Есть	154	56.4		
Positive	Нет	63	71.6	0.094	
	Есть	25	28.4		

Notes: * the comparison of reliability was carried out between the respondents who answered negatively and positively; ** the correlation was carried out depending on respondents having children and the attitude to abortion.

or if the mother's life was in danger (χ2=47,676; P<0.001). When comparing the willingness of female students to have an abortion under different circumstances of pregnancy, depending on religiosity, a statistically significant difference was proved in the following circumstances: in the case of "abortion on demand", regardless of the reason (χ2=11,908; P=0.012), teenage pregnancy (χ2=33,308; P<0.001) and if pregnancy interferes with the mother's career (χ2=35,897; P<0.001). Non-religious students expressed more liberal views [11].

Conclusion. Social research on the study of the attitude of the population to artificial termination of pregnancy (abortion) is relevant. It is especially important to know the opinion of young people of reproductive age, since the attitude to abortion in different societies cannot be called certain. The frivolous attitude of young people towards abortion may lead to significant demographic problems in the country in the future. In our study, the majority of young people not only condemn abortions (31.3%), but also believe that it is possible to allow artificial termination of pregnancy only for strict medical reasons (26%), legislative prohibition is supported by 7.7% of respondents, 29.3% adhere to liberal views on abortion. We did not find a connection between the attitude to abortion and the marital status of the respondents, but the presence of children determines the negative attitude of respondents to the artificial termination of pregnancy.

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THE PRACTICAL IMPLEMENTATION OF THE IMMUNOCYTOCHEMICAL RESEARCH METHOD IN THE DIAGNOSIS OF PRECANCEROUS DISEASES OF THE CERVIX

Patients with cervical pathology were analyzed by cytological, immunocytochemical and molecular genetic tests. Among the selected groups, 78 women were more likely to have low-grade dysplasia, or LSIL (16.3%). In more than half of the cases (60.2%) in women who were tested, positive HPV tests were observed. The majority of positive HPV tests in the were for high oncogenic risk (32%), with 16 (19.1%) and 31 (8.5%) being the most common types. It was found that the frequency of double staining of p16ink4a/Ki67 was noted in women with a high degree of intraepithelial lesion (37%), and the detection of HPV type 16 was prevailed - 19.1%. The number of cases in the group of women without pathology, but with latent HPV infection, cases with a positive HPV test and a negative result of p16/Ki67 protein expression prevailed was 94.1%. It was found that the number of p16-positive cells was higher in women of group 1 with LSIL (55.8%) and group 2 (44.4%) with HSIL. In the group of women without cervical pathology, but with latent HPV infection, indicators of weak expression of p16ink4a were most frequently recorded (57.1%).

Keywords: cytology, diagnostics, human papillomavirus, dysplasia.

Relevance. Currently, there is an increase in the number of patients with cervical cancer (CC) each year. According to the available data available for the year 2021, The incidence of CC in Russia has increased from 113.1 in 2011 to 126.7 per 100 thousand females in 2021. In recent years, the incidence CC has been in the second position after uterine body cancer in the structure of genital cancers in the country [1]. At the same time, in terms of prevalence in women in the age category from 35 to 49 years, it took the second place [3]. According to Minkin G.N., cervical pathology is a prevalent gynecological disease among women in their reproductive years, ranging from 10% to 15% [1,6]. There are a number of background processes that are noted for the development of CC, in particular, candylomas, dysplasia of varying severity and cancer in situ. The accessibility of malignant neoplasms of the cervix and the possibility of using various diagnostic measures should help reduce the incidence of cervical cancer. Early detection and timely treatment of precancerous diseases can prevent most cases of cervical cancer [7]. It is known that the most important factor in cervical carcinogenesis is infection of women with human papillomavirus (HPV). In turn, early sexual activity, frequent changes of sexual partners, non-compliance with sexual hygiene, sexually transmitted diseases, immunodeficiency, smoking and contact with toxic substances create condition for HPV infection, thereby increasing the risk of developing CC [2,6].

More than 15-20% of women are infected with HPV, but only a few develop dysplasia of varying severity or cervical cancer, according to studies [10]. In this regard, early detection of background and precancerous cervical diseases associated with HPV is important. In addition to the detection of high-risk HPV (HR), numerous foreign studies have shown that research data based on markers of proliferation, methylation in HPV infection, and their introduction into clinical practice are carried out [4,9,13].

It is known that cytological examination is the main method of screening for cervical cancer and precancerous diseases. Despite the general availability, reliability, and widespread use of this method, there are several disadvantages: the lack of standard methods for sampling biomaterial for research, which can lead to false negative results in 20-30% of cases, the probability of false positive results in 5-70% of cases, and the problems associated with repeated sampling of material. In recent years, the method of obtaining smears using the method of liquid cytology has been improved, which makes it possible to perform additional molecular types of research, one of which is immunocytochemical examination (ICC) [5].

The immunocytochemical method is a method of molecular immunological diagnostics that allows the identification of cellular and tissue antigen molecules as a result of their binding to antibodies and the formation of an “antigen-antibody” complex. In modern diagnostics, CC ICC is a combination of cytological and enzyme immunoassay methods. It detects the pathological process directly in the cell under study, and also determines the parameters of HPV aggressiveness. The determining factor in the pathological process is the presence of cancer proteins p16ink4a and Ki-67, whose concentrations increase in response to the viral assault [8]. The index of proliferative activity of p16ink4a and Ki-67 expression should be determined by the immunocytochemical method for the differential diagnosis of the severity of cervical lesions, according to the data of the All-Russian public organization "Russian Society of Specialists in the Prevention and Treatment of Tumors of the Reproductive System" [11]. The immunocytochemical analysis of the p16ink4a cancer protein permits the precise distinction between tumor and non-tumor dysplasia, as well as demonstrating a high degree of specificity. It is known that the expression of this protein is associated with mild (CIN I), moderate (CIN II) and severe dysplasia (CIN III), as well as intraepithelial cervical cancer. It's rare to find it in the cells of the squamous epithelium without signs of dysplasia.According to the results of studies by different authors, it seems important to find out if the p16ink4a protein is expressed by immunocytochemical method in addition to the routine method of liquid cytology and Pap smear staining [5,15]. A marker of proliferation, Ki-67, is an antigen of a cancerous tumor that is detected in a cell when it divides, but it is not detected in the resting phase of the cell cycle. The aggressiveness of a malignant tumor and unfavorable prognosis can be predicted by this feature of Ki-67, which is a useful indicator of the prognosis of its behavior [14,16].

There are a number of studies that present data that the diagnostic value is not only the mono-expression of p16ink4a and Ki-67 proteins, but their simultaneous detection (co-expression) in the form of double staining. Some authors say that the cytological method of double staining is recommended for additional examination of HPV-positive patients during primary HPV screening because it makes cytology being a more specific test for sorting HPV-positive women [12,17].

The purpose of the study.To evaluate the results of expression of p16ink4a and Ki-67 protein in cytological samples by immunocytochemical method in women with cervical pathology associated with HPV.

Materials and methods of research: the research used a comprehensive cytological, immunocytochemical and molecular genetic examination of 245 women aged 22 to 56 years and older. It was performed on the basis of the Yakut Scientific Center for Complex Medical Problems. The obtained material from each woman was examined by the method of liquid cytology (LC) on the automated CellPrepPlus system (Korea), as an independent highly informative method that contributes to the improvement and standardization of all stages of cytological examination. Glass staining by the Romanovsky–Giemsa method was used for traditional smears and Pap staining for smears by liquid cytology. The interpretation of the results was in line with the terminology of the Bethesda System, 2015[16], which is based on the introduction of the term SIL (Squamous Interaepithelial Lesion) – squamous intraepithelial lesion. It is divided into categories: NILM (negative for intraepithelial lesion or malignancy) – characterizes the clinical norm, i.e. the absence of pathology or the presence of benign changes due to reparative and reactive changes. LSIL (low-grade squamous intraepithelial lesion): These lesions are changes in the squamous epithelium that are typical of HPV infection and mild dysplasia CIN I. HSIL (high grade squamous intraepithelial lesion) - intraepithelial lesions of the squamous epithelium of a high degree, the group covers moderate and severe dysplasia, CIN II, CIN III, ASCUS- atypical squamous epithelial cells of unclear significance, ASC-H – atypical squamous epithelial cells that do not allow to exclude HSIL, CIS – carcinoma in situ, SIL - squamous intraepithelial lesion.

Immunocytochemical examination of smears for the protein p16ink4a and Ki-67 was performed using the CINtec PLUS kit (Germany). The nuclear and cytoplasmic reactions were evaluated, and the nuclear reaction was compares to the cytoplasmic reaction (pic.3). The results of liquid cytology were compared with the results of traditional cytology.

The HPV typing was conducted using a PCR test referred to as "Kvant-21"

(Moscow, Russia) on the basis of the microbiological laboratory of the NEFU Clinic. The real-time PCR method can quickly tell what type of HPV it is by measuring the amount of virus and comparing it to other samples. It can also tell the difference between 21 different types of HPV (6,11, 16, 18, 26, 31, 33, 35, 39, 44, 45, 51, 53, 56, 58, 59, 66, 68, 73, 82, serotypes). Including high- and low-oncogenic types.

Relative indicators (P) and their errors (m) were calculated and intergroup and intragroup differences were estimated. The calculation was performed using the SPSS Statistics 19 package.The differences were considered statistically significant at $p<0.05$.

Results and discussion. The absence of intracellular lesion (NILM) was detected in 171 women, which was 69.8% of all women studied, according to the results of cytological examination by liquid cytology.The prevalence of squamous intraepithelial lesions of low (LSIL) and high (HSIL) degree was observed in 28.6% of cases, with LSIL detected in 40 cases (16.3%), HSIL detected in 30 cases and accounting for 12.2% of the total number of women studied. In 1 case (0.4%), ASCUS, ASC-H, SIL and CIS were diagnosed. Patients with HPV-associated CIN and patients without cervical pathology, but with a latent form of HPV infection, were selected for the study. A total of 78 women were examined. The average age of women was 44.27+11.7 years. Three distinct groups were established: group 1 comprised patients with LSIL (mean age 47.32+12.1) (n=34), group 2 comprised patients with HSIL (mean age 42.19+12.5) (n=27), and group 3 comprised patients who did not have cervical pathology but had latent HPV-infection with a diagnosis of NILM (mean age 41.47+8.31) (n=17).

Given the role of HPV in the development of cervical cancer, HPV testing is currently the most important moment of screening, [7,13]. The results of the study showed that 47 women had confirmed HPV carrier status, which was 60.2% of all surveyed women. The presence of oncogenic HPV types of high oncogenic risk was detected in 25 women and amounted to 32%. The most common types were highly oncogenic HPV types 16 and 31 (19.1 and 8.5%, respectively). Papillomavirus infection was found to be monoinfection (68%) or coinfection (31.9%) on a quantitative basis. The evaluation of the indicators revealed statistically significant patterns among the analyzed groups, as well as a favorable response to the HPV test (r=0.444, p=0.000).

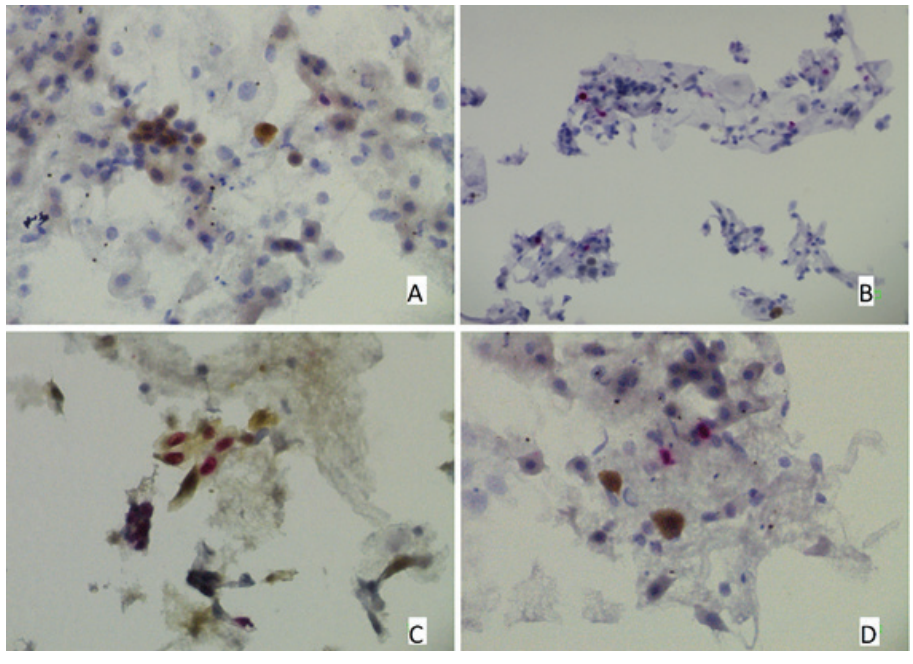

Fig. 1. Positive ICC reaction to p16ink4a and Ki-67 (Pap staining, x200). A- expression of p16ink4a protein, B – expression of Ki-67 protein, C –coexpression16ipk4a/Ki-67, D– monoexpression16ipk4a and Ki-67

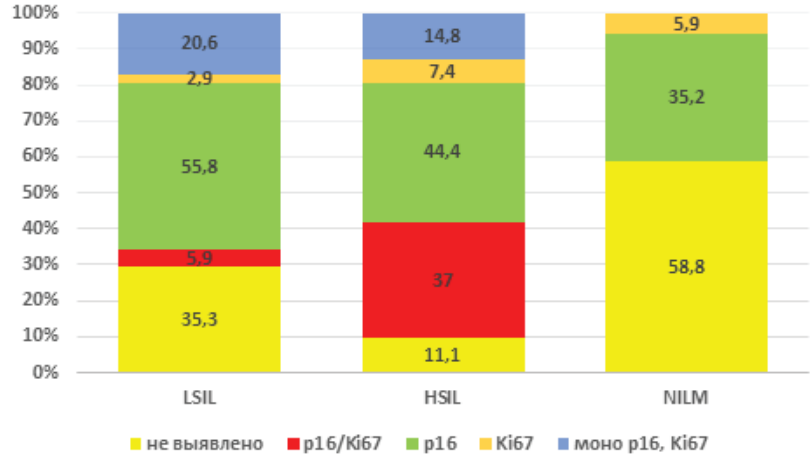

Fig. 2. Evaluation of detection of expression of proteins p16ink4a and Ki67

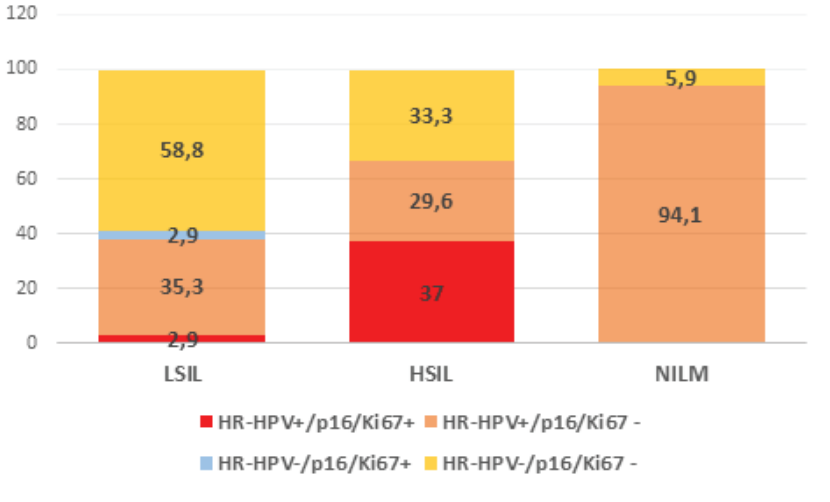

Fig. 3. Combination of HR-HPV infection detection results and p16ink4a/Ki67 protein detection

The simultaneous co-expression of p16ink4a and Ki67 in the same cell indicates a violation of the regulation of the cell cycle characteristic of HPV transformation and the development of dysplasia of cervical epithelial cells. The frequency of positive results from double staining of p16ink4a/Ki67 by immunocytochemical examination in 78 examined patients revealed that co-expression of p16ink4a/Ki67 was detected in 12 examined women, representing a percentage of 15.4%. As depicted in Pic. 1, it can be observed that in patients belonging to group 1 with LSIL, there were two instances of double staining of p16ink4a/Ki67, which accounted for 5.9% of the total cases. In contrast, in group 2 with HSIL, the frequency of p16ink4a/Ki67 cases increased with the severity of dysplasia, reaching a total of 37%. The correlation analysis between the examined groups and the positive p16ink4a/Ki67 test revealed the presence of a negative correlation (r=-0.391, p=0.000). There was no expression of the studied marker in any material among patients without cervical pathology (group 3).

Positive expression of p16ink4a protein was detected in 37 examined women and amounted to 47.4%. It was discovered that the highest prevalence were observed in the first group of women, which was 55.8%. Moreover, in 44.1% of cases, the expression was considered moderate, in 8.8% of cases, it was severe, and in 2.9%, it was considered weakly expressed. In group 2, the p16ink4a protein was found in 44.4% of cases, 26% of them were moderate, 7.4% were severe and 11.1% were weakly expressed. In group 3 - 35.2% of cases, weakly expressed p16 protein expression prevailed the most – 57.1% and in 11.7% of cases – moderate. Thus, a direct correlation was also established between the positive p16ink4a test between the groups of the examined (r=0.401, p=0.000). The expression of the Ki67 protein cell proliferation marker was detected in 2.9% of women from group 1, 7.4% of women from group 2, and 5.9% of women from group 3.

Various combinations of p16ink4a/Ki67 expression with the presence or absence of HR-HPV, depending on the severity of cervix epithelial dysplasia, are presented in Pic.2.The difference between the selected subgroups was statistically significant (r=-0.924, p=0.000).

In group 1, patients with LSIL pathology were most often characterized by the absence of HR-HPV-/ p16/Ki67- -58.8% (20 cases), in 35.3% (12 cases) - HR-HPV+/p16/Ki67- and in 1 case (2.9%, respectively) HR-HPV+ was detected/ p16/Ki67+ and HR-HPV-/p16/Ki67+. In group 2, cases with overexpression of HR-HPV+/p16/Ki67+ prevailed in HSIL - 10 cases (37%), while 8 (29.6%) cases with HR-HPV+/p16/Ki67- and 9 (33.3%) cases with absence of HR-HPV+/p16/Ki67- prevail. It should also be noted that with a high intraepithelial lesion of the cervix, the detectability of HPV type 16 in the presence of p16/Ki67 coexpression was higher and amounted to 19.1%, while the detectability of other HPV types varied from 4.2 to 2.1% of cases. The frequency of cases in the group of women without pathology but with latent HPV infection was dominated by cases with HR-HPV+/p16/Ki67- - 16 cases (94.1%) and in 5.9% with a negative result of the presence of the virus and the expression of the protein p16/Ki67.

It's important to know that if a group of women with latent infection have the p16ink4a marker, they might have dysplasia even if they don't have any symptoms or signs of cancer at the time of the study. This means more research should be done with these women.

Thus, the predominance of NILM in cytological samples (69.8%) compared with intraepithelial lesions of the cervix (28.6%) was revealed. Among the these groups, low–grade dysplasia - LSIL (16.3%) was more common. 60.2% of women had positive HPV tests.The majority of positive HPV tests revealed high oncogenic risk (32%), while HPV of 16 (19.1%) and 31 (8.5%) types were most common. It was discovered that the frequency of double staining of p16ink4a/Ki67 was observed in women with a high degree of intraepithelial lesion (37%), while the detection of HPV type 16 prevailed at 19.1%. The frequency of cases with a positive HPV test and with a negative result of p16/Ki67 protein expression was highest (94.1%) in the group of women without pathology but with latent HPV infection. It was found that the proportion of p16-positive cells was higher among women belonging to group 1 with LSIL (55.8%) and group 2 (44.4%) with HSIL. Among the group of women without cervical pathology, but with latent HPV infection, indicators of low expression of p16ink4a (57.1%) were most often recorded. This may suggest a threat of dysplasia in the absence of clinical and cytological signs of the lesion.

Conclusion. A comprehensive examination of women with various cervical pathologies was carried out with the inclusion of a molecular diagnostic method**.** The evaluation of the expression of the protein p16ink4a and Ki-67 in the tissues of the cervix revealed the presence of precancerous changes.

The aim of early detection of cervical pathology is achieved through cytological and PCR-based diagnostics of the human papillomavirus. Screening diagnostics provide additional opportunities for the prevention of cervical cancer at an early stage, which determines the basis for the prospects of preserving women's health.

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HYGIENE, SANITATION, EPIDEMIOLOGY AND MEDICAL ECOLOGY

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THE STATE OF THE ADAPTIVE POTENTIAL OF THE WORKING POPULATION IN THE ZONE OF INCREASED RADIATION EXPOSURE

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A radiological and medical-biological study of the population of Aldan and Tommot of the Aldan region of South Yakutia, located in the zone of increased natural radiation, was carried out. The annual individual effective exposure dose to the population turned out to be 2 times higher in the city of Aldan (6.22 mSv). The contribution of radon and its decay products in the city of Aldan was 59.5%, in the city of Tommot - 48.3%.

Assessment of the adaptive potential (AP) of the circulatory system of the population showed a high percentage of the occurrence of functional stress of adaptation mechanisms, especially among residents of the city of Aldan (86.5%). The correlation showed a negative role of an increase in the level of triglycerides, urea, the activity of LDH, CK, and a decrease in the activity of alkaline phosphatase on AP. The presence of GBL dysfunction and, especially, fatty hepatosis was also associated with a decrease in AP.

Keywords: radon, radiation, adaptive potential, Yakutia, fatty hepatosis.

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Introduction. Natural sources of ionizing radiation, as a constant physical environmental factor, dominate the collective effective dose, causing the main harm to public health (about 70%), and cause reasonable concern among the population in areas with an unfavorable radioecological situation. In the Republic of Sakha (Yakutia), in the Aldan region, there are 2 uranium-bearing provinces, where the main uranium reserves of the Russian Federation are concentrated. The Elkon uranium ore region with an area of 1,500 km2 is located 50 km east of the administrative center, the city of Aldan, with a population of more than 20,000 people. and 40 km southeast of the city of Tommot with a population of more than 7 thousand people. The federal highway Neryungri -Yakutsk passes through the central part of the district from south to north with a high traffic intensity [2].

According to the Gosatomnadzor of the Far Eastern District of the Russian Federation, in the process of large-scale geological prospecting since 1959, more than 1 million tons of mining and ore mass containing about 2000 tons of uranium have been extracted from the bowels and stored on the day surface in the form of dumps. There were centers of radioactive contamination with high DER values (equivalent dose rate), reaching 1500-2000 μR/h. In 2022, in 40 premises of public buildings in the Aldan district, the values of ERVA (equivalent equilibrium volumetric activity) of radon exceed-

ed sanitary standards. The maximum value of EEVA for 222Rn was 1313±198 Bq/m3.

The proportion of water samples from centralized water supply sources exceeding the intervention level in terms of the content of specific activity of 222Rn was 20.3%. The maximum detected content of 222Rn in the sample was 362.9±40.1 Bq/l. In general, in the surveyed settlements of the Aldan region, the annual individual effective dose to the population varies from 3.02 to 6.92 mSv/year and exceeds the average for the Republic of Sakha (Yakutia) (2.99 mSv/year). In the Russian Federation, the average value of GIEDON is 3.24 mSv/year [4]. A certain contribution to environmental pollution is made by heavy motor traffic on the federal highway. The concentration of lead in the snow cover in places where vehicles are concentrated (gas stations, parking lots) can reach 0.7 g/m2. The ecological situation in the area is 53.5% of the area as satisfactory, 39.6% of the area is tense, and 6.9% of the area is critical [2].

The World Health Organization (WHO) has recognized radon as a human lung carcinogen [25] and is the second leading cause of lung cancer after smoking. Smokers are 25 times more likely to develop cancer than non-smokers. [24]. Data from the official statistics for 2000-2020. show high values of general mortality rates of the population of the Aldan region in comparison with the national indicators (respectively 13.4-14.2 versus 8.6-7.8 per 100 thousand population). The first place is occupied by mortality from diseases of the circulatory system, more than 2 times higher than the data for RS (Y) (741.7 versus 354.0 per 100 thousand population). Mainly from ischemic heart disease (325.9) and cerebrovascular diseases (133.4) [14]. In second place is mortality from neoplasms (in 2019 - 214.0 and in 2020 - 215.5 per 100 thousand of the population), in third - mortality from external causes (in 2019 - 146.9 and in 2020 - 143.3 per 100 thousand population). For 2011-2015 the annual total rate of oncological morbidity of the adult population in the Aldan region of the Republic of Sakha (Yakutia) amounted to 790 diseases per 100,000 people. (for 461 men and 328.2 for women), which is 2 times higher than in the Russian Federation. According to the ranking of the administrative territories of the Republic of Sakha (Yakutia) with a high level of cancer incidence for 2011-2020. Aldan region, with an increase in incidence over 10 years by 31.3%, ranks second [7]. In the structure of the general morbidity of the adult population for 2000-2018. diseases of the respiratory organs, the circulatory system, and the musculoskeletal system were of decisive importance [12].

Thus, the deterioration of the health status of the population in the zone of increased radiation exposure persists. And any shift from health to disease occurs on the basis of a gradual decrease in the body's adaptive reserves [21].

In this regard, complex radioecological and biomedical studies of the population in areas of increased radiation exposure require an assessment of the adaptive potential (AP) and are relevant.

The purpose of this work was to assess the levels of exposure of the working population of the cities of Aldan and Tommot of the Aldan region from natural sources of radiation and to determine the adaptive potential of the circulatory system.

Methods of biomedical research. In the spring season of 2022, researchers and practicing physicians of the Yakut Scientific Center for Complex Medical Problems conducted a survey of the adult and child population of the cities. Aldan and Tommot of the Aldan region with the participation of a therapist, cardiologist, ophthalmologist, neurologist, oncologist, gynecologist, ultrasound doctor, endoscopist and pediatrician. The object of the study were 175 people. adult population working and living in Aldan and Tommot of the Aldan region (Table 1).

The program of the study of the adult population included the following: informed consent of the respondent to conduct research; questionnaire survey to assess the objective state of health; anthropometric examination with measurement of height and body weight with calculation of body mass index, waist and hips; blood donation (according to the protocol of the local bioethical committee of the YSC CMP) from the cubital vein in the morning on an empty stomach after a 12-hour abstinence from food for biochemical and immunological studies. After centrifugation, the serum was stored in a freezer (at -70°C) until analysis.

Blood pressure (BP) was measured twice with an OMRON M2 Basic automatic sphygmomanometer (Japan) in the sitting position with the calculation of mean blood pressure with a maximum permissible measurement error of ±3 mmHg [20]. Arterial hypertension (AH) was defined as BP ≥ 140/90 mm Hg. or taking antihypertensive drugs during the survey period or stopping them less than 2 weeks before the survey [23]

Determination of total cholesterol (TC), high-density lipoprotein cholesterol (HDL cholesterol), triglycerides (TG), glucose, uric acid, urea, creatinine, total protein and albumin, The activity of enzymes of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), creatine kinase (CK), lactate dehydrogenase (LDH) was measured by the enzymatic method on an automatic biochemical analyzer "Labio" using reagents "Analyticon" (Germany). The nosological diagnosis was made by specialists according to the International Classification of Diseases of the 10th revision.

The state of the adaptive reserve of the body was assessed by the method of calculating the adaptive potential (AP) of the circulatory system using its functional indicators and anthropometric data according to the formula of R. M. Baevsky and A. P. Berseneva [1]:

AP \u003d 0.011 (HR) + 0.014 (SBP) + +0.008 (DBP) + 0.014 (V) + 0.009 (BW) - - 0.009 (P) - 0.27,

where AP is the degree of AP, HR is heart rate, SBP is systolic blood pressure, DBP is diastolic blood pressure, B is age, years, BW is body weight, kg, P is height, cm.

According to the results of calculations, the degree of AP was assessed:

1) good adaptation (AP <2 c.u.);

2) satisfactory adaptation (AP = 2.10 units) — sufficient functionality of the circulatory system;

3) functional stress of adaptation mechanisms (AP = 2.11-3.20 units);

4) unsatisfactory adaptation (AP = 3.21-4.30 conventional units) - a decrease in the functionality of the circulatory system with an insufficient, adaptable response to stress;

5) failure of adaptation (AP >4.30 units) — a sharp decrease in the functionality of the circulatory system with the phenomenon of failure of the mechanisms of adaptation of the whole organism.

Methods of radioecological research. Gamma-ray survey of objects was carried out using the SRP-68-01 radiometer according to the method adopted in geology and radiation ecology [8, 13]. The measurement of the dose rate of gamma radiation with a radiometer was carried out both at a height of 1 m from the radiating surface, and near it, within 0.1 m. The DER value was estimated by the arithmetic mean of 3-5 measurements at each survey point. As a result of dosimetric gamma survey, sites were selected for sampling the fine earth of dumps, soils, water and plants.

To determine the content of natural radionuclides (uranium, radium, thorium and potassium) in the prepared sample

samples, nuclear-physical (X-ray spectral, gamma-ray spectrometric), radiochemical (emanation) and physicochemical (laser-luminescent) methods were used, which are widely used in geology and radioecology [16, 17].

As the main spectrometric instrumentation, a GAMMA-01 multichannel analyzer (SPC Aspect, Russia) with a sodium-iodine scintillation detector measuring 150x100 mm, as well as a RKG-AT1320 spectrometric type gamma radiometer was used. A high-performance device ARF-6M was used for X-ray spectral analysis.

Determination of the uranium content in water was carried out by the laser-luminescent method using an AUF-101-"Angara" fluorometer. The emanation method for determining the content of radium includes the decomposition of samples (0.5-5 g) by fusing them with a mixture of caustic soda and soda, and then the isolation of radium from the hydrochloric acid solution by co-precipitation of BaSO4, the subsequent dissolution of the precipitate with 0.6 N hydrochloric acid and the measurement of the radium emanation activity -radon on the Alfa-1M device.

Radonometry was carried out by measuring the volumetric activity of radon (RAR) and the radon flux density (RFR) from the soil surface using radiometers of the RRA-01M-01, Alfarad plus AV and Alfarad plus RP types, as well as the POU-04 sampling device according to the method [13] . Calculation of the radon SPD based on the measurement results was carried out according to the formula: SPD (mBq/s-m2) = (Q - Qf) · (V2+V3)/ T S2, where: Q is the measured RAR (Bq/m3); Qf is the background RSA (Bq/m3); V2 is the volume of the PPA measuring chamber, V2 = 1.60 l; V3 is the free volume of the storage chamber-2 and connecting tubes, V3 = 0.093 l; T is the operating time of the FOU blower, T = 300 s; S2 is the area of radon collection by the accumulation chamber-2, S2 = 0.0016 m2. The repeatability of PPR measurements is 5-fold, while the error in determining RVA and PPR was maximum 30-40%.

The normality of the distribution of features was tested using the Shapiro-Wilk test. Due to the partial asymmetry of the distribution series, nonparametric statistics methods were used. The data for the samples are presented as medians (Me) with a percentile interval (25%; 75%). To assess differences between groups, the Mann-Whitney U-test was used. The level of statistical significance for group comparison was taken at $p<0.05$. The relationship of indicators was assessed using the Spearman correlation coefficient.

Results and discussion. Annual individual effective dose of public exposure from natural sources in the Aldan region of the Republic of Sakha (Yakutia). The effective dose is a value that is used as a measure of the risk of long-term consequences of exposure to both the entire human body and its individual organs and tissues, taking into account their radiosensitivity.

The values of the individual annual effective dose of external exposure of adult residents of the Aldan region were determined from the results of measurements of the dose rate of gamma radiation in residential and public buildings and in open areas within the territories of settlements based on the standard model of the time spent by the population indoors and outdoors - 80 and 20%, respectively. The dose of internal exposure of adults from long-lived natural radionuclides in drinking water and food is calculated taking into account the annual per capita consumption of the relevant food (milk, bread, meat, potatoes, vegetables and fish) in the Republic of Sakha (Yakutia). [5]. At the same time, the content of radionuclides in food products and drinking water, presented in the methodological recommendations [18], was used in the calculations, and the standard annual consumption of drinking water (730 l/ year) was also used. When assessing individual effective doses of public exposure due to radon and its daughter decay products, the value of the dose coefficient is assumed to be 0.028 mSv/year/Bq/m3 radon EA or 9 mSv/h/Bq/m3 radon EEVA, which complies with the UNSCEAR recommendations [9]. The annual individual effective exposure dose to the population from natural sources of radiation in the Aldan region varies from 3.02 to 6.92

Table 1

Sex and age composition of the surveyed population

gender	Aldan	Tommot	Total
Men	44 (33; 52) n=59	62 (41; 64) n=7	45 (34.50; 53) n=66
Women	39 (32; 45) n=58	48 (37; 59) n=51	42 (36; 51.50) n=109
Total	41.35 (33.25; 49) n=107	48.84 (37.75; 60.25) n=66	44 (35; 52) n=175

Table 2

Annual individual effective dose of exposure of the population from natural sources in the Aldan region of the Republic of Sakha (Yakutia)

Type of exposure	Effective dose, mSv/year	%
Aldan		
cosmic radiation	0.40	6.4
External gamma radiation from natural radionuclides	1.80	28.9
Radon and its decay products indoors	3.70	59.5
^{40}K in the body	0.17	2.7
Radionuclides: in food	0.14	2.3
in water	0.01	0.2
Сумма	**6.22**	100
Tommot		
cosmic radiation	0.40	11.4
External gamma radiation from natural radionuclides	1.10	31.2
Radon and its decay products indoors	1.70	48.3
^{40}K in the body	0.17	4.8
Radionuclides: in food	0.14	4.0
in water	0.01	0.3
Сумма	**3.52**	100

mSv/year. Its value in the city of Aldan is almost 2 times higher than in the city of Tommot, while the contribution of radon and its decay products was 59.5% in the city of Aldan, and 48.3% in the city of Tommot (Table 2).

Exposure of the population due to natural sources of ionizing radiation (SIR) includes external and internal exposure. The share of internal exposure due to short-lived daughter products of radon isotopes contained in indoor air accounts for more than 70% [6]. A certain contribution to the dose of internal exposure of the population is made by the oral intake of natural radionuclides with drinking water and food. The dose of internal exposure due to the intake of ^{40}K with water and food is almost the same for all people. Due to the intake of radionuclides of the uranium and thorium radioactive series, it is proportional to the annual intake of radionuclides with drinking water and food.

External exposure of the population is formed mainly due to gamma radiation of natural radionuclides contained in the external environment, as well as cosmic photon and corpuscular radiation. The variability of the levels of public exposure to natural radiation sources depends on the values of internal exposure to radon isotopes and the intake of radionuclides of the uranium and thorium radioactive series with drinking water and food, as well as external gamma radiation. In addition, technogenic radionuclides of global fallout as a result of nuclear weapons testing, as well as technogenic radionuclides introduced into the environment and concentrations of natural radionuclides as a result of various human activities, are practically an integral part of the natural radiation background, which leads to increased levels of both internal and external exposure population. Currently, exposure from natural background radiation continues to be the main source of human exposure in the modern world.

The contribution of natural sources in 2021 was 78.5%. The average annual effective dose in the Republic of Sakha (Yakutia) is 4.80 mSv per year, in the Russian Federation - 3.20 mSv per year [12].

The initial data for calculating individual annual effective doses of exposure to the population due to natural sources of ionizing radiation should include:

- data on the average annual values of the equivalent equilibrium volume activity (EEVA) of radon isotopes in the air of residential and public buildings, as well as in the atmospheric air on the territory of a settlement (district, etc.);

- data on the average values of the dose rate of gamma radiation in residential and public buildings, as well as on the territory of a settlement (district, etc.);

- information on the content of natural radionuclides in the water of sources of drinking water supply for the population:

- data on the main components of the diet of the population, the annual consumption of food products and the values of the specific activity of natural radionuclides in them;

- data on the average annual content of dust (aerosols) in the surface layer of atmospheric air and the specific activity of long-lived natural radionuclides in dust. The average value of the individual annual effective dose of exposure of adult residents of a settlement (district, etc.) from all natural sources of ionizing radiation is determined by the sum of all components:

$$Epr.=0.57+Eext.+1.05Eext.Rn+$$
$$+Eext\ pp.+Eext.pv.+Eext.ing.,$$

where Eext is the dose of external gamma radiation; internal exposure dose: due to Evn.Rn - short-lived decay products of radon isotopes, Evn.pl - due to radionuclides in food, Evn.dv - due to radionuclides in drinking water; Evn.ing. due to inhalation of dust. The term 0.57 in the above formula takes into account the contribution of the ionizing component of cosmic radiation (0.40 mSv) and internal exposure due to 40K [18] to the effective doses of public exposure.Assessment of adaptive potential.

Nonparametric correlation analysis showed a close association of AP with age (0.624; p=0.000) and place of residence (0.355; p=0.000).

In table. 3 shows a significant association of the state of AP with the place of residence. Good (1) and satisfactory adaptation (grade 2) are extremely rare, only 3 Aldans (1.70%). Basically, the surveyed are in a state of functional tension of adaptation mechanisms (grade 3) - 133 people (81.6%) and more often in Aldan.

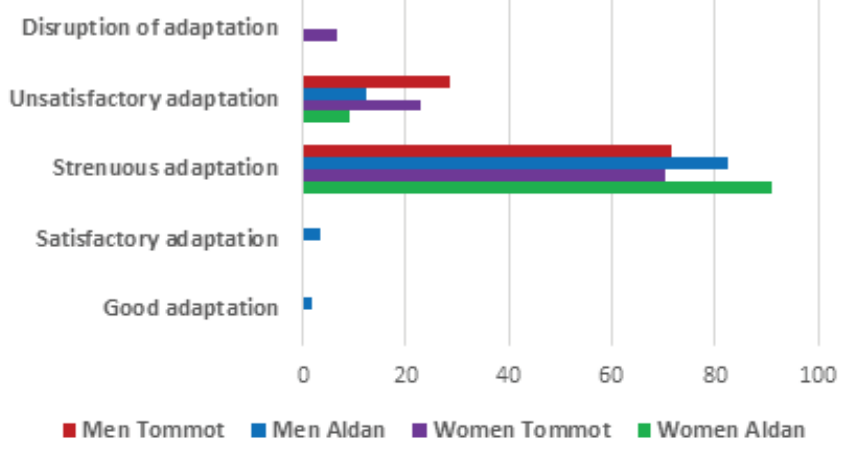
Unsatisfactory adaptation - a decrease in the functionality of the circulatory system with an insufficient, adaptive response to stress is quite common: in 24 people. (13.3%). In percentage terms, the 4th degree of AP is 2 times more common among residents of the city of Tommot. 5th degree of AP - failure of adaptation, i.e. a sharp decrease in the functional capabilities of the circulatory system with phenomena of violation of the mechanisms of adaptation of the whole organism was also detected in 3 tommots (5.7%).

Depending on gender and place of residence, the functional tension of adaptation mechanisms (3rd stage) is more common in men and women in Aldan (figure).

Table 3

The degree of AP among residents of Aldan and Tommot, abs. number/%

Groups	Degree of adaptive potential					Pearson's chi-square	df	p
	1	2	3	4	5			
Aldan	1/0.9	2/1.8	95/**86.5**	12/10.8	-	12723	4	0.013
Tommot	-	-	37/71.2	12/23.1	3/5.7			
Total:	1/0.6	2/1.2	133/81.6	24/14.7	3/1.8			



The state of AP in men and women in Aldan and Tommot, %

Unsatisfactory adaptation (the 4th degree of AP) is higher among residents of Tommot, in addition, adaptation failure was detected in 3 visiting women 36; 60 and 75 years old, also living in Tommot.

According to Table. 4, the average AP in women is worse than in men (p<0.048), while in Tommot, AP is slightly lower in men, and in Aldan, in women.

With age, there is a significant deterioration in AP. Comparison of groups of Aldans under 45 and after 45 showed a decrease in AP by 12.3%, and among Tommots by 20.3%. Comparison of age-standardized groups of Aldans and Tommots under 45 revealed a higher AP value by 3.48% of Tommots, and in groups after 45 years, the difference in AP of Tommots was 10.96%.

Comparison of standardized groups by age and northern experience showed that the AP of the newcomer population of Tommot over 45 years of age and with northern experience of more than 20 years turned out to be worse by 16% than that of the Aldans. In the group born in the area and aged over 45 years, AP was worse by 4% among Tommots.

AP had a close positive correlation with CVS pathology (0.557**; p=0.000) and fatty hepatosis (r=0.478**, p=0.000), which indicates a negative impact of a decrease in the adaptive reserves of the body on the functional state of the cardiovascular and hepatobiliary systems. This is evidenced by the fact that the presence of CVS pathologies was accompanied by a significant tension in the AP, especially pronounced in the residents of the city of Tommot (p=0.001). Нарушение функции печени и желчевыводящих путей у обследованного населения встречается довольно часто (43,9%), из них 18,9% обусловлено жировым гепатозом. В процентном отношении ожирение печени чаще встречается у жителей Томмота.

Non-alcoholic fatty liver disease (NAFLD) is an increasingly common disease, affecting more than 25% of the adult population worldwide, and varies by race and ethnicity [22]. In the Republic of Sakha (Yakutia), the incidence of NAFLD in the Yakut population is 50-60%, among Russians – 20% [10].

The main factors in the development of fatty hepatosis are an unhealthy lifestyle (high-calorie diet combined with insufficient physical activity, diabetes mellitus, thyroid disease, CCC and gallbladder diseases), exposure to the body of chemically active compounds: organic solvents, organophosphate poisons, metal compounds and natural toxins.

Alcoholic fatty liver disease (AFH) is the second most common and relevant liver disease after viral hepatitis.

Of the 17 Aldans with fatty hepatosis, only 4 people admitted to drinking alcohol once a week (23.5%), and among the residents of the city of Tommot - 2 people. (13.3%). Perhaps these data are erroneous due to untruthful answers of the respondents. Nevertheless, the frequency of fatty hepatosis is higher in percentage terms among residents of the city of Tommot than among Aldans (Table 5).

The table 6 shows the association of GBS pathology with CVS pathology (p=0.033) and especially closely with fatty hepatosis (p=0.011).

Statistical analysis of the odds ratio showed that the frequency of fatty

Table 4

Average values of adaptive potential depending on gender, age, place of residence, northern experience and pathologies of CVS and fatty hepatosis

(M±m)			p
All n=163	Men n=64	Women n=99	
2.81±0.03	2.72 ±0.05	2.87±0.04	0.048
Me(Q1;Q3)			
2.73 (2.46; 3.13)	2.65 (2.36; 3.01)	2.79 (2.52; 3.14)	0.054
AP by age			
Age	Aldan	Tommot	
Up to 45 years old	2.52 (2.33; 2.74 n=64	2.61(2.45;2.91) n=20	0.021
over 45 years	2.83 (2.56; 3.14) n=48	3.14(2.89; 3.42) n=31	0.000
	p=0.000	p=0.000	
AP depending on the place of residence			
Location	Aldan	Tommot	
All	2.65 (2.40; 2.92) n=107	2.99 (2.63; 3.34) n=51	0.000
Men	2.60(2.33; 2.93) n=57	3.13 (2.99; 3.77) n=7	0.007
Women	2.66 (2.45; 2.90) n=55	2.91 (2.63; 3.33) n=44	0.001
AP among visitors and local population			
Groups	Aldan	Tommot	
Northern experience >20 years, age over 45 years	n=16 2.87 (2.64; 3.17)	n=17 3.16 (2.93; 3.41)	0.072
born, Age over 45 years	n=14 2.83 (2.48; 3.25)	n=10 2.96(2.82; 3.24)	0.380
AP in patients with CVD pathology			
Groups	Healthy	Sick	
All	2,46 (2,31; 2,64) n=59	2,92 (2,65; 3,23) n=104	0,000
Aldan	2,43 (2,29; 2,64) n=47	2,82(2,52; 3,14) n=65	0,009
Tommot	2,54 (2,42; 2,79) n=12	3,13 (2,88; 3,42) n=39	0,000
p	0,088	0,001	
AP in patients with fatty hepatosis			
Groups	Здоровые	Больные	
All	2.45(2.32; 2.62) n=21	2.85(2.54; 3.16) n=31	0.000
Aldan	n=15 2.43(2.18; 2.64)	n=18 2.76(2.39; 3.16)	0.021
Tommot	n=6 2.51(2.40; 2.68)	n=13 2.89(2.69; 3.18)	0.009
p	0.276	0.150	

Table 5

The frequency of fatty hepatosis among the surveyed population, abs.h. /%

	Aldan		Tommot	
	Abs. num.	%	Abs. num.	%
Total	n=112	100	n=58	100
Hepatosis	17	15.1	15	25.8
Hepatosis drinkers once a week	4	23.5	2	13.3

Table 6

Association of CVS and GBS pathologies

Cardiovascular pathology	GBS pathology				X^2	df	p	OR (95% confidence interval)	p
	No		Yes						
	Abs. num.	%	Abs. num.	%					
No	42	43.8	22	28.7	4.527	1	0.033	1.98 (1.041-3.744)	0.036
Yes	54	56.3	56	71.8					
Fatty hepatosis									
No	21	84.0	6	18.2	6.463	1	0.011	3.10 (1.207-8.000)	0.019
Yes	84	59.2	27	81.8					

hepatosis is 4.5 times more common in people with cardiovascular pathology than in people without such a pathology (table 6).

Fatty hepatosis is one of the factors in the development of atherosclerosis: in persons with cardiovascular diseases, fatty hepatosis occurs 3.10 times more often. The complication of NAFLD is a risk factor for atherosclerotic cardiovascular disease, which is the leading cause of death in patients with NAFLD. According to a scientific statement from the American Heart Association, NAFLD and NASH are becoming increasingly common conditions that are underdiagnosed and underestimated as risk factors for morbidity and mortality from CVD [22].

A direct correlation was found between AP and the level of TG (0.249; p=0.001), the activity of LDH enzymes (0.258; p=0.001), CK (0.162; p=0.039), and urea (0.298; p=0.000), which indicates a negative role in reducing the adaptive reserves of the body of hypertriglyceridemia, metabolic disorders of carbohydrates and proteins. A negative relationship was found with ALP activity (-0.231; p=0.003). A decrease in ALP activity was registered among residents of the city of Tommot. This fact does not agree with the fact that one of the factors for reducing the activity of alkaline phosphatase is radiation, since the largest annual individual effective dose was recorded in the city of Aldan (6.22 μSv), and the smallest - in the city of Tommot (3.52 μSv).

Thus, a comparative calculation of the annual individual effective dose of exposure of the population of the city of Aldan and the city of Tommot of the Aldan region showed a higher value of annual individual effective dose to the population in the city of Aldan. The adaptive potential of the inhabitants of the Aldan region is mainly in a state of functional tension of the mechanisms of adaptation, more often among the inhabitants of the city of Aldan. However, a further decrease in AP is more common among residents of the city of Tommot. The decrease in AP is directly related to an increase in the level of triglycerides, urea, the activity of energy metabolism enzymes LDH, CK, with a decrease in the activity of alkaline phosphatase, which indicates the negative role of dyslipidemia, impaired carbohydrate and protein metabolism in reducing the adaptive potential of the circulatory system. In addition, a decrease in AP was associated with dysfunction of the hepatobiliary system, especially with fatty hepatosis. Fatty hepatosis, detected three times more often in those examined with pathology of the cardiovascular system, is a negative risk factor for reducing AP and the associated risk of developing atherosclerotic cardiovascular diseases.

Conclusion: The high frequency of functional stress of the mechanisms of adaptation of the population of the city of Aldan in conditions of increased natural radiation, and especially poor adaptation, failure of adaptation, frequent occurrence of fatty hepatosis among the inhabitants of the city of Tommot, undoubtedly require the study of the complex impact of negative factors.

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PHYSICAL DEVELOPMENT OF PRESCHOOL CHILDREN IN MUNICIPAL DISTRICTS OF THE REPUBLIC OF SAKHA (YAKUTIA)

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The relevance of studying the state of children's health is closely related to the issues of their physical development, which is one of its main indicators. Our article presents data from the analysis of the anthropometric indicators of preschool children living in the regions of the Republic of Sakha (Yakutia). The study was conducted in 17 municipal districts representing 5 socio-economic zones of the republic: arctic, eastern, western, central, southern. A total of 643 pupils of preschool educational institutions aged 3 to 6 years were examined. The study group on physical development consisted of children with an actual age of 3 years in the amount of 208 people and children of 6 years old in the amount of 127 people.

Keywords: body weight, height, body mass index (BMI), physical development, anthropometry, head circumference, subcutaneous fat, obesity, tall stature, short stature.

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Introduction. The physical development of children is one of the main indicators reflecting the health of the younger generation and the nation as a whole. According to the definition of the Union of Pediatricians of Russia, physical development is understood as a dynamic process of growth (increase in body weight and length, development of organs and body systems) and biological maturation of a child. Healthy growth and development means that children are supported to thrive in areas including physical, cognitive, language and emotional areas that play an important role in children's lives from birth to adulthood [4, 6]. Monitoring height and weight helps to correctly diagnose diseases and implement therapeutic and preventive measures.

Not only genetic predisposition, but

Table 1

Physical development of children in municipal districts of the Republic of Sakha (Yakutia), z-score, %

Соц.-эконом. зона	z <-3	-3 ≤ z < -2	-2 ≤ z < -1	-1 ≤ z ≤ +1	+1 < z ≤ +2	+2< z ≤ +3	z > +3
	3 years, «height-for-age»						
RS(Ya)	0	4.63	17.77	63.27	8.53	3.83	1.95
Arctic zone	0	5.88	5.88	82.35	5.88	0	0
Eastern zone	0	8.33	25.00	41.66	25.0	0	0
Western zone	0	4.29	21.43	62.86	7.14	4.29	0
Central zone	0	4.65	23.23	62.79	4.65	1.55	3.10
Southern zone	0	0.00	13.33	66.67	0.00	13.33	6.67
	3 years. «body mass index for age»						
RS(Ya)	0.29	0.29	6.35	61.60	22.39	4.30	4.77
Arctic zone	0	0	0	70.59	23.53	0	5.88
Eastern zone	0	0	0	66.66	25.00	0	8.33
Western zone	1.43	1.43	11.42	55.71	20.00	8.57	1.43
Central zone	0	0	7.03	61.72	23.44	6.25	1.53
Southern zone	0	0	13.33	53.33	20.00	6.67	6.67
	3 years. «head circumference for age»						
RS(Ya)	0	2.508	7.706	51.254	20.946	10.43	3.376
Arctic zone	0	5.88	11.76	47.06	17.65	5.88	11.76
Eastern zone	0	0	0	50.00	25.00	25.00	0
Western zone	0	0	4.29	47.14	31.43	12.86	4.29
Central zone	0	0	2.48	52.07	23.98	1.74	0.83
Southern zone	0	6.66	20	60	6.67	6.67	0
	3 years. «circumference of the middle part of the shoulder»						
RS(Ya)	0.58	3.16	11.17	60.71	16.99	8.05	1.85
Arctic zone	0	5.88	11.76	58.82	17.65	5.88	0
Eastern zone	0	0	0	58.33	8.33	25	8.33
Western zone	1.43	1.43	27.14	68.57	14.29	0	0
Central zone	0.9	1.80	3.60	71.17	18.02	2.70	0.9
Southern zone		6.67	13.33	46.67	26.67	6.67	0
	6 years. «height-for-age»						
RS(Ya)	0	5.21	18.11	73.04	8.58	2.86	2.86
Arctic zone	0	0	6.67	86.67	6.67	0	0
Eastern zone	0	0	25.00	75.00	0	0	0
Western zone	0	8.69	26.09	65.22	0	0	0
Central zone	0.95	1.90	20.00	68.57	4.76	3.81	0
	6 years. «body mass index for age»						
RS(Ya)	0.19	1.33	3.99	44.72	16.46	8.37	4.09
Arctic zone	0	6.67	0	40.00	33.33	6.67	13.33
Eastern zone	0	0	0	50.00	25.00	25.00	0
Western zone	0	0	8.89	68.89	11.11	6.67	4.44
Central zone	0.95	0	11.43	67.62	13.33	3.81	2.86

also environmental factors, such as nutritional conditions, upbringing, the presence of diseases, social, climatic and other factors, have a significant impact on the process of growth and development of children [4, 5].

In recent years, a significant number of overweight and underweight children have been registered, there is a tendency to disharmonious development of children, an increase in the frequency of various deviations from normal developmental parameters [1, 2, 3].

Impairment of physical development is the result of long-term exposure to inadequate nutrition, lack of child care, poor environmental and socio-cultural conditions. This is associated with higher morbidity and mortality, mental retardation, poor educational achievement, and reduced intellectual ability, all of which are strong predictors of human capital and social progress.

Childhood malnutrition is estimated to be the largest contributor to the global burden of disease, killing millions of children in developing countries and causing high health care costs [6].

In some regions of Russia, due to the diversity of climate and geographical areas, nationalities and ethnic groups, differences in the social and economic situation, regional indicators of the physical development of children have been developed. In the Republic of Sakha (Yakutia) in the 2000s. research was conducted and regional standards for the physical development of children under the age of 7 were developed.

The purpose of this work is to identify, compare and evaluate the physical development of preschool children in 5 socio-economic zones of the republic: arctic, eastern, western, central, southern.

Materials and methods. To assess the physical development of children, anthropometry was carried out, the following somatometric indicators were measured: height, body weight, head circumference, circumference of the middle part of the shoulder, subcutaneous fat of the back and shoulder according to the AnthroWHO-2007 program guidelines. Preliminarily representatives of all identified children received informed consent.

According to the requirements, anthropometric measurements were carried out on a naked child, in the “at attention” position (the child stands upright, tucking up his stomach and straightening his shoulders, lowering his arms along the body, putting his heels together, toes apart, the head is set in the “horizontal” position - the lower edge of the orbit and the upper edge of the tragus of the ear are in the same horizontal plane) in the first half of the day, since the body length decreases by 1-2 cm by the end of the day due to the flattening of the arches of the foot, intervertebral cartilage, a decrease in muscle tone, and body weight increases on average almost per 1 kg.

Height was measured in a standing position at the height of inhalation on a vertical electronic stadiometer REP-1 Napolny, with an error of ±2 mm. The counting was carried out from the platform along the vertical rack.

Body weight was measured on a ВМЭН-150-100-И-Д-А electronic scale. These scales are easy to use. Allow to measure the weight of people up to 150 kg. Measurement accuracy up to 100 g. Before weighing, the scales were checked and adjusted.

The data of anthropometric measurements were entered into an individual card. All anthropometric data of the subject were accompanied by mandatory information, such as: individual number, date of examination, gender, year, month and date of birth (with subsequent calculation of age on the day of the examination).

All obtained somatometric data were entered into the WHO Antrho (from 0 to 5 years old) and WHO Antrhoplus for personal computers (from 5 to 19 years old) computer programs. This program allows you to calculate individual indicators of height, body weight, BMI and evaluate them in accordance with the current WHO standards on a percentile scale and on a Z-score, with the criteria of underweight, short stature -2SD, over-

Table 2

The results of the assessment of the physical development of rural and urban children of preschool age

Index	Urban	Rural	p
3 year			
Height	98,55 (95-102,64)	97,3(94-100,5)	0,008
Weight	15,18(14,0-16,5)	15,0(14,0-16,55)	0,574
Body mass index	15,64(14,86-16,69)	15,85(14,6-16,97)	0,993
6 year			
Height	118(114,85-121,35)	115(111,9-118,65)	0,0001
Weight	21,15(19,32-23,6)	20,55(18,7-23,1)	0,023
Body mass index	15,17(14,39-16,39)	18,05(15,91-20,5)	0,0001

Table 3

Height and weight indicators with an interval of 20 years

Indicators	Age	Data of Zakharova N.M. (1976)		Data of Zakharova N.M. (2001)		Own research (2022)	
		Д	М	д	М	д	м
Height (cm)	3.0 4.0 5.0 6.0	92.29 98.64 104.92 109.98	92.54 99.80 104.76 111.36	93.38 99.47 104.91 111.01	94.52 100.38 105.17 111.80	97.75±3.97 104.13±4.33 112.12±4.36 116.15±4.77	99.20±4.02 103.87±3.08 112.53±4.43 118.03±4.18
Weight, (kg)	3.0 4.0 5.0 6.0	14.35 15.85 17.27 18.94	14.88 16.17 17.64 19.40	14.32 15.79 17.76 19.33	15.00 16.30 17.42 19.67	15.30±1.81 17.46±2.33 19.90±2.59 21.4±2.86	15.78±1.68 17.10±1.84 19.96±2.30 22.5±3.19

weight +1SD, obesity, tall stature + 2SD.

Research results. The study involved 17 municipal districts representing 5 socio-economic zones of the republic: arctic, eastern, western, central, southern. In total, 643 pupils of preschool educational institutions from 3 to 6 years old were involved, of which children with an actual age of 3 years in the amount of 208 and 6 years in the amount of 127 were included in the study group on physical development.

We carried out an assessment of physical development in the socio-economic zones of the republic in which children lived. The proportion of children meeting WHO standards in general was 88.3% at the age of 3 years, and 65.2% at the age of 6 years. The table below presents data on territorial administrative zones (Table 1).

At the age of 3 years, short stature is most often found in the regions of the Eastern zone (8.33%), and tall stature in the regions of the Southern zone (20%). Underweight is more common in areas of the Western zone (2.84%), and in this zone the largest number of overweight children (8.57%). Obese children are more common in areas of the Southern zone (6.67%).

At the age of 6 years, the prevalence of short stature is higher in the areas of the Western zone (8.51%). The proportion of tall children is higher in the regions of the Central Zone (5.72%). Overweight children are more common in areas of the Eastern zone (25%), with obesity in areas of the Arctic (13.33%) and Western zones (4.26%).

In addition, we compared the main parameters of the physical development of rural children with urban children (Table 2).

The analysis showed that rural children in both age groups lag behind in height. At the age of 6 years, the lag in physical development in rural children increases ($p < 0.0001$). At the same time, the body mass index is higher than that of urban residents, against the background of low growth and almost equal weight categories.

In order to identify the acceleration of development rates, a comparison was made of the average values of the height-weight indicators of the physical development of boys and girls aged 3 to 6 years with an interval of more than 20 years, the data are presented in table 3.

As can be seen from the table, children are now taller and larger in body weight, which shows the acceleration of their development compared to the previous generation and confirms the fact of acceleration.

Conclusion. According to the results of the study, it was revealed that the physical development of most children correspond to the WHO standard for height and body weight. However, there are a number of children with deviations towards deficiency or overweight. It is also worth paying attention to the differences in the physical development of children depending on the socio-economic zone of residence, which confirms the need to take into account this aspect. Such differences may be related to the climate, economic situation and other factors. In addition, given the dynamics of growth and weight indicators of preschool children over the past 20 years, the need to update the standards of physical development of children of this age group is obvious.

In general, the study allows us to assess the physical development of children and identify problem areas that require additional attention and correction.

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TOPICAL ISSUE

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RISK FACTORS FOR THE DEVELOPMENT OF PNEUMONIA IN PATIENTS WITH IMMUNO-INFLAMMATORY RHEUMATIC DISEASES RECEIVING GIBP DURING COVID-19 PANDEMIC

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A retrospective analysis of the database of the register of patients with immuno-inflammatory rheumatic diseases receiving treatment with biologic disease-modifying anti-rheumatic drugs in the Novosibirsk region was carried out, which included 318 patients, 94 of whom had indications of pneumonia during the period from 01.04.2020 to 31.12.2020.

There was a statistically significant increase in the risk of developing pneumonia over the age of 60 years and in patients receiving rituximab, while the therapy with TNF-α inhibitors significantly reduced the risk of developing pneumonia.

Keywords: COVID-19, pneumonia, immune-inflammatory rheumatic diseases, biologic disease-modifying anti-rheumatic drugs.

One of the severe manifestations of a new coronavirus infection is pneumonia. Pathological changes in the lungs in patients with COVID-19 are presented in the form of pulmonary edema, diffuse alveolar damage with the formation of hyaline membranes, reactive hyperplasia of type II alveolocytes, protein aggregates, fibrinous exudate, monocytic and macrophage infiltration of the alveolar spaces [19,20]. Immunohistochemical study revealed infiltration of the alveoli with CD68+ cells, CD20+B cells, and CD8+T cells [19]. The pathogenesis of the development of pneumonia is associated with the peculiarities of the virus to bind through the ACE2 receptor, about 83% of which ACE2 is localized on type 2 alveolocytes. After the penetration of the virus into the lung tissue, activation of macrophages and monocytes, activation of Th1 cells that produce GM-CSF and IL-6 occurs. GM-CSF, in turn, activates CD14+CD16+ monocytes to produce IL-6, TNF-α, etc. [18]. The cytokine storm in COVID-19 is characterized by overproduction of IL-6 and TNF-α. SARS-CoV-2 activates NF-κB and interacts with ACE2 on the cell surface, resulting in a decrease in ACE2 expression followed by an increase in AngII. In addition to NF-κB activation, the type 1 angiotensin receptor axis can also induce the expression of TNF-α and the soluble form of IL-6Ra (sIL-6Ra). IL-6 binds to sIL-6R to form the IL-6-sIL-6R complex, which can activate STAT3 [17]. Both NF-kB and STAT3 are able to activate IL-6 production. IL-6 not only binds to sIL-6R but can also bind to the membrane-bound IL-6 receptor (mIL-6R), resulting in a pleiotropic effect on adaptive and innate immune cells and the development of a cytokine storm [16,17].

Interstitial lung disease is often found among patients with immuno-inflammatory rheumatic diseases (IIRD), especially with rheumatoid arthritis, systemic sclerosis, polymyositis, etc., which often requires correction of antirheumatic therapy, as well as the appointment of antifibrotic drugs [3], while the X-ray picture according to computed tomography is similar to changes in the lungs with coronavirus pneumonia [11]. According to D. Galarza-Delgado et al. patients with IIRD who are on biological disease-modifying antirheumatic drugs (bDMARDs) are not susceptible to a more severe course of coronavirus infection, apart from patients treated with rituximab [7]. J. Loarce-Martos et al. also described a more severe course of coronavirus infection with a high risk of bilateral lung injury and mortality among patients treated with rituximab [14]. According to the analysis of the course and outcomes of a new coronavirus infection pursuant to the register of patients receiving bDMARDs, Russian colleagues discovered dependence of the severity of the course of COVID-19 on age, comorbidities [5]. Previously, we also published the results of an anal-

ysis of the outcomes of the course of COVID-19 among patients receiving bDMARDs in the Novosibirsk region, which demonstrated the effect of age on the severity of the disease, as well as the use of rituximab [1].

Despite the data already available to date, the study of the risks of developing a severe course of coronavirus infection in patients on immunosuppression requires further study, in particular, the identification of risk factors for the development of pneumonia in patients with an initially high risk of developing interstitial lung disease.

The purpose of the study is to examine the risk factors for the development of pneumonia in patients with immunoinflammatory rheumatic diseases receiving bDMARDs

Materials and methods. A retrospective analysis of the database of the register of patients with IIRD who received bDMARDs under the Territorial Program of State Guarantees of Free Medical Assistance to Citizens in the Novosibirsk Region was performed.

Patient information included demographics, medical history, disease activity, and therapy received. Data on COVID-19 included clinical manifestations, laboratory data confirming the diagnosis of a new coronavirus infection, and the outcome of the disease.

Statistical analysis. Statistical data processing was carried out on a personal computer using the NumPy, Pandas, SciPy, MatPlotLib and Seaborn libraries for the Python language. Analysis of the joint distribution of binary features was carried out using Fisher's exact test, and the odds ratio (OR) indicator was used as a quantitative assessment of risk change between groups. Comparison of quantitative characteristics was performed using the Mann-Whitney test. The median and the values of the first and second quartiles were used to describe quantitative variables. The description of the qualitative features is given with the indication of the number of patients and their percentage in the corresponding group.

Results. The study included 318 patients with IIRD in the registry of patients receiving bDMARDs, 94 of which had symptoms of a new coronavirus infection (Table 1).

As it can be seen, the majority of the patients are women, while the distribution of diagnoses corresponded to the prevalence of nosologies in the population. The groups of patients who had a new coronavirus infection and those without COVID-19 did not differ in age, duration of the disease, or duration bDMARDs therapy. Most of them received therapy with the anti-B-cell drug rituximab, which is due to the predominance of rheumatoid arthritis nosology in the patient registry; ,TNF-α inhibitors were in second place in terms of frequency of use, followed by IL-17A inhibitors, the smallest number of patients received IL-6 inhibitors and IL12 / 23 axis inhibitors.

The diagnosis of a new coronavirus infection in the group of patients was established according to the then-current "Interim Guidelines: Prevention, Diagnosis and Treatment of a New Coronavirus Infection (COVID-19)" of the Ministry of Health of the Russian Federation, version 6-1 of 28.04.2020.

An analysis of the distribution of age-sex and clinical characteristics of patients by the presence/absence of pneumonia showed that coronavirus pneumonia was more common in women (48%) than in men (37.5% of cases), while pneumonia was more common at an older age (median age was 53 years against 44). Among various nosologies, patients with RA were more likely to develop pneumonia (69% of cases). In the group of patients with proven pneumonia, more than half of the patients received glucocorticoids, while among bDMARDs, in the group of patients treated with rituximab, there was a high incidence of pneumonia (81% of cases).

To analyze the risk factors for the development of pneumonia (Table 2), gender, age of patients, duration of the disease, the diagnosis with which patients were observed, and the therapy received were analyzed. It was shown that age over 60 significantly increased the risk of developing pneumonia (OR = 7.44; p = 0.006). At the same time, out of the analyzed diagnoses, patients with ankylosing spondylitis and psoriatic arthritis have a statistically significantly lower risk of developing pneumonia compared to patients with rheumatoid arthritis (OR=6.05,

Table 1

Clinical characteristics of patients with rheumatic diseases receiving bDMARDs

Parameters	Meaning	
	Without COVID-19	COVID-19
The number of patients, n	224	94
Age, years	49 (39.0; 59.0)	46.5 (38;56)
Sex, n (%)		
Men	78 (34.8)	24 (25.5)
Women	146 (65.2)	70 (74.5)
Diagnosis		
RA	133 (59.4)	49 (52.1)
AS	71 (31.7)	29 (30.9)
PsA	12 (5.4)	12 (12.8)
Other	8 (3.5)	4 (4.3)
Duration of disease, years	15 (7;21)	13 (7; 20)
DMARDs, n (%)		68 (72.3)
Methotrexate	150 (66.9)	42 (44.7)
Sulfasalazine	50 (22)	11 (11.7)
Leflunomide	30 (13.3)	9 (9.6)
Hydroxychloroquine	10 (4.46)	8 (8.5)
Glucocorticoids	10 (4.46)	14 (14.9)
Duration of DMARDs treatment, years	4 (1;7)	3 (1.05;5)
Duration of DMARDs treatment		
Under a year	49 (21.9)	13 (13.8)
Over a year	175 (78.1)	81 (86.2)
bDMARDs		
Rituximab (Acellbia)	121 (54.0)	43 (45.7)
Certolizumab pegol (Cimzia)	32 (14.3)	13 (13.8)
Secukinumab (Cosenthix)	26 (11.6)	11 (11.7)
Adalimumab (Humira)	12 (5.4)	9 (9.6)
Golimumab (Simponi)	15 (6.7)	6 (6.4)
Etanercept (Enbrel)	7 (3.1)	4 (4.3)
Abatacept (Orencia)	1 (0.4)	3 (3.2)
Ixekizumab (Tals)	0 (0)	2 (2.2)
Sarilumab (Kevzara)	0 (0)	1 (1.1)
Olokizumab (Artlegia)	1 (0.4)	1 (1.1)
Ustekinumab (Stelara)	0 (0)	1 (1.1)
Tocilizumab (Actemra)	3 (1.3)	0 (0)
Netakimab (Efleira)	6 (2.7)	0 (0)

p<0.001). Despite a higher incidence of pneumonia among patients treated with glucocorticoids, risk factor analysis did not reveal an effect of hormone intake on the development of pneumonia. At the same time, rituximab therapy significantly increased the risk of developing pneumonia (OR = 15.45, p <0.001), while taking a TNF-α inhibitor reduced the risk of developing pneumonia (OR = 0.1; p <0.001).

Discussion. The results presented in the work confirm the effect of rituximab on the severity of the course of coronavirus infection and associated pneumonia. A number of authors associate the revealed effects with the depletion of B-cells. Earlier studies have shown that iatrogenic depletion of B-lymphocytes increases infectious risks [15]. In the review by E.L. Nasonov [4] the currently existing views on the role of B cells in the course of coronavirus infection were demonstrated. Based on these results, it was concluded that there was a need for a careful approach to the indications for prescribing anti-B-cell drugs during the pandemic of a new coronavirus infection, the need to temporarily cancel treatment if SARS-CoV-2 infection was suspected and the disease was developing.

Another important issue related to the use of rituximab in COVID-19 pandemic is the vaccination of patients with IIRD. According to the current recommendations, patients with IIRD should be vaccinated against COVID-19 in order to minimize the high risks of a severe course of the disease [2,13]. The question of the effectiveness of vaccination in patients who have already received rituximab and have B-cell depletion is relevant. Michael Markus Bonelli et al. showed that in patients treated with rituximab, there was a decrease in the humoral response with a preserved T-cell response [22], while therapy with TNF-α inhibitors had practically no effect on antibody production [6]. Currently, there are data on the possibility of using monoclonal antibodies to SARS-COV-2 to reduce the risk of severe coronavirus infection in immunocompromised patients [21].

Age is an undeniable risk factor for severe COVID-19 and pneumonia. Fei Zhou et al. showed that the mortality of patients with severe pneumonia and respiratory distress syndrome occurred in an older age group and is a risk factor associated with in-hospital mortality [9].

As it is known, therapy with TNF-α inhibitors in patients with IIRD is associated with infectious risks. However, data from various studies show that therapy with TNF-α inhibitors in patients with inflammatory joint diseases does not increase, but reduces the risk of a severe course of coronavirus infection [8,10,12], which is consistent with our results.

Over the past three years of the pandemic, the SARS-CoV-2 virus has mutated, which affected not only the contagiousness, but also the severity of clinical manifestations. Currently, there is a lower incidence of severe cases and a decrease in mortality in the population, however, whether the susceptibility, as well as the severity of the course of COVID-19, has changed in patients with IIRD receiving bDMARDs, requires further study.

Conclusion. Rituximab therapy during the COVID-19 pandemic significantly increases the risk of a severe course of a new coronavirus infection, the development of pneumonia, and the likelihood of death. Treatment with TNF-α inhibitors reduces the risk of developing pneumonia associated with SARS-COV-2.

All patients treated with rituximab are recommended to be vaccinated, as well as to receive virus-neutralizing monoclonal antibodies to SARS-COV-2.

The work was carried out within the framework of RICEL Subject No. FWNR-2023-0009.

Table 2

Analysis of the qualitative characteristics of patients as potential risk factors for pneumonia in COVID-19

Parameters	The presence of feature, N1 / N2	The absence of feature, N1 / N2	OR	p
Sex: male	9 / 15	36 / 33	0.55	p = 0.244
Age > 60	11 / 2	34 / 46	7.44	p = 0.006
Daignosis RA AS psA	 33 / 15 8 / 21 2 / 10	 12 / 33 37 / 27 43 / 38	 6.05 0.28 0.18	 p < 0.001 p = 0.008 p = 0.028
Duration of the disease (years) > 5	36 / 39	9 / 9	0.92	p = 1.000
DMARDs	36 / 31	8 / 15	2.18	p = 0.149
Glucocorticoids	9 / 5	35 / 41	2.11	p = 0.253
Inhibitors of TNF-α	5 / 27	40 / 21	0.10	p < 0.001
Inhibitors of IL-6	0 / 2	45 / 46	0.00	p = 0.495
Inhibitors of IL-17	4 / 9	41 / 39	0.42	p = 0.235
Anti-B-cells therapy	34 / 8	11 / 40	15.45	p < 0.001
Anti-T-cells therapy	1 / 2	44 / 46	0.52	p = 1.000

* N1 – pneumonia, N2 – without pneumonia, OR – odds ratio.

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POLYMORPHISM OF THE MICROSOMAL EPOXIDE HYDROLASE *EPHX1* GENE (RS1051740) IN FREQUENTLY ILL CHILDREN FROM AN INDUSTRIAL AREA IN SOUTHERN SIBERIA

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The aim of the study was to analyze frequency of the EPHX1 gene polymorphism (rs1051740) associated with diseases of the upper airways and elevated manganese levels in biological media of children from an industrial area located in the Southern Siberia.

Materials and methods. We examined children aged 4-7 years who permanently lived in an industrial area in the Southern Siberia. It was a monotown with its economy dominated by a large non-ferrous metallurgy plant. The test group was made of 60 children who were often sick (more than 6 times a year) for a long time. The reference group included 39 conditionally healthy children with manganese levels in their blood being within the reference range. We identified frequency of polymorphism of the microsomal epoxide hydrolase EPHX1 gene (rs1051740) and the cytochrome C level using PCR and ELISA accordingly. The statistical significance was taken at p<0,05.

Results and discussion. Average manganese levels were 1,8 times significantly higher in blood of the children from the test group than in the reference one. We established statistically significant authentic differences in frequencies of the EPHX1 gene genotypes and alleles (rs1051740) between the test and reference groups (the C/C genotype was 3,2 times more frequent; the C allele, 1,5 times, p<0,05). Cytochrome C levels were 2,2 times lower in blood serum in the test group against the reference one.

Conclusions. The study established several peculiarities in children who often had diseases of the upper air ways (the test group). They had elevated manganese levels in their blood, higher than its safe level; the serum cytochrome C level was lower in them; they more frequently had the C/C genotype (OR=4,05, 95% CI=1,26-13,05) and the C allele (OR=1,98, 95% CI=1,09-3,60) of the EPHX1 gene (rs1051740). Many authors believe polymorphism of this gene to be a risk factor able to cause respiratory

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diseases. Deficiency of microsomal epoxide hydrolase 1, combined with the candidate gene polymorphism, disrupts detoxification, promotes accumulation of non-conjugated chemical exogenous factors (manganese), inhibits anti-oxidation and weakens the immunity. All this makes children who live in the analyzed town fall sick with respiratory diseases more frequently.

Keywords: xenobiotic biotransformation, the *EPHX1* gene polymorphism (rs1051740), respiratory pathology, detoxification genes, manganese contamination in biological media.

Introduction. At present, a lot of attention is paid to investigating contributions made by exogenous chemical factors to population incidence, children included. Occurrence and growth in environmental diseases, including respiratory ones, depend on quality of ambient air and levels of pollution in it [8]. Ambient air in industrial regions located in the Southern Siberia tends to be polluted with a wide range of harmful chemicals [3,11]. Manganese is a frequent component in emissions from non-ferrous metallurgy plants; its aerosols primarily target the respiratory organs [7]. Manganese plays an important role in many enzymatic processes in the body but if it is introduced through inhalation in excessive quantities, it can produce cumulative toxic effects [10].

An issue regarding interactions between genetic and environmental factors is no less relevant. Individual reactions to the same environmental exposures may vary significantly between keeping good health and falling sick. Therefore, it is necessary to identify certain indicators for assessing risks of various diseases, respiratory ones included. The lungs are protected from inhaled toxic chemicals and reactive oxygen species mostly by genes and enzymes that belong to the xenobiotic transformation system. The microsomal epoxide hydrolase *EPHX1* gene is located on the chromosome 1 (1q42.1). Its polymorphism rs1051740 leads to tyrosine being replaced with hystidine in the 113 location (Tyr113His) thereby reducing activity of the enzyme by 50% in C/C homozygotes and by 25% in heterozygotes. The gene plays a key role both in detoxification (with cytochrome) and development of chronic respiratory diseases; it is a protective enzyme that acts against potentially harmful small molecules penetrating the body from the environment [9,11].

The aim of this study was to analyze frequency of the *EPHX1* gene polymorphism (rs1051740) in children from an industrial center located in the Southern Siberia who were often sick with respiratory diseases.

Materials and methods. We examined children aged 4-7 years who permanently lived in a large industrial center in the Southern Siberia. It was a monotown with its economy dominated by a large non-ferrous metallurgy plant. The study was accomplished in conformity with the international standards established in the Declaration of Helsinki. The legal representatives of the examined children gave their written consent to medical examinations and tests. The test group was made of 60 children who were often sick (more than 6 times a year) for a long time. The reference group included 39 conditionally healthy children.

Manganese levels were identified in children's blood by mass spectrometry with inductively coupled plasma in accordance with the Methodical Guidelines MUK 4.1.3230-14 [MUK 4.1.3230-14 Measurement of mass concentrations of chemical elements in biological media (blood, urine) with mass spectrometry with inductively coupled plasma].

Patients' genotypes were identified by using buccal epithelium as a test material. DNA was extracted with DNK-Sorb-AM kit manufactured by the Rospotrebnadzor's Central Scientific Research Institute for Epidemiology. The Tyr113His polymorphism of the *EPHX1* gene (rs1051740) was analyzed with PCR in real time using a reagent kit provided by Syntol scientific production company and a CFX96 amplifier.

Cytochrome C levels were identified in children's blood serum by ELISA tests using a

Human Cytochrome C Platinum ELISA kit (eBioscience) and an Elx808 microplate reader.

The data were statistically analyzed with STATISTICA 6.1. The results were given as a mean value of an indicator (X), standard deviation (SD) and standard error of mean (SEM); the significance level was below 0,05. Genetic analysis of the data relied on using the Gen-Expert online calculator. The genotype distribution was tested for conformity with the Hardy – Weinberg equilibrium. We considered whether alleles or genotypes were associated with susceptibility to respiratory diseases using values of odds ratio (OR) with 95% confidence interval (95% CI). The statistical significance was taken at $p<0,05$.

Results and discussion. Manganese levels in blood of the children from the test group were higher than the reference ones and varied between 0,014 and 0,033 μg/cm^3. Manganese levels were within their reference range in the reference group where they varied between 0,006 and 0,013 μg/cm^3. Average manganese levels were 1,8 times higher in the test group against the reference one ($p=0,0001$) (Table 1).

We examined frequency of the Tyr113His polymorphism of the microsomal epoxide hydrolase 1 gene responsible for xenobiotic transformation and did not established any deviations from the Hardy – Weinberg equilibrium in the analyzed groups ($p=0,06$ for the test group; $p=1,00$ for the reference group).

The comparative analysis established statistically significant differences in frequency of genotypes and alleles. The C/C genotype was 3,2 times more frequent and the C allele was 1,5 times more frequent in the test group against the same indicators in the reference one ($p<0,05$). We established the following frequencies of genotypes and alleles in the children who were often sick for a long time and had elevated manganese levels in their blood: T/T, 35%; T/C, 33%; C/C, 32%; T, 52%; C, 48%. These frequencies were different in the conditionally healthy children with manganese levels in their blood within the reference range: T/T, 46%; T/C, 44%; C/C, 10%; T, 68%; and C, 32%, accordingly. The C/C genotype and the C allele of the *EPHX1* gene Tyr113His (rs1051740) created elevated risks of respiratory diseases in the test group (C/C: OR=4,05, 95% CI=1,26-13,05; C: OR=1,98, 95% CI=1,09-3,60) (Table 2).

Table 1

Manganese levels in blood of the children from an industrial region in the Southern Siberia who are often sick for a long time

Indicator	Reference range	The test group (n=60), X±SD	The reference group (n=39), X±SD	Statistical significance, p
Manganese, μg/cm^3	0.006-0.014	0.020± 0.005	0.011± 0.002	0.0001

Table 2

Frequency of the Tyr113His polymorphism of the microsomal epoxide hydrolase 1 gene in the children from an industrial region in the Southern Siberia who are often sick for a long time

Ген	Genotypes/ alleles	The test group (n=60)	The reference group(n=39)	OR (95% CI)	Statistical significance, p
EPHX1 (rs1051740)	T/T	0.35	0.46	0.63 (0.28-1.43)	0.04
	T/C	0.33	0.44	0.65 (0.28-1.48)	
	C/C	0.32	0.10	4.05 (1.26-13.05)	
	T	0.52	0.68	0.50 (0.28-0.91)	0.02
	C	0.48	0.32	1.98 (1.09-3.60)	

Table 3

Expression of cytochrome C protein in blood serum of the children from an industrial region in the Southern Siberia who are often sick for a long time

Indicator	Reference range	The test group (n=60), X±SEM	The reference group (n=39), X±SEM	Statistical significance, p
Cytochrome C, ng/ml	0.1-0.5	0.143±0.012	0.316±0.067	0.004

Cytochrome C is a protein that activates xenobiotic detoxification, participates in metabolism and cell breathing. We identified its levels in both groups and established its weaker expression in the children from the test group where tis levels were 2,2 times lower against the reference group (0,143±0,012 ng/ml; p=0,004) (Table 3).

Respiratory diseases are a vital challenge for contemporary healthcare since they occupy a leading rank place in incidence, especially among children [1,9]. Children's airways have a peculiar structure that makes them more susceptible to airborne technogenic chemicals.

Enzymes of the xenobiotic transformation system and anti-oxidant protection have a significant role in protecting the lungs from toxic environmental exposures. Roles that genes can possibly have in development of diseases are established by investigating single nucleotide polymorphisms (SNPs). It is single nucleotide polymorphisms that are responsible for new functional properties of proteins. It seems rightfully relevant to identify specific genetic markers responsible for susceptibility or resistance to respiratory pathologies as predictors of health disorders [4].

According to many studies, such markers include the microsomal epoxide hydrolase 1 gene and its polymorphisms, for example, rs1051740, which is associated with respiratory diseases such as bronchial asthma, pneumonia [2], chronic obstructive pulmonary disease (COPD) [12], bronchopulmonary dysplasia, and common respiratory diseases. Our studies give evidence that Tyr113His polymorphisms of the *EPHX1* gene (rs1051740) accompany frequent diseases of the upper airways in children from an area with heavy technogenic pollution due to elevated manganese levels in biological media.

Cytochrome C levels can be considered a marker showing apparent disorders of energy metabolism and detoxification. This protein is necessary for proper functioning of the respiratory chain. Cytochrome C is a complex protein located on the internal mitochondrial membrane; it participates in the electron transport chain, inhibits lipid peroxidation, and contributes to xenobiotic neutralization [4]. We established statistically significant differences in cytochrome levels in blood serum of the children with elevated manganese concentrations in blood against the reference group (p<0,05).

Manganese is highly capable of creating chemical complexes by binding sulfhydryl groups in glutathione and blood plasma proteins and of inducing lipid peroxidation in cellular membranes. As a result, reactive oxygen species occur and this leads to developing oxidative stress and impairs functional state of various organs. Our studies established that elevated manganese contamination in biological media caused frequent respiratory diseases in children living in an area with technogenic pollution in the Southern Siberia, with the C/C genotype and the C allele of the *EPHX1* gene (rs1051740) Tyr113His [6].

Conclusions. We examined children who lived in an industrial area in the Southern Siberia and were often sick with respiratory diseases; as a result, we established higher frequency of the *EPHX1* gene polymorphism (rs1051740) in them in comparison with conditionally healthy children.

The children from the test group had authentically higher frequency of the C/C genotype (OR=4,05, 95% CI=1,26-13,05) and the C allele (OR=1,98, 95% CI=1,09-3,60) of the *EPHX1* gene (rs1051740); this can be a risk factor of respiratory diseases. An association between respiratory diseases and the candidate gene SPNs was also combined with elevated manganese contamination in biological media and cytochrome C deficiency (both are pathogenetically associated with microsomal epoxide hydrolase expression) in comparison with the conditionally healthy children with manganese levels in their blood remaining within its reference range.

Therefore, identification of the *EPHX1* gene (rs1051740) polymorphism reflects a risk of frequent respiratory diseases in children living in an area with heavy technogenic pollution in the Southern Siberia. We can recommend using the Tyr113His replacement of the *EPHX1* gene (rs1051740) with the C/C genotype and the C allele as a diagnostic indicator when planning and implementing activities aimed at reducing respiratory incidence among children and at preventing severe clinical forms of respiratory diseases and pathological processes in the airways (bronchial asthma, COPD, and pneumonia).

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ANALYSIS OF ASSOCIATIONS OF CANDIDATE GENES POLYMORPHISM WITH THE DEVELOPMENT OF KNEE OSTEOARTHRITIS IN OBESE PATIENTS

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Background. Knee Osteoarthritis (OA) is a chronic disease with multifactorial pathogenesis. Risk factors for developing knee OA include age, genetic predisposition, and obesity. The share of genetic factors in the development of the disease accounts for up to 50 %. Despite the obvious association between obesity and knee OA, studies that reveal the role of genetic factors in the development of the disease in the interaction with obesity or overweight are extremely limited. **The aim of the study:** To study the association of candidate genes polymorphic loci *GDF5* (rs143384), *NFAT5* (rs6499244), *WWP2* (rs34195470), *SBNO1* (rs1060105, rs56116847) with the development of knee OA in patients with obesity. **Materials and methods**. The sample for the study included 322 obese individuals: 255 patients with OA of the knee and 67 people in the control group. Genotyping of DNA samples from all study participants was performed using standard real-time PCR on CFX96 amplifier (USA). Associations of genetic markers with knee OA in obese patients were assessed using the odds ratio and 95 % confidence interval. **Results.** Analysis of the associations of the studied polymorphic loci with the development of knee OA in obese patients revealed significant differences only for the *GDF5* (rs143384) gene polymorphism. It was established that the frequency of the G/G genotype rs143384 in obese knee OA patients was 14.12 %, which is 1.8 times less compared to the control (25.36 %, p = 0.043, OR = 0.48). **Conclusions.** The modifying role of obesity on the nature of the rs143384 polymorphic marker *GDF5* gene associations with the developing knee OA in the population of the Russia Central Chernozem Region was shown. The G/G genotype rs143384 was found to be a protective factor in the development of knee OA in obese patients.

Keywords: knee osteoarthritis, *GDF5*, candidate genes, obesity.

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Introduction. Knee Osteoarthritis (OA) is a chronic joint disease that is accompanied by progressive softening and destruction of cartilage, the growth of new cartilage and bone material at the articular margins, as well as the formation of areas of sclerosis and cysts in the subchondral bone [2]. Knee OA or gonarthrosis is widespread due to an increase in life expectancy of the population, as well as an increase in obesity [16]. According to S. Safiri et al. (2020) the prevalence of OA of the knee and hip joints worldwide is 3754.2 per 100 thousand population [10]. The social and economic importance of OA is high. Hip and knee OA ranks 11th in terms of disability in the world and 38th in terms of the number of years lived with disability [24].

Knee OA is a chronic disease with multifactorial pathogenesis [1, 16]. The leading risk factors for the development of knee OA are age, genetic predisposition, and obesity [16]. In obese individuals, the risk of developing OA is three times higher than in individuals with normal weight [6]. It is known that in patients with knee OA, who are obese or

overweight, progression of the disease is more often observed. The role of obesity in the pathogenesis of gonarthrosis is reduced not only to an excessive load on the joint, but also to the secretion of many biologically active substances by the adipose tissue itself, for example, adipokines, which have various negative effects on the joint [21]. Thus, adipokines enhance catabolic processes in cartilage tissue and increase the synthesis of pro-inflammatory mediators (IL-1β, IL-6, TNF-α, etc.) in joint tissues [21].

Osteoarthritis and obesity result from the interaction of many genetic and environmental factors [7, 23], and these diseases share common pathophysiological mechanisms. The share of the genetic determinant in the development of osteoarthritis is up to 50% [25]. It should be noted that, despite numerous data indicating a significant relationship between obesity and the development/progression of knee OA, genetic studies that reveal the role of individual single nucleotide polymorphisms (SNPs) in the development of the disease when interacting with obesity or overweight are limited, which determines the relevance of further research in this direction.

The aim of the study: to study the association of candidate genes polymorphic loci *GDF5* (rs143384), *NFAT5* (rs6499244), *WWP2* (rs34195470), *SBNO1* (rs1060105, rs56116847) with the development of knee OA in patients with obesity.

Material and methods. The sample for the study included 322 obese individuals: 255 patients with knee OA and 67 people in the control group. Body mass index (BMI) was calculated by the standard method as the ratio of body weight (kg) to the square of height (m). According to WHO, obesity was determined in individuals with a BMI ≥ 30 kg/m^2. The sample for the study was formed on the basis of the City Hospital No 2 of Belgorod.

The inclusion criteria for the group of patients were as follows: a) diagnosed primary knee OA, b) age at least 40 years, c) 2-4 radiological stages of the disease according to the Kellgren-Lawrence classification, d) the presence of pain in the knee joint according to the visual analog scale of more than 40 points, e) the presence of voluntary informed consent to the study. The control group included individuals without any pathology of the musculoskeletal system (examined during preventive examinations).

Criteria for exclusion from the study groups: a) non-European origin, residence and / or birth outside the Central Chernozem region of Russia, b) the presence of severe forms of diseases of the cardiovascular, endocrine systems, renal and hepatic pathology, oncology, history of joint injuries, congenital malformations of the musculoskeletal system.

In each patient, the radiological stage of the disease was determined according to the Kellgren-Lawrence scale: 98 (38.43 %) patients had the 2nd radiological stage of the disease, 114 (44.71%) patients had the 3rd stage, 43 (16.86 %) patients had the 4th stage of OA of the knee joint. All study participants underwent peripheral venous blood sampling (≈ 4–5 ml) and DNA was isolated using the standard phenol-chloroform method.

For this study, five single nucleotide polymorphisms of candidate genes (rs1060105 and rs56116847 of the *SBNO1* gene on chromosome 12, rs34195470 of the *WWP2* gene and rs6499244 of the *NFAT5* gene of chromosome 16, rs143384 of the *GDF5* gene of chromosome 20) were selected, which showed significant associations for the genome- wide level ($p \leq 5 \times 10^{-08}$) with knee OA in samples of European origin [5, 8, 14-15, 19], which have a pronounced functional significance and have a minor allele frequency of at least 5%. The selection of candidate gene polymorphisms was performed from the genome-wide research catalog (GWAS) (https://www.ebi.ac.uk/gwas/). The functional significance of the studied SNPs was assessed using modern databases of functional genomics – online resources HaploReg (v4.2) and GTExPortal [3].

Genotyping of DNA samples from all participants in the study was performed using standard real-time PCR on a CFX96 amplifier, including positive and negative controls.

Separately, in the group of patients with knee OA and in the control, an assessment was made of the correspondence between the observed distribution of genotypes of the studied SNPs and the theoretically expected distribution according to the Hardy–Weinberg pattern. Deviations were considered statistically significant at $P_{HWE} \leq 0.05$. Comparative analysis of the frequencies of alleles and genotypes of the studied polymorphic loci between the group of patients and the control group was performed in 2 x 2 contingency tables using the χ^2 test with Yates' correction for continuity. The association of genetic markers with knee OA was assessed using odds ratio (OR) and 95% CI (95% confidence interval for OR). $P < 0.05$ was taken as a statistically significant level.

Results and discussion. The studied groups of patients with knee OA and controls with obesity did not differ in sex and age composition, as well as height ($p > 0.05$) (Table 1). However, patients with OA of the knee had a higher BMI compared to the control group ($p = 0.0001$).

Table 1

Characteristics of patients with osteoarthritis and the control group with obesity

Index		OA patients with obesity [n = 255]	Control group with obesity [n = 67]	*p*
Men, n [%]		86 [33.73]	26 [38.80]	0.53
Women, n [%]		169 [66.27]	41 [61.20]	
Average age, years	Me [Q25 – Q75]	53.0 [50.0 – 56.0]	54.0 [50.0 – 58.0]	0.12
	Min/Max	40.0/68.0	42.0/70.0	
	M [σ]	52.55 [5.48]	53.54 [6.15]	
Height, cm	Me [Q25 – Q75]	167.0 [162.0 – 173.0]	165.0 [160.0 – 173.0]	0.20
	Min/Max	156.0/193.0	156.0/180.0	
	M [σ]	167.89 [7.23]	166.78 [7.67]	
BMI, kg/m^2	Me [Q25 – Q75]	33.87 [31.60 – 36.73]	31.92 [31.10 – 34.34]	**0.0001**
	Min/Max	30.00/47.05	30.04/38.67	
	M [σ]	34.50 [3.50]	32.72 [2.32]	

Примечание. Me – медиана; Q25-Q75 – интерквартильный размах – 25-й и 75-й процентили; Min/Max – минимальное/максимальное значения; M – среднее значение; σ – стандартное отклонение; *p* – уровень статистической значимости.

It was found that for the distribution of genotypes over all polymorphic loci of candidate genes *GDF5* (rs143384), *NFAT5* (rs6499244), *WWP2* (rs34195470), *SBNO1* (rs1060105, rs56116847) in the group of patients with knee OA, as well as in the control group, the Hardy-Weinberg pattern (P_{HWE} > 0.05) (Table 2).

In a comparative analysis of the frequencies of alleles and genotypes of the studied polymorphic loci of candidate genes between the group of patients and controls, significant differences were established only for the rs143384 gene *GDF5*. It was found that the frequency of the G/G genotype rs143384 of the *GDF5* gene in the control group was 1.8 times higher than in the group of patients (p = 0.043) (Table 2).

In our work, it was found that the G/G genotype rs143384 of the *GDF5* gene is a protective factor in the development of knee OA (OR = 0.48). To date, according to five published genome-wide studies, the rs143384 *GDF5* polymorphism has been associated with knee OA [5, 8, 14-15, 19]. Three studies [5, 15, 19] showed that allelic variant A of this polymorphic locus is a risk factor for the development of knee OA (OR = 1.07 – 1.10). In a genome-wide study by U. Styrkarsdottir et al. (2019) established an association of the allelic variant G rs143384 of the *GDF5* gene with gonarthrosis in a sample of European origin (OR = 0.91) [14]. Similar results were obtained in the largest GWAS meta-analysis to date of C. Henkel et al. (2023) (a sample of about 700 thousand individuals with OA and a control group was studied), which also showed the association of the G allele of this polymorphism with idiopathic knee OA in Caucasians (OR = 0.94) [8]. Thus, we can note that our results on the protective role of the G/G rs143384 genotype of the *GDF5* gene in the development of knee OA in obese patients are fully consistent with the data presented in independent sources on this issue.

A number of genome-wide studies have revealed a relationship between the studied *GDF5* gene polymorphism and weight and various anthropometric parameters that may be related to overweight and obesity [4, 9, 13]. The allelic variant G rs143384 of the *GDF5* gene is associated with a lower distribution of adipose tissue in the body in women (β = -0.044, p = 1 x 10^{-40}) [9], waist-to-hip ratio adjusted for BMI (β = -0.035, p = 3 x 10^{-28}) [13]. At the same time, in GWAS S. Sakaue et al. (2021) the G allele rs143384 is associated with an increase in body weight in Europeans and Asians (β = 0.028, p = 3 x 10^{-57}) [4].

Table 2

Distribution of frequencies of alleles and genotypes of the studied polymorphic loci of candidate genes in patients and in the control group with obesity

Polymorphism	Allele, genotype	OA patients with obesity n [%]	Control group with obesity n [%]	OR [95% Cl]	p
rs1060105	C	401 [78.63]	110 [82.09]	0.80 [0.48–1.34]	0.447
	T	109 [21.37]	24 [17.91]	1.25 [0.75–2.10]	
	C/C	157 [61.57]	46 [68.66]	0.73 [0.40–1.35]	0.355
	C/T	87 [34.12]	18 [26.87]	1.41 [0.75–2.69]	0.328
	T/T	11 [4.31]	3 [4.47]	0.96 [0.24–4.49]	1.000
	H_o/H_e [P_{HWE}]	0.341/0.336 [1.000]	0.269/0.294 [0.422]	-	-
rs56116847	G	334 [65.49]	83 [61.94]	1.17 [0.77–1.76]	0.507
	A	176 [34.51]	51 [38.06]	0.86 [0.57–1.30]	
	G/G	116 [45.49]	24 [35.82]	1.50 [0.83–2.71]	0.200
	A/G	102 [40.00]	35 [52.24]	0.61 [0.34–1.08]	0.096
	A/A	37 [14.51]	8 [11.94]	1.25 [0.52–3.09]	0.733
	H_o/H_e [P_{HWE}]	0.400/0.452 [0.072]	0.522/0.472 [0.447]	-	-
rs6499244	T	279 [54.71]	81 [60.45]	0.79 [0.53–1.19]	0.275
	A	231 [45.29]	53 [39.55]	1.27 [0.84–1.90]	
	T/T	73 [28.63]	26 [38.81]	0.63 [0.34–1.15]	0.145
	A/T	133 [52.16]	29 [43.28]	1.43 [0.80–2.55]	0.249
	A/A	49 [19.21]	12 [17.91]	1.09 [0.52–2.33]	0.947
	H_o/H_e [P_{HWE}]	0.522/0.496 [0.449]	0.433/0.478 [0.448]	-	-
rs34195470	G	278 [54.51]	75 [55.97]	0.94 [0.63–1.41]	0.839
	A	232 [45.49]	59 [44.03]	1.06 [0.71–1.58]	
	G/G	74 [29.02]	19 [28.36]	1.03 [0.55–1.96]	1.000
	A/G	130 [50.98]	37 [55.22]	0.84 [0.47–1.50]	0.630
	A/A	51 [20.00]	11 [16.42]	1.27 [0.59–2.78]	0.626
	H_o/H_e [P_{HWE}]	0.510/0.496 [0.706]	0.552/0.493 [0.457]	-	-
rs143384	A	302 [59.22]	72 [53.73]	1.25 [0.84–1.87]	0.296
	G	208 [40.78]	62 [46.27]	0.80 [0.54–1.19]	
	A/A	83 [32.55]	22 [32.85]	0.98 [0.54–1.82]	1.000
	G/A	136 [53.33]	28 [41.79]	1.59 [0.89–2.84]	0.123
	G/G	36 [14.12]	17 [25.36]	0.48 [0.24–0.98]	**0.043**
	H_o/H_e [P_{HWE}]	0.533/0.483 [0.096]	0.418/0.497 [0.220]	-	-

Примечание. H_o/H_e – наблюдаемая/ожидаемая гетерозиготность, P_{HWE} – уровень значимости отклонения от закона Харди – Вайнберга.

Growth differentiation factor 5 (GDF5) is a morphogenetic protein of cartilage origin and a member of the TGFβ superfamily, which plays an important role in skeletal formation [18]. C. M. Coleman et al. (2013) note that overexpression of *GDF5* in human mesenchymal stem cells leads to increased chondrogenesis *in vitro* [11]. In the work of K. Kania et al. (2020) revealed a high expression

of *GDF5* in cartilage during recovery after unilateral destabilization of the medial meniscus in mice [20]. E. Hinoi et al. (2013) observed an increase in *GDF5* gene expression in brown adipose tissue in obese mice [12]. Experimental studies in mice have shown that systemic overexpression of *GDF5* in adipocytes reduces non-alcoholic fatty liver disease induced by a high-fat diet [22]

The associations of rs143384 of the *GDF5* gene with knee OA may be based on the pronounced regulatory effects of polymorphism in the human body, which we established in silico (data from the HaploReg (v4.2) online resource): it is located in an evolutionarily conservative region, in the region of histones that mark active H3K9ac promoters in adipose tissue (adipose nuclei, cultured adipocyte cells derived from mesenchymal stem cells), in the region of histones, marking active enhancers of H3K27ac in fatty nuclei, in the area of hypersensitivity to type I DNase in 16 tissues and the region of one Ascl2 DNA regulatory motif. It is also known that rs143384 *GDF5* is associated with the level of expression of 21 genes in more than 30 different tissues and organs (data from the GTExPortal online resource), including those involved in the pathogenesis of gonarthrosis (adipose tissue, thyroid gland, etc.). It should be noted that 6 of these 21 genes (*CPNE1, EDEM2, GDF5, PROCR, RPL36P4, UQCC1*) are expressed in subcutaneous and visceral adipose tissue. Also, the G allelic variant of the rs143384 locus is associated with high expression of two genes *GDF5* and *RPL36P4* in adipose tissue (β = -0.14 – -0.34 for the opposite A allele).

Thus, we can note the important role of the *GDF5* gene in the formation and development of the skeleton, as well as its participation in the processes occurring in adipose tissue, which ultimately can determine the relationship between the G/G genotype rs143384 of the *GDF5* gene and knee OA in obese patients.

Conclusion. Our study shows the modifying role of obesity on the nature of associations of the rs143384 polymorphic marker of the growth differentiation factor 5 gene with the development of knee OA in the population of the Central Chernozem Region of Russia. It has been established that the G/G genotype rs143384 of the *GDF5* gene is a protective factor in the development of knee OA in obese patients.

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BREAST CANCER: MORBIDITY AND MORTALITY STATISTICS

An analysis was made of the primary incidence of breast cancer (BC) among women in the Irkutsk region for 2011–2021. and mortality from it in comparison with data for the Russian Federation in order to assess the situation. According to the results of the analysis, an increase in the relative indicators of the primary incidence of breast cancer in the region by 25%, in the Russian Federation – by 17%. At the same time, the upward trend in indicators is more pronounced in the region: the average growth rate is +2.2% against +1.8% in Russia. In 2021, the region recorded an excess of the all-Russian primary incidence of breast cancer by 9% and in all age groups by 1.1–1.3 times, except for women 75–79 and over 85 years old. During the study period, mortality from breast cancer in the Irkutsk region changed little, while in the Russian Federation there is a downward trend with an average annual rate of 1.5%. Standardized indicators characterizing the true situation with breast cancer indicate that the Irkutsk region is not well: the mortality rate for 2011–2021 exceeded the national average by 9%, and in 2021 the region ranked 6th among the subjects of the Russian Federation in terms of primary morbidity and 8th in terms of mortality.

Keywords: breast cancer, primary morbidity, mortality, relative and standardized rates.

Introduction. Breast cancer (BC) is the most common localization of malignant neoplasms among women. In terms of new cases, 2.2 million were detected worldwide in 2020 [5]. In Russia, breast cancer is diagnosed annually in ~70 thousand women and more than 20 thousand die. Malignant neoplasms of the breast continue to be a huge medical and social problem, causing enormous damage at the state, regional [1, 6] and individual levels. The fight against an increase in the incidence of breast cancer depends on the state of women's health, including reproductive, genetic predisposition, awareness of the female population about risk factors, prevention measures, and the quality of medical care [4, 7, 8, 10, 11]. As is known, certain factors increase the risk of developing breast cancer: age after 40 years, obesity, low physical activity, heredity, the harmful effects of radiation, alcohol, smoking, etc. [4, 7, 8, 10, 12].

The purpose of the study: to assess the situation in the Irkutsk region in terms of the level of primary incidence of breast cancer in women in 2021 and mortality from it in comparison with 2011 and data for Russia.

Materials and research methods. The data of the statistical collections "Malignant neoplasms in Russia (morbidity and mortality)" of the Ministry of Health of the Russian Federation for 2011–2021 were used; mortality tables 5TC "Main cumulative characteristics by causes of death" of Rosstat for 2011 and 2021. The methods of comparative analysis of relative, standardized (world standard), long-term averages and average growth/decrease rates were used in the work, the statistical significance of differences in indicators was determined using a 95% confidence interval (95% CI); ranking of indicators of 85 constituent entities of the Russian Federation in descending order. The calculations were carried out using standard Windows programs (version 10.0).

Results and discussion. In women of the Irkutsk region in 2011, 1018 primary cases of breast cancer (BC) were registered, in 2021 - 1237 cases, a total of 12598 cases were detected for the first time during this period. The incidence rate for newly diagnosed breast cancer increased by 24.7% from 78.1 in 2011 (95% CI 73.3; 82.9) to 97.4 per 100,000 in 2021 (95% CI 91.9; 102.8). The average long-term level of primary incidence of breast cancer in the region for 2011–2021 amounted to 88.6 per 100 thousand, the average annual growth rate is +2.2%. During the study period, the standardized rate of primary cancer incidence of breast cancer in the region increased from 50.6 to 59.2 per 100 thousand (+17.0%), the long-term average was 55.6 per 100 thousand - fig. 1. The decline in 2020 is certainly related to the COVID-19 pandemic, after which the upward trend resumed, but the indicators did not return to pre-Covid levels.

In the Russian Federation, the primary incidence rate of women with breast cancer in 2011 was recorded at the level of 74.9 (95% CI 74.3; 75.5); in 2021 - 89.3 per 100 thousand (95% CI 88.6; 89.9), i.e. increased by 19.2%. The long-term average for the Russian Federation for the study period was 84.6 per 100 thousand, the annual growth rate is +1.8%. In the US, the primary incidence of breast cancer continues to rise, but more recently over the course of 2010–2019. the indicator increased annually by 0.5% [9]. The standardized primary incidence rate of breast cancer among women in the Russian Federation increased by 11.5% from 45.2 in 2011 to 50.4 per 100 thousand in 2021 with a long-term average of 49.3 per 100 thousand (Fig. 1). The authors of the studies draw attention to the continuing growth of cancer incidence among the population, including breast cancer [1, 2, 6, 9].

In 2021, the primary incidence rate of breast cancer among women in the Irkutsk region at 97.4 per 100 thousand (95% CI 91.9; 102.8) was 9.1% higher than in the Russian Federation - 89.3 per 100 thousand (95% CI 88.6; 89.9). In 2011, although this indicator exceeded that in the Russian Federation by 4.3%, the difference was statistically insignificant. The average long-term rate of primary incidence of breast cancer in women in the Irkutsk region statistically significantly exceeds the figure for the Russian Federation by 4.7%. The upward trend in the primary incidence of breast cancer in the region is characterized by large fluctuations and faster rates: +2.2% per year against +1.8% in the Russian Federation. During the study period, the percentage of women living in the Irkutsk region who were diagnosed with stage IV breast cancer for the first time decreased from 12.5 (95% CI 10.5; 14.5) to 10.4 (95% CI 8.7; 12.1), but was statistically significantly higher than in the Russian Federation: 2011 - 9.1% (95% CI 8.9; 9.3); 2021 - 8.1% (95% CI 7.9; 8.3).

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Fig. 1. Dynamics of breast cancer incidence rates in women in the Irkutsk region and the Russian Federation for 2011–2021: a) relative; b) standardized (per 100 thousand)

According to the data for 2021, the Irkutsk region in terms of relative and standardized indicators of the primary incidence of breast cancer among 85 subjects occupied the 26th and 6th rating positions, respectively.

The highest levels of primary morbidity among women in the Irkutsk region in 2011 were recorded at the age of 60 to 74 years. But in 2021, a group of women aged 80-84 years was added to these risk groups by age (Table 1), in which a high growth rate of +60% was registered. During the study period, high growth rates were noted in women aged 30–49 (from +21.3 to +122.5%), in connection with which the multiplicity of exceeding the all-Russian indicators increased (Table 1). Thus, higher rates of primary incidence of breast cancer in women of the region, compared with similar levels in the country, have developed in almost all age groups. The region is no exception in this situation [6].

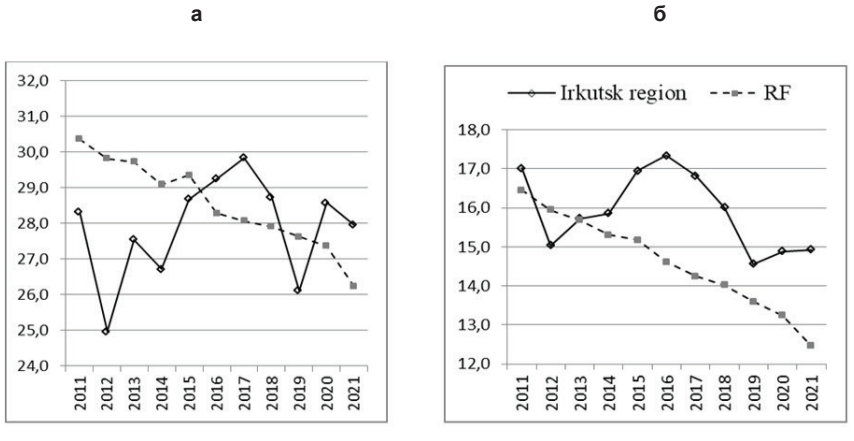
In the Irkutsk region for 2011–2021 3964 women died from breast cancer. The mortality rate from breast cancer remained practically at the same level: 28.3 per 100 thousand in 2011 (95% CI 25.4; 31.2); 2021 - 27.9 (95% CI: 25.0; 30.8). The absence of a pronounced dynamics of women's mortality from breast cancer in the region confirms the value of the average annual rate - minus 0.1%. The decrease in the standardized mortality rate from breast cancer during the study period was not statistically significant: from 17.0 (95% CI: 15.2; 18.9) to 14.9 per 100 thousand (95% CI: 13.3; 16.6). Unstable dynamics of relative and standardized mortality rates from breast cancer in the region is shown in fig. 2.

In general, in Russia there is a clear trend towards a decrease in the mortality of women from breast cancer [3], with an average annual rate of decline of 1.5%. In the United States, the rate of decline in mortality from breast cancer slowed down - 1.3% per year from 2011 to 2020, and in the previous decade 1.9% [9]. For 2011–2021 in the Russian Federation, the relative mortality rate of women from breast cancer decreased by 13.6% from 30.4 (95% CI 30.0; 30.7) to 26.2 per 100 thousand (95% CI 25.9; 26.6). The decrease in the standardized mortality rate of women in the Russian Federation from breast cancer is also statistically significant: from 16.5 (95% CI 16.2; 16.7) to 12.5 per 100 thousand (95% CI 12.3; 12.7). The line of dynamics of standardized indicators for the Russian Federation is smoother compared to the line of relative indicators, and almost straight (Fig. 2).

Table 1

Incidence rates of breast cancer in women in the Irkutsk region and the Russian Federation in 2011 and 2021 by individual age groups (per 100.000; frequency of excess. number of times)

age groups	Irkutsk region		RF		$P_{Irkutsk\ region}/P_{RF}$	
	2011	2021	2011	2021	2011	2021
25-29	6.6	5.8	4.6	5.5	1.4	1.1
30-34	12.3	27.4	15.4	20.3	0.8	1.3
35-39	30.7	52.9	33.5	48.1	0.9	1.1
40-44	54.6	111.7	65.4	83.7	0.8	1.3
45-49	121.0	146.8	97.9	119.4	1.2	1.2
50-54	140.8	136.5	124.3	129.6	1.1	1.1
55-59	167.0	194.7	151.8	152.7	1.1	1.3
60-64	223.7	214.3	187.5	181.5	1.2	1.2
65-69	216.5	240.3	193.1	205.3	1.1	1.2
70-74	222.0	241.7	181.1	217.3	1.2	1.1
75-79	187.9	160.7	162.0	191.8	1.2	0.8
80-84	139.6	223.4	132.2	161.3	1.1	1.4
85+	158.6	100.3	119.1	105.1	1.3	1.0



Fig/ 2. Dynamics of mortality rates of women in the Irkutsk region and the Russian Federation from breast cancer for 2011-2021 a) relative; b) standardized (per 100 thousand)

The average long-term mortality rate from breast cancer in the Irkutsk region for 2011–2021 does not exceed the level in the Russian Federation - 27.9 versus 28.5 per 100 thousand. However, the standardized mortality rate from breast cancer in the region for the study period statistically significantly exceeds the national indicator by 8.9%: 15.9 (95% CI 15.0; 16.8) versus 14.6 per 100 thousand (95% CI 14 .4; 14.7). The Irkutsk region, as before [3], belongs to the disadvantaged subjects of the Russian Federation in terms of standardized indicators of female mortality from breast cancer, ranking 8th among the subjects of the Russian Federation in 2021.

In a more detailed analysis of the standardized mortality rates of women in the Irkutsk region from breast cancer for 2011–2021. among women from 15 to 59 years old, a decrease is recorded from 14.7 to 10.4 per 100 thousand, among women over 60 years old, on the contrary, an increase in the indicator from 77.6 to 80.5 per 100 thousand.

In 2011, the average age of women who died from breast cancer at the age of 15–59 years was 51.45 years; in 2021 – 49.90 years; over the age of 60 years – 73.29 and 72.48 years, respectively, i.e. over the past 10 years, women of working age and the elderly began to die from breast cancer earlier - with a difference of 1.55 and 0.81 years, respectively.

Conclusion. According to the results of the analysis, an increase in the relative indicators of the primary incidence of breast cancer in the Irkutsk region by 24.7% was revealed, in the Russian Federation – by 17.0%. At the same time, the upward trend in indicators is more pronounced in the region: the average growth rate is +2.2% against +1.8% in Russia. According to official data for 2021, an excess of the primary incidence of breast cancer in the Russian Federation by 9.1% was registered in the region, as well as in all age groups by 1.1–1.3 times, excluding women 75–79 and over 85 years old.

Breast cancer is a preventable cause of death, so the mortality rate largely depends on the timeliness of its detection [6]. In the Irkutsk region, although the proportion of women with diagnosed breast cancer in advanced stage IV has decreased from 12.5% in 2011 to 10.4% in 2021, this indicator still differs from that of the Russian Federation – 9.1 and 8.1% respectively. According to WHO, in recent years, high-income countries have made progress in the treatment of breast cancer and mortality rates have begun to decline [4, 9]. But other middle- and low-income countries have yet to achieve similar results [5].

During the study period, mortality from breast cancer in the Irkutsk region changed little, while in the Russian Federation there is a downward trend with an average annual rate of 1.5%. Standardized indicators characterizing the true situation with breast cancer indicate that the Irkutsk region is not well: the mortality rate for 2011-2021 exceeded the national average by 9%, and in 2021 the region ranked 6th among the constituent entities of the Russian Federation in terms of morbidity and 8th in terms of mortality. Thus, if for the Russian Federation the results in reducing mortality from breast cancer over the past 10 years can be called positive, then for the Irkutsk region, such success, unfortunately, has not been achieved.

The increase in the primary incidence of breast cancer indicates the aging of the female population and the improvement in the quality of medical care for the diagnosis of this disease. Taking into account the absence of noticeable shifts in mortality in the region from breast cancer, the level of care for patients is still insufficient and requires additional research. No one can dispute the importance of early detection and subsequent treatment of breast cancer. But due to the fact that not all risk factors for breast cancer are subject to adjustment [4, 7], the role of primary cancer prevention is increasing [4].

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BLOOD INTEGRATED INDICATOR OF OXIDATIVE STRESS IN INFANTS BORN TO HIV-INFECTED MOTHERS

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A single-center cross-sectional study was conducted from March 2020 to January 2021 and included 142 neonates born with a gestational age of more than 37 0/7 weeks. The aim of the work was an individual assessment of the oxidative stress index (OSI) in newborns of HIV-infected mothers. Materials and methods: the state of the LPO–AOD system was assessed by determining the content of lipid peroxidation oxidation substrates with conjugated double bonds (DB), diene conjugates (DC), ketodienes and conjugated trienes (KD and CT), TBA-active products in the blood (TBA-AP), retinol, α-tocopherol, reduced (GSH) and oxidized glutathione (GSSG), superoxide dismutase (SOD) activity. OSI was used to evaluate differences in lipid peroxidation processes in HIV-exposed uninfected newborns. Results: The index of oxidative stress in the group of HIV-exposed uninfected newborns was detected 1.98 times higher compared to healthy newborns and was 2.5 and 1.26, respectively. The value of the oxidative stress coefficient indicates a significant imbalance in the LPO-AOP system reflecting the enhancement of the lipid peroxidation processes. An increase in the concentration of substrates and products of peroxidation leads to the destruction of lipids, and, consequently, to the degradation of the cell membrane complex. Conclusion: The integral oxidative stress index is recommended as a general indicator that characterizes the level of lipid peroxidation products and antioxidant protection factors in a particular group. Also, OSI is more sensitive indicator than separated components of the system, which objectively represents the LPO–AOD system malfunctioning, and provides the comprehensive view to the both lipid peroxidation processes and the level (effectiveness) of protective factors. To summarize, the results of the study point to the need to further investigations aimed to determination of prognostically significant markers in PVEN, which could be involved or could indicate the pathological processes both in the early neonatal period and in the later stages of infant development.

Keywords: newborns, HIV-infected pregnant women, HIV infection, oxidative stress, integral index.

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Oxidative stress (OS), resulting from increased free radical processes and a decrease in the buffer capacity of antioxidant defense (AOD), is one of the main factors in the pathogenesis of many diseases [1, 2, 3, 4, 5, 6]. In clinical practice, both in the analysis of pathological conditions and in the detection of disorders caused by environmental factors, integral indicators are used, since they are more sensitive in assessing the balance of LPO–AOD processes than comparing separate markers [3, 4, 5]. This is due to the fact that the complexity and multicomponent nature of the LPO–AOD system makes it difficult to quantify oxidative stress and, accordingly, interpret the results. The methodology for individual assessment of the Oxidative stress index (OSI) as a single systemic indicator for characterizing disorders in the LPO–AOD system is one of the most relevant today.

On the one hand, over the past decades there has been observed a significant decrease in mother-to-child HIV-transmission frequency to 1.5%. [7, 8]. On the other hand, advanced methods of providing medical care to HIV-positive pregnant women and modified approaches for prescribing antiretroviral therapy (ART) have significantly increased the number of perinatally HIV-exposed uninfected infants [9]. Underestimation of the clinical status and risks specific to this cohort of children leads to an increase in adverse neonatal outcomes. Perinatally HIV-exposed uninfected children have a higher incidence of morbidity and mortality, an increased risk of a complicated course of infectious diseases, immunological disorders and disorders of neuropsychic development [14]. It is assumed that unfavorable outcomes are associated with the direct and indirect effects of HIV, exposure to ART and with comorbidity [10]. A large number of studies have been devoted to the study of immunological disorders in perinatally HIV-exposed uninfected children. It has been shown that at birth there is impairment of the activation and differentiation of T- and B-cells and increase in some markers of inflammation and activation of monocytes, which persist up to 6 months of life and are not associated with the inflammatory status of the mother. It is also suggested that oxidative stress may be a potential cause of long-term persistence of inflammatory markers in perinatally HIV-exposed uninfected children. In addition, maternal ART can cause fetal mitochondrial toxicity, which in turn leads to the development of oxidative stress. It has also been shown that the effects of mitochondrial toxicity in a child can persist for a year after intrauterine exposure

to antiretroviral drugs [11, 12, 13]. At the same time, the monitoring integrative indicators of oxidative stress in perinatally HIV-exposed uninfected children remains relevant to determine prognostically significant factors in the development of pathological processes both in the early neonatal period and at later stages of child ontogenesis.

The aim of this work was an individual assessment of OSI in newborns of HIV-infected mothers.

Materials and methods. A single-center cross-sectional study was conducted from March 2020 to January 2021 and included 142 neonates born in the Irkutsk City Perinatal Center with a gestational age of more than 37 0/7 weeks.

All newborns were divided into 2 groups: the main group included 62 children from HIV-seropositive mothers, the control group consisted of 80 full-term newborns from HIV-seronegative mothers.

Criteria for inclusion in the main group: infants born alive at a gestational age of more than 37 0/7 weeks; confirmed HIV infection in mothers; informed consent signed by parents and / or legal representatives. *Criteria for exclusion from the main group:* parents and/or legal representatives refuse to take part in the study at any stage; a newborn from a discordant couple; a child with hemolytic disease of the newborn; a child with chromosomal diseases; infants born to PCR-confirmed SARS-CoV-2 positive mothers or confirmed mother`s vaccination against COVID-19 in the third trimester of pregnancy; the patient does not meet the inclusion criteria.

Depending on the HIV viral load in HIV-infected mothers before childbirth (at a gestational age of 34 weeks or more), newborns of the main group were divided into two subgroups: in the 1st subgroup of children (n=41), the HIV viral load in their mothers had a detectable value (more than 50 copies/ml), in the 2nd subgroup of children (n=21) the mothers` HIV viral load was undetectable (less than 50 copies/ml).

Criteria for inclusion in the control group: newborns born alive at a gestational age of more than 37 0/7 weeks; HIV-negative mothers, informed consent signed by parents and / or legal representatives of the child. *Criteria for exclusion from the control group:* children with hemolytic disease of the newborn, chromosomal diseases; infants born to PCR-confirmed SARS-CoV-2 positive mothers or confirmed mother`s vaccination against COVID-19 in the third trimester of pregnancy; the patient does not meet the inclusion criteria. The groups of newborns were comparable on gestational age, anthropometric parameters at birth and Apgar score ($p > 0.05$).

The study was conducted in accordance with the principles of the WMA Declaration of Helsinki (2013 edition); the experiment was approved by the Biomedical ethics committee at the Federal state public scientific institution «Scientific center for family health and human reproduction problems» (extract from the Minutes of the meeting No. 2 dated 04.03.2021). Informed consent for the inclusion in the study was signed by the parents and/or legal representatives of the child.

All mothers of newborns of the main group were prescribed antiretroviral therapy (ART) by the infectiologist of the regional AIDS Center to prevent the transmission of HIV infection from mother to child during pregnancy (the first stage of PMTCT). The particular type of ART regimen was prescribed considering its effectiveness, dynamics of the viral load (VL) and the absence of contraindications. Prevention of transmission of HIV infection during childbirth (intravenous administration of zidovudine) was performed in all mothers of newborns of the main group (second stage of PMTCT) (Clinical guidelines "HIV infection in pregnant women", 2021). The need of prophylactic ART scheme in newborns of the main group depended on the risk of HIV transmission from mother to child (the third stage of PMTCT). Children were prescribed zidovudine monotherapy for a period of 4 weeks in case of undetectable HIV-VL in mother before delivery (at a gestational age of 34 weeks or more). At mother`s HIV-VL level of more than 50 copies/ml the risk of HIV transmission was considered high and newborns of the main group were prescribed three-component ART for a period of 4 weeks (zidovudine, lamivudine and nevirapine) (Clinical guidelines "HIV infection in children", 2020).

All children of the main group were HIV-negative according to PCR testing. After birth all newborns of the main group were formula fed to prevent HIV infection. Children from the control group were breastfed.

Blood plasma and erythrocyte hemolysate were used as biological samples. Blood was collected on the 3rd day of life in the morning before feeding from a peripheral vein (back of the hand) using disposable vacuum systems with a 23G needle into tubes with EDTA-K3. All children underwent analgesia (non-pharmacological methods of pain correction - non-nutritive sucking and 20% glucose solution per os).

To assess the intensity of lipid peroxidation processes, the concentrations of the substrates with double bonds (DB) and oxidation products: diene conjugates (DC), ketodienes, conjugated trienes (KD and CT), active products of thiobarbituric acid (TBA-AP) were measured. The activity of the components of the antioxidant defense system was assessed by the value of total antioxidant activity of blood serum (AOA), based on the concentrations of natural endogenous antioxidants (α-tocopherol and retinol) oxidized (GSSG), reduced (GSH) glutathione and superoxide dismutase (SOD) activity. The measurements were carried out using spectrophotometric and fluorometric methods.

The acknowledged method of calculating the oxidative stress index (OSI) was used in order to individual assessment of oxidative stress and was based on the ratio of pro- and antioxidant factors [8].

$$OSI = ((ДСi/ДСn) \times (TBA\text{-}AP\ i)/(TBA\text{-}AP\ n) \times (KD\_CTi/KD\_CTn)) / ((SODi/SODn) \times (\alpha\text{-}TOCOFEROLi/\alpha\text{-}TOCOFEROLn) \times (RETINOLi/RETINOLn) \times (GSHi/GSHn)),$$

note: «i» – the levels of indicators of the examined patients; «n» - levels of indicators of the control group.

Normally, the oxidative stress index tends to 1 [8]. The value of OSI > 1 is considered as an increase in the degree of oxidative stress. The greater the value of the oxidative stress index, the more intense the processes of lipid peroxidation and the less effective the AOD system.

The data was processed using the Statistica 6.10 software (StatSoft, Inc.). The visual-graphic method and Kolmogorov-Smirnov`s criterion with Lilliefors and Shapiro-Wilk`s corrections were used to determine the proximity to the normal law of distribution. The equality of general variances was tested with Fisher's F-test. The nonparametric Mann-Whitney test was used to compare the main and control groups. Differences were considered significant at $p<0.05$.

Results and discussion. Since there were no significant differences between groups of newborns when comparing the indicators of the "LPO-AOD" system, depending on the mothers` HIV viral load in, the newborns were combined into one group. Also, the groups of newborns were comparable on maturity and Apgar score. Intergroup differences during the period of early postpartum adaptation and early neonatal period were not found in the compared groups. At the time of delivery and the course of pregnancy, the

mothers of the control group and the HIV group were comparable and did not have statistically significant differences.

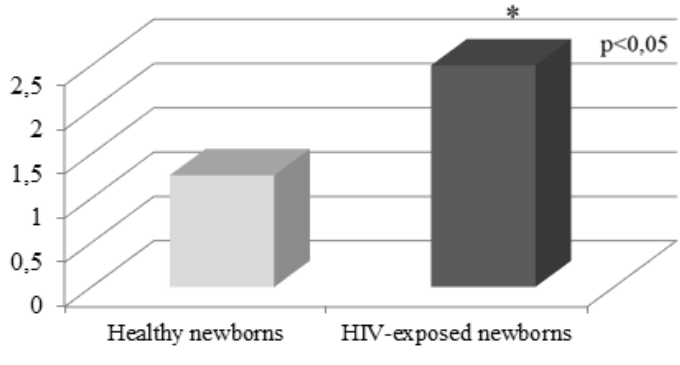
In the blood of HIV-exposed uninfected newborns, a statistically significant increase in substrates with double bonds (DB), the level of primary (DC) and final products (TBA-AP) of lipid peroxidation were found. The antioxidant defense system was characterized by decrease in the levels of total blood AOA, SOD and α-tocopherol with increased retinol concentration (Table). The index of oxidative stress in the group of HIV-exposed uninfected newborns was detected 1.98 times higher compared to healthy newborns and was 2.5 and 1.26, respectively (Figure).

cal in various pathological conditions, the mechanisms of their development may have specific features. Accumulation of free radical oxidation products in chronic diseases causes damage of the vascular endothelium with subsequent development of the endothelial dysfunction [3, 5]. In acute inflammation the development of oxidative stress is characterized by the rate of accumulation and an excessive increase in the concentration of metabolites, when the body does not have time to mobilize its own antioxidant reserve, which contributes to the aggravation of free radical oxidation reactions and hyperactivation of the immune system [2, 4].

The integral oxidative stress index is recommended as a general indicator that

Parameters of the LPO-AOD system in healthy and HIV-exposed newborns

Index	Healthy newborns. n=80	HIV-exposed uninfected newborns. n=62
DB. units	1.96 (1.39; 2.47)	2.12 (1.94; 2.62)
KD и CT. units	0.56 (0.42; 0.79)	0.76 (0.53; 0.98)
DC μmol/l	1.09 (0.72; 1.44)	1.33 (1.16; 1.68)
TBK-AP. μmol/l	1.07 (0.71; 1.52)	1.35 (1.13; 1.74)
AOA. units	1.52 (1.29; 1.75)	1.29 (1.15; 1.57)
retinol. μmol/l	0.49 (0.36; 0.6)	0.61 (0.46; 0.69)
α-tocopherol. μmol/l	9.29 (7.33; 11.26)	7.58 (6.53; 9.42)
GSH. mmol/l	2.22 (1.94; 2.41)	2.15 (1.74; 2.61)
GSSG. mmol/l	1.95 (1.62; 2.19)	1.85 (1.54; 2.14)
SOD. units	1.47 (1.39; 1.52)	1.42 (1.33; 1.52)



Oxidative stress index in groups of healthy and HIV-exposed newborns

The value of the oxidative stress coefficient indicates a significant imbalance in the LPO-AOP system reflecting the enhancement of the lipid peroxidation processes. An increase in the concentration of substrates and products of peroxidation leads to the destruction of lipids, and, consequently, to the degradation of the cell membrane complex. Despite the fact that free radical processes are typi-

characterizes the level of lipid peroxidation products and antioxidant protection factors in a particular group. Also, OSI is more sensitive indicator than separated components of the system, which objectively represents the LPO–AOD system malfunctioning, and provides the comprehensive view to the both lipid peroxidation processes and the level (effectiveness) of protective factors.

To summarize, the results of the study point to the need to further investigations aimed to determination of prognostically significant markers in PVEN, which could be involved or could indicate the pathological processes both in the early neonatal period and in the later stages of infant development. In order to improve the medical care of PVEN, it is necessary to continue research and develop clinical recommendations for managing this category of newborns at all stages of nursing and treatment.

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ANALYSIS OF THE C.757A>G P.(ILE253VAL) VARIANT OF THE *SLC26A4* GENE IN *GJB2*-NEGATIVE PATIENTS WITH HEARING LOSS IN YAKUTIA

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In this work, we searched for the missense variant c.757A>G p.(Ile253Val) of the *SLC26A4* gene in *GJB2*-negative patients with hearing loss (n=201) and in the control group of hearing individuals (n=103) in Yakutia. As a result, this variant was detected with a frequency of 2.02% among patients, in the control group - 1.94%. To interpretation the clinical significance, a frequency analysis of this variant and *in silico* evaluation were performed, the results of which are in favor of the likely benign of the c.757A>G p.(Ile253Val) variant of the *SLC26A4* gene, as indicated by the high frequency of occurrence in population samples, and the fact that this missense substitution theoretically does not violate the structural stability of the pendrin protein (SLC26A4).

Keywords: variant c.757A>G p.(Ile253Val), *SLC26A4* gene, pendrin (SLC26A4), sensorineural hearing loss.

Introduction. Hearing impairment (HI) is one of the most common congenital pathologies. The prevalence of congenital and childhood hearing loss (HL) and deafness in the world is estimated at 1.33 per 1000 newborns [19]. It is known that up to 50% of cases of congenital deafness have a hereditary etiology [6, 19]. About 70% of genetic causes of HL are thought to be nonsyndromic, with the remaining 30% being syndromic. At the same time, approximately 75% of all cases of nonsyndromic HL and deafness occur in autosomal recessive forms [6, 19].

The contribution of the *SLC26A4* gene to the etiology of autosomal recessive forms of HL is considered one of the most significant, and the proportion of its pathogenic variants among all identified causative variants of other genes in a number of regions of the world is in second place after the *GJB2* gene [5, 7, 9, 10, 16, 31], and in some and in first place (Pakistan) [12]. Pathogenic variants of the *SLC26A4* gene are associated with both autosomal recessive deafness type 4 (DFNB4, OMIM #600791) and Pendred syndrome (PS, OMIM #274600). The *SLC26A4* gene is located on chromosome 7 at the locus 7q22–q31, contains 21 exons, and encodes a transmembrane protein known as pendrin (PDS), which is expressed in the inner ear, thyroid gland, kidneys and airway epithelium [21-24, 28, 30]. Pendrin consists of 780 amino acids forming 12–14 TM segments, including a segment of the intracellular functional STAS domain (Sulfate Transporter and Anti-Sigma factor antagonist) [15, 29].

Earlier in Yakutia, using direct Sanger sequencing of the coding region of the *SLC26A4* gene, an analysis of its mutational contribution was carried out among six patients with HI and inner ear anomalies (IP-1, IP-2, and EVA) [1]. Five variants were identified in the *SLC26A4* gene: c.85G>C p.(Glu29Gln), c.757A>G p.(Ile253Val), c.2027T>A p.(Leu676Gln), c.2089+1G>A (IVS18 +1G>A) and c.441G>A p.(Met147Ile). Pathogenic biallelic variants of the *SLC26A4* gene were detected in four patients (4/6) and monoallelic *SLC26A4*-variants of in two patients (2/6). It should be noted that all monoallelic patients were Yakuts and carried the same heterozygous variant of the *SLC26A4* gene - c.757A>G p.(Ile253Val) [1]. In the ClinVar database, this variant is not annotated (https://www.ncbi.nlm.nih.gov/snp/rs773657545/#publications). However, in the Deafness variation database (DVD), the c.757A>G p.(Ile253Val) variant is classified as likely pathogenic

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(LP), and its allele frequency according to gnomAD is very rare, at only 0.008754% (https: //gnomad.broadinstitute.org/variant/7-107315546-A-G?dataset=gnomad_r2_1). Since the second mutant allele was not found in two patients with the heterozygous variant c.757A>G p.(Ile253Val) of the *SLC26A4* gene, a mutational search was performed in these patients in the genes associated with the hypothesis of digenic inheritance in the *FOXI1* and *KCNJ10* genes, which also did not reveal causative variants in these genes [1]. Due to the "absent" heritability in single-heterozygous patients with c.757A>G p.(Ile253Val) of the *SLC26A4* gene, to assess its pathogenetic role, it is relevant to search for this variant in extended cohorts of patients with hearing impairments and in control groups of hearing individuals.

In this regard, the aim of this work was to analyze the frequency of occurrence of the c.757A>G p.(Ile253Val) variant of the *SLC26A4* gene among *GJB2*-negative patients with HL, in comparison with the control group of hearing individuals in Yakutia.

Materials and methods. ***Study sample.*** *Patients.* The study sample consisted of genomic DNA samples of 201 patients (of which 198 were unrelated) with deafness and/or hearing loss from Yakutia, who had earlier, as a result of the analysis of nucleotide changes in the *GJB2* gene, pathogenic variants in the biallelic state, causing autosomal recessive deafness type 1A (DFNB1A, OMIM 220290) was not detected (*GJB2*-negative) [25]. Among patients, men accounted for 42.3% (n=85), women - 57.7% (n=116), mean age 26.63±17.51 years. Ethnic composition: Yakuts - 58.7% (n=118), Russians - 21.9% (n=44), of mixed - 13.9% (n=28) and other ethnicities - 5.5% (n=1). Audiological examination of the state of hearing was carried out using impedancemetry (AA222, Interacoustics, Denmark) and tone audiometry (GSI61, Grason Stadler inc., USA). Air conduction thresholds were obtained at 0.125, 0.25, 0.5, 1, 2, 4 and 8 kHz. The severity of hearing loss was defined as mild (25–40 dB), moderate (41–70 dB), severe (71–90 dB), or profound (above 90 dB).

Control group. In the control group of the studied, the search for the analyzed variant was carried out in 103 individuals with normal hearing from the population sample of Yakuts living in different regions of the republic. Hearing status was assessed using a clinical and audiological examination, including threshold tone audiometry.

Molecular genetic analysis. Genomic DNA samples were isolated by phenol-chloroform extraction. The search for the c.757A>G p.(Ile253Val) variant localized in exon 6 of the *SLC26A4* gene was carried out using PCR-RFLP analysis. For amplification of a 251 bp fragment primers (F) 5'-CAGAGAGTAGGTTTC-TATCTCAGGC-3' and (R) 5'-CCCTG-GAGCAAGAAGCAACA-3' were used. For RFLP analysis, restriction endonuclease *Hpa*I (restriction site GTT↑AAC/CAA↓TTG) was used. RFLP analysis was performed by electrophoresis in 4% agarose gel with registration in the Molecular Imager Gel Doc XR Sistem gel documentation system (Bio-Rad, USA). In the work, reference sequences were used: Gene ID: 5172 (*SLC26A4* - solute carrier family 26 member 4 [Homo sapiens (human)]): NM_000441.2, NP_000432.1, (https://www.ncbi.nlm.nih.gov/gene/5172); Ensembl ID: ENSG00000091137, ENSP00000494017, ENST00000644269 (https://www.proteinatlas.org/ENSG00000091137-SLC26A4).

In-silico analysis. *Database.* To search for genetic information (on the clinical significance and phenotypic relationship of variants, prevalence, publications, evaluation of *in silico* predictive programs for the classification of missense variants), the following databases were used: OMIM® (https://www.omim.org/), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), Deafness variation database (DVD, https://deafnessvariationdatabase.org/).

AlphaFold System. AlphaFold was used, which is a computational algorithm that can regularly predict protein structures with atomic accuracy, even in cases in which no similar structure is known [14]. AlphaFold produces a confidence metric for amino acid residues, as a predicted local distance difference test (pLDDT), on a scale of 0 to 100 [14]. An expected value of pLDDT>90 is taken as the high accuracy cut-off (blue), pLDDT>70 indicates low confidence and corresponds to a generally correct backbone prediction (turquoise color, good backbone prediction), pLDDT≤70 indicates that we should also add substantial coverage for sequences without a good template in PDB (yellow color, should be considered with caution), and pLDDT<50 indicates very low confidence (orange, should not be interpreted) [13]. Full details are available at: https://www.ebi.ac.uk/about.

PyMol program. PyMol (PyMOL Molecular Graphics System) - graphics program that provides 3D visualization of proteins, small molecules, molecular surfaces and trajectories. Full details are available at: https://pymol.org/2/#products.

Evaluation of the clinical significance of c.757A>G p.(Ile253Val) of the SLC26A4 gene by ACMG. The classifi-

Table 1

Allele frequency c.757A>G p.(Ile253Val) of the SLC26A4 gene in patients with hearing loss/deafness in comparison with population samples

Country (Region)	Sample of patients*	AF	Population sample**	AF	References
Russia (Yakutia)	8/198	2.02% (8/396)	4/103	1.94% (4/206)	This work
China	1/284	0.17% (1/568)	-	-	[4]
China	1/2352	0.02% (1/4704)	-	-	[18]
China (Tibet)	1/114	0.43% (1/228)	4/106	1.88% (4/212)	[3]
China	1/227	0.22% (1/457)	0/200	0% (400)	[20]
China	1/371	0.13% (1/742)	-	-	[11]
Total	13/3546	0.18% (13/7092)	8/409	0.97% (8/818)	-
χ^2	0.18% (13 из 7095)		0.97% (8 из 818)		$\chi^2 = 17.49$. $p<0.001$

Note: * Number of patients with c.757A>G p.(Ile253Val) of the SLC26A4 gene per total number of patients with hearing loss/deafness, ** - Number of individuals with c.757A>G p.(Ile253Val) of the SLC26A4 gene per total number of individuals from population samples. AF - allelic frequency; "-" - no data.

Table 2

Clinical characteristics of patients with the c.757A>G p.(Ile253Val) variant of the *SLC26A4* gene

Country (Region)	*SLC26A4*-genotypes of patients with c.757A>G p.(Ile253Val)	EVA/other anomalies	Hearing	Evaluation	References
China	c.[757A>G];[wt]	-	deafness	VUS	[4]
China	c.[757A>G];[wt]	-	-	-	[18]
China (Tibet)	c.[757A>G];[wt]	norm/ hypoplastic cochleavestibular and semicircular canals	deafness	benign	[3]
China	c.[757A>G];[wt]	EVA	deafness	benign	[20]
China	c.[919-2A>G];[757A>G]	norm	norm	VUS	[11]
Russia (Yakutia)	c.[757A>G];[wt] c.[757A>G];[wt]	EVA+IP-1 IP-1/IP-2	deafness deafness	likely pathogenic	[1]

Note: EVA – enlarged vestibular aqueduct; IP-1 – Incomplete Partition Type 1, IP-2 – Incomplete Partition Type 2; VUS – variant uncertain significance. «-» - no data.

cation of the clinical significance of the c.757A>G p.(Ile253Val) variant of the *SLC26A4* gene was carried out according to the standards of the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) [26], adjusted for genetic HL [8].

Ethical approval. All examinations provided in this study have been conducted with the informed written consent of all participants. This study was approved by the local Biomedical Ethics Committee of Federal State Budgetary Scientific Institution "Yakut Science Centre of Complex Medical Problems" (Yakutsk, Protocol #7, August 27, 2019).

Results and discussion. ***Search for the c.757A>G p.(Ile253Val) variant of the SLC26A4 gene in GJB2-negative patients and in the control group.*** The search for c.757A>G p.(Ile253Val) of the *SLC26A4* gene in a sample of *GJB2*-negative patients (n=201) with HI in Yakutia revealed this variant in the heterozygous state in 8 Yakut patients. None of the patients had this variant in the homozygous state. In the control group of hearing individuals from the population sample of Yakuts (n=103), this variant was found in 4 individuals, also in the heterozygous state. Thus, the allelic frequency of the c.757A>G p.(Ile253Val) variant of the *SLC26A4* gene was practically the same in both study groups, among unrelated patients it was 2.02% (8/396), and in the control group it was 1.94 % (4/206) (Table 1). For a comparative analysis of the allelic frequency c.757A>G p.(Ile253Val) of the *SLC26A4* gene, we analyzed the literature, which described cases of detection of this variant. As a result, the variant we analyzed was previously identified only in isolated cases among

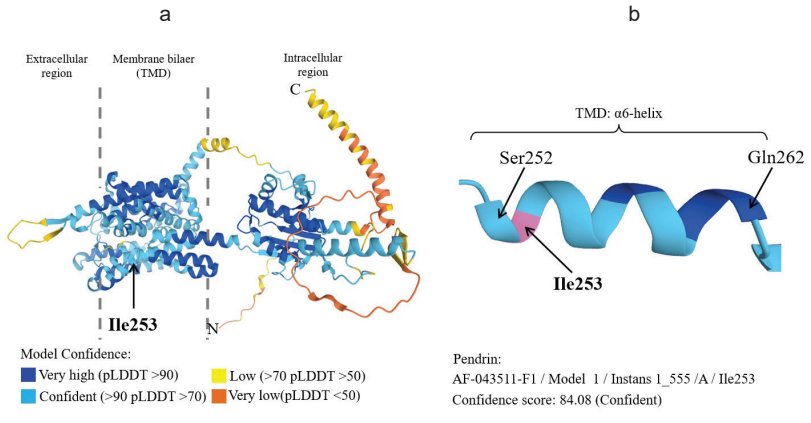

Fig. 1. Three-dimensional (3D) spatial structural model of the human pendrin (SLC26A4) as predicted by Alphafold 2.0. A. General view (https://alphafold.ebi.ac.uk/entry/O43511). Color represents regions of the monomer with varying confidence in conformation prediction (see bottom). Dashed lines indicate extra- and intracellular regions of the monomer. The arrows indicate the analyzed amino acid residues (Ile253). B. Close-up of the location of Ile253 in the α6-helix of the TMD of pendrin (shown in pink), consisting of 10 amino acid residues (from Ser252 to Gln262).

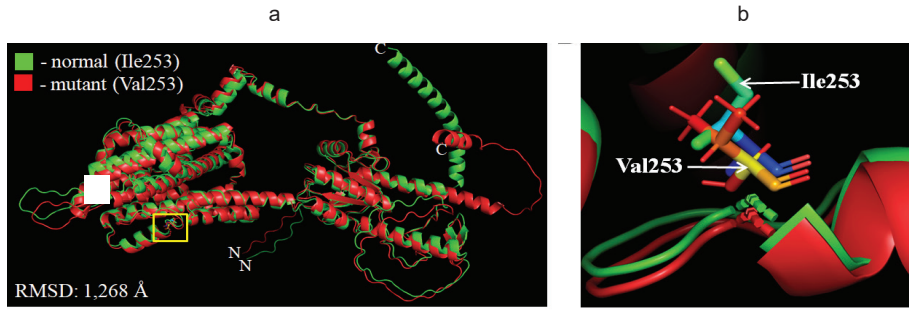

Fig. 2. Alignment of mutant p.(Ile253Val) and normal structures of pendrin (SLC26A4) in PyMOL. A. The yellow square marks the localization site of the amino acid residue at position 253, in the norm Ile (green), in the mutant Val (red). B. Close-up view of the side chains of the amino acid residues Ile253 and Val253, originating from the C-atoms of the main chain.

patients with HI in China [3, 4, 11, 18, 20]. In general, the allelic frequency of c.757A>G p.(Ile253Val) of the *SLC26A4* gene in patients with HL/deafness in the studied regions of the world was 0.18% (13/7095). However, in a comparative analysis of the identified overall frequency of occurrence of the variant among the deaf population, in comparison with the frequency in population samples, it turned out that the c.757A>G variant occurred 5 times more often in popula-

Table 2

Classification of the clinical significance of the missense variant c.757A>G p.(Ile253Val) of the *SLC26A4* gene according to the ACMG recommendation

	ACMG Category			
	Pathogenic (P, LP)		**Benign (B, LB)**	
1	**Population Data**			
	Criteria for classifying pathogenic variants	Evidence	Criteria for classifying benign variants	Evidence
	Strong: very strong – PVS(1) strong – PS(1-4)	-	Strong: stand-alone – BA(1) strong – BS(1-4)	-
	Moderate: PM(1-4)	**PM2_Supporting:** Low MAF in population databases (<0.0007 [0.07%] for autosomal recessive) [8]		
	Supporting - PP(1-5)	-	Supporting - BP(1-6)	-
2	**Functional Data**			
	Strong: very strong – PVS(1) strong – PS(1-4)	-	Strong: stand-alone – BA(1) strong – BS(1-4)	-
	Moderate: PM(1-4)	-		
	Supporting: PP(1-5)	**PP2:** Missense variant in a gene that has a low rate of benign missense variation and in the ClinVar database: out of 389 missense changes, P/LP/VUS-variants account for 94% (https://www.ncbi.nlm.nih.gov/clinvar/?term=SLC26A4%5Bgene%5D&redir=gene) (PP2 - Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease [26])	Supporting: BP(1-6)	-
3	***In silico* Predictions**			
	Strong: very strong – PVS(1) strong – PS(1-4)	-	Strong: stand-alone – BA(1) strong – BS(1-4)	-
	Moderate: PM(1-4)	-		
	Supporting: PP(1-5)	**PP3:** 8 in silico programs (MT, DANN, MetaLR, Polyphen-2, LRT, MutationTaster, PhyloP, GERP++) predict pathogenic effect (https://franklin.genoox.com/clinical-db/variant/snp/chr7-107315546-A-G?app=assessment-tools; https://deafnessvariationdatabase.org/gene/SLC26A4) (PP3 - Multiple lines of computational evidence support a deleterious effect on the gene or gene product [26])	Supporting: BP(1-6)	**BP4:** 1) 4 in silico programs (MUT Assesor, SIFT, GenoCanyon, fitCons) predict benign effect (https://franklin.genoox.com/clinical-db/variant/snp/chr7-107315546-A-G?app=assessment-tools; https://deafnessvariationdatabase.org/gene/SLC26A4) (BP4 - Multiple lines of computational evidence suggest no impact on gene or gene product [26]) 2) Alignment of the mutant (p.Ile253Val) and native protein structures showed that the analyzed substitution does not affect the functional significance of pendrin
Classification	PM2 + PP2 + PP3 = insufficient to classify pathogenic		BP4 = insufficient to classify benign	

tion samples (0.97%) than in people with disabilities hearing (0.18%) ($\chi 2 = 17.49$, $p<0.001$) (Table 1).

When analyzing the clinical characteristics of seven patients with the c.757A>G p.(Ile253Val) variant of the *SLC26A4* gene, previously detected in other studies [1, 3, 4, 11, 18, 20], it was found that in most of them (6 out of 7) the variant was found in single-heterozygous condition, the second mutant allele was not detected (Table 2). Only one case is known (1 out of 7), where the c.757A>G p.(Ile253Val) variant was detected in a compound heterozygous state with the *SLC26A4*-variant c.919-2A>G during neonatal screening for deafness in China [11]. However, the authors report that at the time of audiological testing, the child with genotype c.[919-2A>G];[757A>G] had no HL, however, they do not exclude the possibility of progression of HI with age [11].

Due to the absence of certain, strong evidence for unambiguous classification of the clinical significance of c.757A>G (clinical and functional evidence), we performed an *in silico* analysis of this variant using the AlphaFold neural network algorithm.

In-silico analysis of the c.757A>G p.(Ile253Val) variant of the SLC26A4 gene using the AlphaFold neural network algorithm. Since at present, the crystal model of the spatial structure of the human pendrin protein (SLC26A4) has not yet been experimentally established, a native model of the three-dimensional spatial structure was obtained using the AlphaFold system (Fig. 1A). AlphaFold predicted a qualitative model of pendrin with high accuracy (most of the protein regions were predicted with >90pLDDT>70), which allowed us to obtain the necessary information about the architecture of the monomer structure. Thus, in the resulting AlphaFold-model, the analyzed amino acid position Ile253 is localized in segment 6 (α6-helix), which is represented by 10 amino acid residues from 252 to 262 (Fig. 1B).

According to the topology of the pendrin protein (SLC26A4), which is composed of 14 transmembrane segments (α-helices in the form of columns 1-7 and 8-14, forming channel pores), segment 6 (α6-helix) is located in the gate domain, which in turn consists of outer helices, 5-7 and 12-14 segments [2, 15, 17, 27, 29]. It is important to note that the analyzed p.Ile253Val substitution is not located in the critical region of the core domain, the disruption of which can lead to improper substrate transport or the appearance of toxic conformations (misfolded proteins) [2, 17, 29]. It is known that mutant amino acid residues located in the core domain can cause disturbances in the conduction of the SLC26A4 ion channel and cause diseases associated with the pendrin protein. However, it is equally important to note that another pathogenic missense variant is currently annotated in the ClinVar database, which is also located in the 6th protein segment - c.754T>C p.(Ser252Pro) (https://www.ncbi.nlm.nih.gov/clinvar/variation/1065210/). This sequence (Ser252) of the polypeptide chain of helix 6, followed by the region we analyzed (p.Ile253Val), is adjacent to the loop of the extracellular region of pendrin, therefore a mutational change in this region can lead to functional impairment of the protein.

Further, using the PyMOL program tool based on the native structure of the SLC26A4 protein, modeled by the AlphaFold 2.0 program, we aligned the three-dimensional folding of the mutant and normal pendrin chain (Fig. 2). As a result of alignment of the three-dimensional folding of the mutant and normal chains using the PyMOL program, the obtained RMSD value: 1,268 Å is within the full similarity criterion (<2 Å) and indicates that the studied missense variant does not lead to a change in the spatial structure of the synthesized protein. This similarity of the two compared structures, with a slight difference, is probably due to the physicochemical properties of the considered amino acid residues in the polypeptide chain. It is known that the amino acids isoleucine and valine belong to the same functional group of amino acids with hydrophobic uncharged side radicals and their isoelectric point is approximately the same (Ile: p*I* = 6,1, -COOH = 2,4, $-NH_3$ = 9,7; Val: p*I* = 6,0, -COOH = 2,3, $-NH_3$ = 9,6).

Evaluation of the clinical significance of c.757A>G p.(Ile253Val) of the SLC26A4 gene. In total, the data obtained made it possible to collate the traits (allelic frequency of occurrence, *in silico* assessment) to classification the clinical significance of the c.757A>G p.(Ile253Val) variant of the *SLC26A4* gene according to the ACMG criteria [8, 26], which are presented in table 3. The resulting combining of criteria does not allow it to be considered as a pathogenic or benign variant, thus, it is interpretation as a variant of uncertain significance - VUS.

Conclusions. The results of this study indicate that the c.757A>G p.(Ile253Val) variant of the *SLC26A4* gene is to be likely benign, since this is indicated by a high frequency of occurrence in population samples, and the fact that this missense substitution theoretically does not violate the structural stability of the pendrin protein (SLC26A4). However, given the rare occurrence in the world, the lack of genotype-phenotypic and functional data, currently the c.757A>G p.(Ile253Val) variant is classification as a variant of uncertain significance according to the ACMG criteria.

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ENDOTHELIAL DYSFUNCTION IN THE PATHOGENESIS OF INFLAMMATORY PERIODONTAL DISEASES

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Manifestations of endothelial dysfunction that occur in response to microbial invasion in inflammatory periodontal diseases may underlie the occurrence and progression of these diseases. The aim of the study was to determine the level of secretion of adhesive molecules of the selectin family and the superfamily of immunoglobulins in the gingival/periodontal pocket and their relationship with marker periodontal pathogens.

For the study, flushes of the gingival pocket (a total of 88 samples) of patients with chronic generalized periodontitis and intact periodontitis were obtained. The content of soluble forms of the adhesion molecules sICAM-1, sVCAM, sE-selectin, and sL-selectin was determined by ELISA. Marker periodontal pathogens were isolated by real-time PCR. The study revealed changes in the adhesiveness of molecules in individuals with chronic generalized periodontitis (CGP): the concentrations of sL-and sE-selectin molecules in the gingival/periodontal pocket discharge in patients with CGP increased by an average of 80,4% (p=0.045) and 63,6% (p=0,038), respectively. While the concentrations of adhesive proteins of the superfamily of immunoglobulins sICAM-1 and sVCAM in individuals with CGP exceeded the corresponding concentrations of the control group to a greater extent: 9,7 (p=0,022) and 18,1 (p=0,023) times, respectively. The frequency of detection of periodontal pathogenic bacteria genes was 96,4% in patients with CGP and 28,6% in the group with intact periodontitis. Statistically significant correlations of moderate and high degree were found between the content of sVCAM and T. forsythia (r=0,683, p=0,02) and A. actinomycetemcomitans (r=0,621, p=0,04), as well as sICAM-1 and P. gingivalis (r=0,628, p <0,001) and A. actinomycetemcomitans (r=0,821, p=0,04) in the group of patients with CGP. In the examined patients with intact periodontitis, weak negative correlation between sL-selectin and T. denticola was found (r=-0,482, p=0,03). Thus, elevated concentrations of the soluble adhesive molecules sICAM-1, sVCAM, sE-and sL-selectin may indicate endothelial cell alteration due to persistent inflammatory process caused by virulence factors of specific subgingival bacterial flora.

Keywords: chronic generalized periodontitis, periodontal pathogenic bacteria, inflammation, adhesion molecules, endothelial dysfunction.

Introduction. Pathogenetic aspects of the onset and progression of inflammatory periodontal diseases (IPD) include an imbalance in the microbiota of oral biotopes, as well as shifts in the immune response system: changes in the secretion of inflammatory markers - cytokines, antimicrobial peptides, acute phase proteins, and secretory immunoglobulins of the gingival fluid. The main bacteria involved in the development and progression of IPD, including chronic generalized periodontitis (CGP), include gram-negative anaerobic flora: *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola,* and *Prevotella intermedia* [3, 6, 8]. Being distinguished by high adhesive, invasive and toxic properties, these representatives of the bacterial community contribute to damage to the membranes of the cell walls of endotheliocytes, penetration into the vascular bed and toxigenic effect on the vascular endothelium. Manifestations of endothelial dysfunction that occur in chronic periodontitis, accompanied by a violation of its antiplatelet, anticoagulant, and fibrinolytic properties, may underlie the occurrence and progression of this disease [4].

Thus, the study of markers of endothelial dysfunction in the pathogenesis of chronic generalized periodontitis is of particular interest. A key role in changing the adhesive properties of the vascular wall is played by a complex system of membrane proteins expressed on the surface of endotheliocytes – intercellular adhesion molecules that include integrins, adhesive receptors of the immunoglobulin superfamily, selectins, cadherins, and homing receptors of leukocytes. Thus, the intercellular adhesion molecule-1 (sICAM-1), a member of the immunoglobulin superfamily and a functional ligand for the leukocyte integrin LFA-1 (Lymphocyte Function-Associated Antigen-1), is a marker that triggers inflammatory reactions and is expressed earlier and in a larger volume than HLA-DR. Studies have shown that the vascular endothelial adhesion molecule 1 (sVCAM), also a member of the immunoglobulin superfamily, is not permanently expressed on the endothelium, but can be synthesized in response to stimulation by bacterial lipopolysaccharides, TNF-α and IL-1 [2, 9], as well as IFN-γ and IL-4. The endothelial-leukocyte adhesion molecule-1 (sE-selectin) and the leukocyte adhesion molecule-1 to endothelial cells (sL-selectin) contribute to the formation of the first, not yet strong contacts of inactivated polymorphonuclear leukocytes with the endothelium at the sites of inflammation, mediate the initial interaction of leukocytes with endothelial cells, and the level of their expression on the endothelium is associated with inflammation [2, 4, 6].

The aim of the study was to determine the level of secretion of adhesive molecules of the selectin family and the superfamily of immunoglobulins in the gingival/periodontal pocket and their relationship with marker periodontal pathogens.

Materials and methods. A dental and clinical laboratory study was conducted on 88 people aged 18 to 45 years, who were undergoing outpatient treatment by a dentist on the basis of the dental polyclinic in Severodvinsk, Arkhangelsk region. The comprehensive study included the determination of dental status, immunological, as well as molecular genetic analysis and sociological research with questionnaires. The medical study was conducted in compliance with the rules of the international standard GCP and protocol approved by the local Ethics Committee of NSMU (Protocol No. 08/11 of 28.11.2018). Two groups were formed: the first group consisted of patients with a diagnosis of "chronic periodontitis" (n=56), including mild (n=32), moderate (n=24) severity of periodontitis in accordance with ICD 10: K05. 31-chronic generalized (mild, moderate) periodontitis; the second-the control group with intact periodontitis (n=32). The main criteria for inclusion in the groups were informed consent of patients, the age category of 18-45years, the presence of chronic periodontitis of mild and moderate severity and satisfactory oral hygiene. The criteria for exclusion from the study were: other inflammatory diseases in the oral cavity, pregnancy, and the postpartum period.

During the study, the discharge of the gingival/periodontal pocket (DGP/DPP) obtained by aspiration using a sterile syringe tube, then the resulting material was centrifuged at 1500 rpm with an exposure for 20 minutes. At the same time, samples of clinical material were frozen and stored at a temperature of -80˚C for further molecular genetic and immunological analyses.

Using an enzyme-linked immunosorbent assay (ELISA) the concentration of soluble forms of adhesion molecules sICAM-1, sVCAM, sE-selectin, and sL-selectin was determined in thawed samples separated by DGP/DPP sL- selectin in accordance with the manufacturer's instructions (Hycult Biotech, the Netherlands). The optical densities and contents of the tablet cells were studied and recorded on photometric device "Multiscan EX" (Thermo Fisher Scientific, USA). Calculations were carried out according to the manufacturer's instructions using calibration curves formed on the basis of measurement standards.

Marker periodontal pathogenic microorganisms were determined in real time using the method of polymerase chain reaction (RT-PCR). Periodontal pathogenic bacteria of the first order included: *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia,* periodontal pathogenic representatives of the second order- *Treponema denticola, Prevotella intermedia, Candida albicans.* The study was conducted in accordance with the requirements presented by the manufacturer on the Dt-light detecting amplifier ("Periodontoscreen", LLC "DNA-Technology", Russia).

Statistical processing of the results of the study with the distribution of data by the studied parameters was carried out using the special software package "STATA v. 12" («Stata Corp», USA). Significant differences (p<0,05) were also determined using the Student's t-test and correlation assessment was performed using the Pearson method.

Results and discussion. Gingival fluid is an exchange medium that promotes the migration of white blood cells from blood vessels through the epithelium to the gingival groove and influenced by bacterial chemotactic factors. The process of periodontal inflammation is characterized by a number of factors: an increase in the number of migrating leukocytes, endothelial dysfunction due to increased adhesiveness, which is probably necessary to create obstacles to the penetration of periodontal pathogenic microflora into the groove epithelium and underlying periodontal tissues [7]. In our study, the content of soluble adhesion molecules sICAM-1, sVCAM, sE-selectin, and sL-selectin in patients with chronic periodontitis was significantly higher than in the control group, which confirms this assumption. Thus, the concentrations of soluble forms of adhesion molecules sICAM-1, sVCAM, sE-selectin, and sL-selectin in chronic periodontitis exceeded those in the control group. Compared with patients with intact periodontitis, the concentration of sL-and sE-selectin molecules in the periodontal pocket discharge in patients with chronic generalized periodontitis increased by an average of 80,4% and 63,6%, respectively. While the concentrations of adhesive proteins of the family of the superfamily of immunoglobulins sICAM-1 and sVCAM in individuals with CGP exceeded the corresponding concentrations of the

control group to a greater extent: by 9,7 and 18,1 times, respectively (Table 1).

When assessing the level of expression of adhesive molecules depending on the severity of chronic periodontitis, it was found that in the subgroup with a mild course (n=32), the concentrations of sL-selectin and sE-selectin were lower by 55,9% (p=0,048) and 28% (p=0,032) than in the subgroup with a moderate course (n = 32). The results of the study showed a high degree of severity (n=24) and were 6,0 ng/ml [5,8; 6,3] and 4,3 ng/ml [4,0; 4,8], respectively. Concentrations of soluble forms of adhesion molecules of the immunoglobulin superfamily were also lower in patients with mild severity CGP: the concentration of sICAM-1 was 72,5 [69,7; 73,1] ng/ml, which is 27% (p=0,05) lower than in the group with moderate severity, while the concentration of sVCAM was 62,7 [56,4; 68,0] ng/ml, which is 24,6% (p=0,036) lower than in the group with moderate severity of chronic periodontitis.

The shifts in the secretion of adhesion molecules in the gingival/periodontal pocket secreted in our study correlate with the data on the study of serum concentrations of adhesive molecules in chronic periodontitis [6]. Thus, the results of the conducted studies indicate that patients with chronic generalized periodontitis develop systemic disorders associated with the violation of the adhesive properties of the vessel wall, manifested in an increase in serum concentrations of sICAM-1, sVCAM, sE-and sL-selectin caused by endotheliocyte alteration [2].

To determine the application points of intercellular adhesion molecules in the pathogenesis of endothelial dysfunction in the development of inflammatory and destructive changes in periodontal tissues, we evaluated markers of periodontal pathogenic microorganisms of the gingival/periodontal pocket. In patients, with chronic periodontitis, periodontal pathogenic bacterial flora was detected in 96,4% of cases. The oral microbiome is in constant dynamic balance: normal microbial flora provides processes of colonization resistance and reparative regeneration, while the appearance of periodontal pathogenic bacteria contributes to the formation of shifts in the homeostasis of the oral ecosystem and the formation of an inflammatory and destructive process. It is the microbial factor that underlies the occurrence and progression of IPD: an inflammatory reaction from the connective tissue and endothelium leads to a violation of the integrity of the gingival epithelium, subsequently causing the formation of deep periodontal pockets

Table 1

Expression level of adhesion molecules of the secreted DGP/DPP in patients with chronic periodontitis and in patients with intact periodontitis M [Q1; Q3]

Adhesion molecules, (ng/ml)	Group 1 (chronic periodontitis)	Group 2 (intact periodontal disease)	Statistical significance level
sL-selectin	9.2 [5.8; 14.7]	5.1 [2.9; 10.1]	p=0.045
sE-selectin	5.4 [4.0; 6.7]	3.3 [0.4; 4.8]	p=0.038
sICAM-1	83.0 [69.7; 98.5]	8.6 [2.5; 11.4]	p=0.022
sVCAM	76.2 [56.4; 82.8]	4.2 [2.1; 8.3]	p=0.023

Table 2

Correlation matrix of soluble adhesion molecules in DGP/DPP washes and markers of periodontal pathogenic microorganisms of the gingival pocket

Indicators	sL-selectin	sE-selectin	sICAM-1	sVCAM
Periodontitis				
P. gingivalis	r=0.289 (p=0.04)	r=0.322 (p=0.034)	**r=0.628** (p<0.001)	**r=0.542** (p=0.05)
T. forsythia	r=0.263 (p=0.03)	r=0.434 (p=0.03)	r=0.142 (p=0.03)	**r=0.683** (p=0.02)
A. actinomycetemcomitans	r=0.371 (p=0.02)	r=0.283 (p=0.006)	**r=0.821** (p=0.04)	**r=0.621** (p=0.04)
Associations of periodontal pathogens	r=0.388 (p=0.31)	r=0.189 (p=0.05)	r=0.112 (p=0.02)	r=0.311 (p=0.04)
Control				
T. denticola	**r=-0.482** (p=0.03)	r=0.134 (p=0.36)	r=0.179 (p=0.2)	r=0.212 (p=0.4)
P. intermedia	r=0.424 (p=0.33)	r=0.017 (p=0.26)	r=0.122 (p=0.08)	r=0.165 (p=0.3)

[1, 4, 8]. Among the isolated periodontal pathogens in the group with CGP, the highest frequency of occurrence was found in periodontal pathogenic bacteria of the first order: *A. actinomycetemcomitans* in 85,7% of cases, *P. gingivalis* in 78,6% of cases, *T. forsythia* in 57,1% of cases. Periodontal pathogens of the second order were also identified: *P. intermedia* in 53,6% of cases, *T. denticola* in 46,4% of cases. *C. albicans* was detected in 4,5% of cases. Associations of periodontal pathogens were found in 16 people (28,6%) in the subgroup with moderate chronic periodontitis: the most common association *was A. actinomycetemcomitans* and *P. gingivalis* in 14,3% of cases. Associations of *P. gingivalis* with *T. forsythia* were found in 8,9% of cases. The periodontal pathogenic microflora identified in patients is characterized by high adhesive, invasive, and toxic properties; these representatives of the bacterial community contribute to damage to cell wall membranes, penetration into the vascular bed, and toxigenic effects on the vascular endothelium [9]. Thus, the leukotoxin *A. actinomycetemcomitans* causes destruction of the phagocytosis object due to the interaction of polymorphonuclear leukocytes and CD11a/CD18 monocytes, accelerates monocyte lysis by activating caspase-1 [3]. The virulence of *P. gingivalis* has a damaging effect on the vascular endothelium: the adhesion and invasion of this periodontal pathogen leads to the generation of reactive oxygen species in endotheliocytes, causing oxidative stress that damages cells [2]. Also, according to some authors, *P. gingivalis* aggression factors lead to the destruction of alpha-tubulin and beta-1-integrin, as well as a decrease in ERK1/2 activation, which probably can contribute to pro-apoptotic effects. Proteo- and glycolytic enzymes *of T. forsythia* binde microorganisms to red blood cells, polymorphonuclear leukocytes and fibroblasts.

At the same time, the *T. forsythia* BspA surface antigen stimulates the production of proinflammatory cytokines in mononuclear cells when interacting with CD14 and TLR4 [3, 8].

Studies conducted in patients with intact periodontitis showed that the frequency of detection of markers of periodontal pathogenic species was 28.5%: *T. denticola* was isolated in 9,4%, *P.intermedia* in 6,3% of cases. At the same time, periodontal pathogenic bacteria of the first order and periodontal pathogens were not identified in the associations.

To conduct a deep pathogenetic assessment of the relationship between the identified types of periodontal pathogens and the content of soluble forms of gingival fluid adhesion molecules, a correlation analysis of the results obtained during the study was performed (Table 2).

Molecules of the superfamily of immunoglobulins sVCAM and sICAM-1 perform selective leukocyte adhesion, promoting the accumulation of mononuclear cells during the transition of the acute phase to the chronic stage with leukocyte endothelial reaction. ICAM-1 and the LFA-1 receptor ensure the production of T-lymphocytes, and the combined action of T-cell receptor and CD2 affects the change in the state of LFA-1 with a further increase in binding to ICAM-1. At the same time, such increase in molecules in the gingival fluid indicates a persistent inflammatory process caused by microbial flora, which is reflected in the revealed correlations of sVCAM and sICAM-1 with first-order periodontal pathogens *P. gingivalis, T. forsythia* and *A. actinomycetemcomitans* [2, 5].

It should be noted that the permeability of the epithelium contributes to the rapid entry of external molecules and cells of the immune system into the gum, and also ensures the delivery of various substances in both directions [7]. A number of researchers indicate that sL-selectin creates conditions for the formation of the phenomenon of "rolling" neutrophils inside vessels with leukocyte adhesion to the endothelium, which, in turn, leads to their accumulation in the inflammatory zone. In this case, metalloproteinases promote the cleavage of sL-selectin with a further decrease in the control of sL-selectin-mediated adhesion. The inducible adhesive molecule sE-selectin is synthesized and expressed by endothelial cells after stimulation with proinflammatory cytokines or bacterial endotoxins [4, 5, 6]. Probably, an increase in the expression of adhesive molecules of the selectin family may reflect their significant expenditure during immune reactions aimed at periodontal pathogen eradication, which is reflected in the revealed negative correlation of the average strength of sL-selectin and *T. denticola* in the control group.

Conclusion. The detected elevated concentrations of the soluble adhesive molecules sICAM-1, sVCAM, sE- and sL-selectin, to a greater extent in moderate CGP, may indicate endothelial cell alteration and endothelial dysfunction, accompanied by a violation of its antiplatelet, anticoagulant, and fibrinolytic properties due to a persistent inflammatory process caused by virulence factors of a specific subgingival periodontal pathogenic flora, mostly of the 1st order.

Thus, the soluble cell adhesion molecules sVCAM and sICAM-1, sE-selectin and sL-selectin are additional laboratory markers for determining the severity of the inflammatory process in periodontal tissues in patients with chronic periodontitis.

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ARCTIC MEDICINE

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COMPARATIVE ANALYSIS OF THERMAL IMAGING PICTURES OF NORTHERNERS IN THE AGE ASPECT

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Four hundred and fifty-two male residents of Magadan Region were examined to study ontogenetic features in their thermal imaging pictures. Based on the subjective age which ranged 15-74, four groups were formed: adolescents, young men, mature men, and elderly men. The indicators of thermography, hemodynamics, gas exchange and energy metabolism were analyzed We could see the temperatures in both individual zones of the body and weighted averages through all analyzed spots drop in increasing reliance on age. The daily energy consumption at rest, oxygen consumption per kg of the body weight, as well as the oxygen utilization factor were also maximal in the group of young men and minimal in older men. The average group indices of peripheral vascular resistance increasingly varied from adolescents to the elderly, while the cardiac output values dropped in more than half within the same age range.

Keywords: thermography, metabolism, hemodynamics, North, men, adaptation.

Introduction. Human body temperature is known to be a key indicator of vital activity [12]. Metabolism is crucial in endothermic animals as it maintains the basic body temperature within a few tenths of a degree Celsius which is the temperature homeostasis regardless of meteorological conditions but at very high energy costs [22]. Body temperature measurement is one of the oldest quantitative assessments of metabolic balance [25]. In fact, infrared thermography is a non-contact and non-radiation imaging method aimed at studying the physiological possibilities associated with thermal homeostatic actions of the body expressed through thermoregulation of the skin [15]. Modern thermal imaging is a scientifically based method which is widely used in science, technology, and national economy for registering the distribution of the body surface temperature with the possibility of converting the obtained two-dimensional thermal images into a digital form suitable for qualitative and quantitative analysis [27]. The method enables to convert infrared thermal radiation into electric signal which is amplified and then reproduced on the LCD screen as a color picture of the temperature distribution [23]. The homoeothermic body is described by the core-shell model [16]. When it comes to temperature, the core of the body is relatively stable, but the shell of the body (surface tissues and skin mainly) contributes to the regulatory process [24]. It is hypothesized that the skin, as the physical boundary of this model, is a kind of mirror reflecting internal thermodynamic processes.

A person's age is important in the balance between the body heat production and heat loss. The body's ability to maintain internal temperature comes down with increasing age [12].

Following on from the above, this study used infrared thermography in order to identify age-associated changes in Northern men based on thermal imaging pictures taken from different zones of the body.

Materials and Methods. Four hundred and fifty-two Caucasian males, all permanent residents of Magadan Region, were examined. The subjects were divided into four groups: adolescents, n=95 (average age 16.2±0.4 yrs, body length 179.1±0.5 cm, body mass 70.5±0.5 kg, body mass index 20.7± 0.4 kg/m^2), young men, n=192 (19.2±0.5 yrs, 178.9±0.7 cm, 66.4±1.2 kg, 21.7±0.2 kg/m^2), mature men, n=109 (38.2±0.7 yrs, 180.1±0.5 cm, 84.1±0.9 kg, 25.9±0.4 kg/m^2), elderly men, n=56 (67.2±1.1 yrs, 172.9± 0.8 cm, 83.9±1.3 kg, 27.9±0.3 kg/m^2).

The thermographic survey was performed in the initial standing position, using a thermal imager of FLIR SC620 (Sweden). The device provided long-wave (7.5-13 microns) visualization with a sensitivity of at least 0.1 °C and a spatial resolution of 640 x 480 pixels. The research was carried out in compliance with the European Thermographic Association standards [11]. We analyzed the pictures obtained with the thermal imager from eight zones, the anterior and posterior parts of the body (Fig. 1): C_1 – the average temperature of the left subclavian area (°C), C_2 – the right subclavian area (°C), C_3 – forehead (°C), C_4 – chest (°C), C_5 – abdominal area (°C), C_6 – upper back (°C), C_7 – shoulder blades / interscapular area (°C), C_8 – lower back / lumbar (°C). For each selected area, the average surface temperature was calculated, which was more representative for that area than its minimum and maximum values. The advantage of infrared systems in comparison with other methods of temperature measurement is that it allows for the simultaneous analysis of a large number of image elements (pixels) in a short period of time, after which real-time processing is possible [14].

We used a Spirolan-M metabolograph (Russia) to explore the level of energy metabolism. The energy consumption at rest, per day (kcal/day), the percentage ratio of energy consumption to the proper level (%), oxygen consumption rate, QO_2 (mL/kg), and oxygen utilization factor, O_2UF (mL/L) were determined.

Cardiovascular system characteristics were studied using a Nissei DS-1862 tonometer (Japan). Cardiac output (CO, mL/min) and total peripheral vascular resistance (TPVR, dyn^2 s cm^{-5}) were calculated.

The investigations were made according to the principles of the Helsinki Declaration; the research protocol was approved by the local Bioethics Committee SRCenter "Arktika" FEB RAS. Male volunteers of different ages were permanent residents of the North, all having similar living conditions and motor activity regimes. Special attention was given to a detailed explanation of the upcoming studies and the participant's informed written consent was obtained.

The results of the studies were processed using the Statistica 7.0 software package. The distribution of measured variables was tested for normality using the Shapiro-Wilk test; all quantita-

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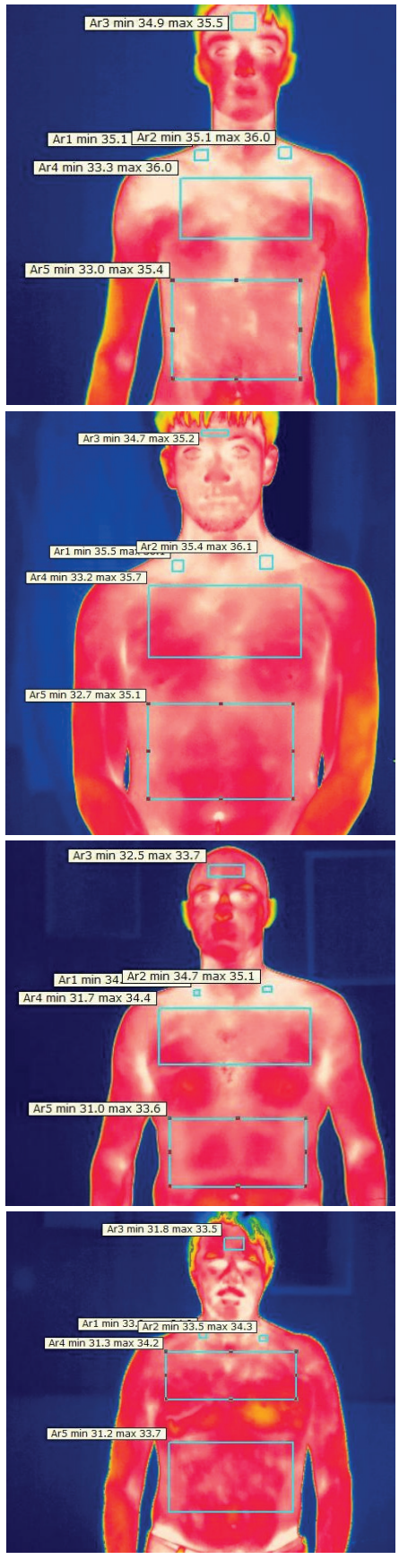
tive data were normally distributed. The processing results are presented as the mean value (M) and its error (±m). The statistical significance of the differences was determined using the Scheffe criterion. The critical significance level (p) in the work was assumed to be 0.05; 0.01; 0.001.

Results and Discussion. The Table shows thermal imaging picture characteristics of the examined male subjects at different ages. The variables of the weighted average temperatures and mean values, as well as all the located surveys (with the exception of C_5 zone – the abdominal surface), the maximum temperatures were seen in the young men (34.91 °C), and the minimum values in the elderly men (33.69 °C). The cumulative temperature drop, thus, amounted to 1.22 °C. The differences (the shift) between temperature extremes grow starting from the upper areas of the ventral part of the body and, conversely, come down in the vertical direction of the dorsal side of the body. So, if we compare the values throughout the groups of young men and the elderly, the temperature difference from the left subclavian area to abdominal area increases from 1% to 5%, while from the upper back to the lower back the dynamics declines (from 6% to 3%).

The highest temperatures among all body areas were recorded in subclavian areas. This was characteristic of all the surveyed groups, regardless of the subjective age. We found the asymmetry values for the left and right subclavian areas: for the mature men the shift was only 0.01 °C, for adolescents 0.03 °C, while for the elderly and young men it was 0.07 °C and 0.09 °C, respectively. The minimum values were seen in the lumbar area, and this was observed throughout the three age-specified groups – from adolescents to men of mature age. The exception was made by the elderly people since their minimum temperatures were found in the abdominal area.

The number of indistinguishable intergroup temperature values of different parts of the body was recorded when comparing: adolescents vs. young men (3 areas), adolescents vs. mature men (2 areas), and young men vs. mature men (1 area). For the rest comparisons, differences were found in all the tested areas of the skin surface. At the same time, when we studied the dynamics for each of the surveyed areas, for which linear (without exception for any group) inter-age differences were observed, the forehead area stood for the front of the body (a decrease in temperature by 0.78 °C (or 2%)) from adolescents to the elderly men), and the back temperature dynamics could be seen in the area under the neck (a decrease of 1.65 °C (5%)) and in the interscapular area of the back (1.48 °C (4%)).



Subjective thermal imaging pictures representing different surveyed groups: adolescent (1), young adult (2), mature man (3), old man (4).

By subjective cardiovascular analysis, we could see the average values of the cardiac output (CO, L/min) feature as follows: 5281.5 ± 90.5 ml for the adolescents, 5699.4 ± 67.2 ml for the early adults, 3715.9± 41.4 ml for the mature men, and 2636.5± 68.5 ml for the elderly men. Such a pronounced lessening in the average CO variables observed with the increasing age obviously occurred together with a growth in the average indices of total peripheral vascular resistance (TPVR, dyn^2 s cm^{-5}) exhibited throughout the groups: 1406.5±28.5, 1468.8± 22.3, 2341.1±31.8, and 3519.7±78.2 for the group of adolescents, early adults, mature men, and the elderly, respectively.

We explored the metabolic picture within the groups and obtained the following average numerical values. The oxygen consumption rate, QO_2 (mL/kg) was equal to 4.23±0.11, 4.28±0.06, 3.58±0.1, and 3.28±0.12 for the group of adolescents, early adults, mature men, and the elderly, respectively. The percentage of energy consumption to the proper level (%) was registered as 1905±60.2 kcal per day (108±2.85% of the norm), 2202±33.3 kcal (121±1.74%), 2067±52.3 kcal (112±3%), and 1787±59.9 (116±3.85%) for the group of adolescents, early adults, mature men, and the elderly, respectively. Finally, the oxygen utilization factor, O_2UF (mL/L) was 23.93±0.43, 36.2±0.2, 29.93±0.77, and 26.03±0.66 for the group of adolescents, early adults, mature men, and the elderly, respectively.

The temperatures of the examined skin areas, as well as their weighted average temperatures were also age-associated becoming lower with the increasing age, from the early adulthood to the extreme old age. Figure shows cases of thermograms by the subjects at different ages.

Our obtained data are comparable with the results of a study [20] which performed an experiment on time computer modeling of the temperature picture that featured the skin on the palm, foot, forearm and lower leg: the authors reported that an older person demonstrated lower temperatures than an average person at a younger age owing to slower basic metabolic rates, cardiac output per min, as well as body mass and body surface area which are characteristic of older ages.

Some authors reported in their recent survey [12] that the elderly (over ≥60

Characteristics of a thermal imaging portrait of males of different ages

	Adolescents (1)	Young men (2)	Mature men (3)	Elderly men (4)	1-2	2-3	3-4	1-3	2-4	1-4
C_1	35.37±0.07	35.59±0.09	35.29±0.05	35.10±0.08	p<0.05	p<0.01	p<0.05	p=0.35	p<0.001	p<0.01
C_2	35.34±0.07	35.68±0.12	35.30±0.05	35.03±0.07	p<0.05	p<0.01	p<0.01	p=0.63	p<0.001	p<0.01
C_3	34.77±0.08	35.14±0.13	34.44±0.07	33.99±0.11	p<0.05	p<0.001	p<0.001	p<0.01	p<0.001	p<0.001
C_4	34.52±0.08	34.61±0.14	34.11±0.08	33.57±0.12	p=0.57	p<0.01	p<0.001	p<0.001	p<0.001	p<0.001
C_5	34.26±0.09	33.95±0.23	33.60±0.10	32.67±0.15	p=0.20	p=0.18	p<0.001	p<0.001	p<0.001	p<0.001
C_6	35.00±0.08	35.39±0.11	34.26±0.09	33.35±0.16	p<0.01	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001
C_7	34.52±0.08	35.03±0.12	33.87±0.10	33.04±0.17	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001
C_8	33.81±0.11	33.89±0.18	33.48±0.11	32.74±0.17	p=0.70	p<0.05	p<0.001	p<0.05	p<0.001	p<0.001
Avrg temp.	34.70±0.07	34.91±0.11	34.29±0.09	33.69±0.12	p=0.11	p<0.001	p<0.001	p<0.05	p<0.001	p<0.001

Note. Average temperature, °C: C_1 – average temperature of the left subclavian region. C_2 average temperature of the right subclavian region. C_3 – average forehead temperature. C_4 – average breast temperature. C_5 – average abdominal temperature. C_6 – average upper back temperature. C_7 – average temperature in the area of the shoulder blades. C_8 – average lower back temperature

years old) exhibited, on average, 0.23 °C lower temperatures than young people (under <60 years old). In our study, the elderly subjects' upper body surface temperatures in weighted average units decreased by 0.6 °C as compared to mature men, by 1.22 °C compared to early adults, and by 1.01 °C in comparison with the examined adolescents.

In other research [9], significantly higher temperatures were read in the chest, forehead, upper back, and subclavian areas, which is associated with the temperature of internal organs that emit heat as a result of their normal metabolic processes, as well as with a low thickness of subcutaneous fat. As opposed to the above average variables, we received slightly higher forehead temperatures when measuring with an infrared thermometer: subjects under the age of 21 reported 34.43 °C, 41-50-year old subjects – 34.0 °C, and those aged 61-70 had 32.6 °C temperature [7]. Similarly, the Moscow research showed lower values of the weighted average temperatures in the back area (32.3±1.0 °C in adolescents, p<0.001, 31.7±0.12°C in mature men, p<0.001) and chest temperatures (32.2±2.1°C in adolescents, p<0.001) as compared to our results [2]. Our earlier studies also indicated significantly higher average skin surface temperatures as opposed to those in young men living in more favourable climatic and geographical conditions (Moscow, Shchepin (Poland) [4].

Symmetrical hyperthermia of the supraclavicular regions is normally characteristic of the optimum thermal imaging picture of the anterior surface of the chest [26]. The author reported 0.25 °C as maximum temperature of the symmetry [26]. In our study, the temperature difference between the left and right supraclavicular areas in all the examined groups was comparable to that shift (0.025 °C), but there was an increase in thermal asymmetry in the group of elderly men and young men up to 0.07 °C and 0.09 °C. A detailed analysis for better understanding of these processes requires further research.

Physiological thermoregulation encompasses all the mechanisms and processes used by the human body to keep warm. Obviously, physiological changes that develop in the human body with age can also involve a decrease in temperatures from adolescents to the elderly owing in particular to the total body fat growth with increasing age. It has been shown that the percentage of the body fat is the proportion of fat in body mass with low thermal conductivity (38% compared to muscles) and it helps to prevent heat loss. The reason is that a higher proportion of the body fat increases a person's ability to keep heat, which leads to lessening in the average skin temperature [18] since the thermal insulation property of adipose tissue is considered to be one of the most important factors affecting individual thermal patterns [13]. Our previous studies confirmed significantly higher (25.9 ± 0.5%) total fat amount in the elderly people than that of both the mature men (20.6± 0.3%, p <0.001) and the young adults (10.9± 0.2%, p<0.001) [5].

After E.B. Akimov and co-authors [1], some processes which cause differences in subjective temperature pictures can be classified. The first process implies the influence of local blood flow provided by both the density of skin capillaries (anatomical factor) and the tone of vasoconstrictor smooth muscles (factor of autonomic regulation in the vascular tone). It is also necessary to consider the metabolic activity of tissues and the entire body [1]. It is known that the cardiovascular system provides Blood Pressure maintenance and also contributes to thermoregulation as it distributes and dissipates heat throughout the body [20]. Under thermally neutral conditions, the body heat production and loss are equal, and the skin surface temperature is only controlled by the skin tissue blood flow rate [11]. The subcutaneous areas contain venous plexuses which strongly affect skin temperatures and heat transfer from the skin to the environment [3]. Such changes in skin surface temperatures occur primarily owing to changes in peripheral blood flow with the blood working as a conductor in heat exchange between the core of the body and its shell [21]. The level of blood perfusion and the tonic state of the surface vessels are among the main factors influencing the surface temperature distribution [3]. Some researchers also confirmed that a lower cardiac output and a reduced ability to redistribute blood from the visceral circulation lead to a slowdown in skin blood flow in older people [10].

Indeed, our results are in line with the above results since significantly higher CO indices were recorded in the group of young men, which was fully comparable with skin temperatures both in various areas and its weighted average,

while the minimum variables for both CO and temperatures were characteristic of the elderly men. It is also possible that a smaller cardiac output in the elderly may reduce their heat exchange [17]. At the same time, the narrowing of peripheral vessels because of the higher sympathetic activity is a stable physiological reaction that minimizes the heat flow from the core of the body into the environment. This peripheral reaction is of particular importance as it provides the first line of protection by minimizing convective heat loss. Our results also confirmed these conclusions as we indicated ageing as a factor accelerating the total peripheral vascular resistance: the smallest values of TPVR were characteristic of adolescents and young men with a significant growth in mature and elderly subjects. Apparently ageing is also a driving factor for lower average temperatures.

By analysis of subjective gas exchange and metabolic rates, we concluded that men at early adulthood demonstrated highest values of oxygen consumption and metabolic indicators (kcal per day) as opposed to examinees of different ages and to the standard values as well, which could be seen in significantly higher variables on their thermal pictures. As for the elderly men, they tended to be significantly lower in gas exchange, within the normative ranges though, with the lowest average temperatures in different areas of the body and the entire thermal picture.

The cutaneous blood flow is also connected with the autonomic nervous system, which regulates vasoconstriction and vasodilation of capillaries to maintain homeostasis [19]. The skin blood circulation is controlled by the adrenergic sympathetic nervous system. In particular, vasomotor sympathetic nerves in the distal extremities are only contractile nerve endings that operate the body temperature by constricting or dilating blood vessels to reduce or expand their lumen [11]. Our previous results showed the lessening in autonomic functions with the increasing age, which was associated with the lowered activity of the parasympathetic link in autonomic nervous system shifting the sympathetic-vagal balance in order to produce the state of relative sympathetic activity [6]. That can also be a driving factor for the lowered average values of the examined elderly men's thermal pictures. Since a lower skin temperature reduces the thermal gradient between the skin and the environment, and because of the rate of the body heat loss that depends on the magnitude of this gradient, the lower skin temperature effectively reduces the heat loss of the entire body and lessens the drop in the core temperature [8]. Such thermoregulation readjustments can be observed in the elderly men. These are accompanied by sufficient daily energy consumption and its percentage ratio to the proper level, which is within the normative range, with a simultaneous decrease in blood flow rate due to vasoconstriction mechanisms caused by the prevailing sympathetic link of the autonomic nervous system. As for younger adults, higher metabolic and resting oxygen consumption rates at high hemodynamic values allow them for maintaining a higher skin temperature and, apparently, show their metabolic adaptation [28].

Conclusion. Our conducted studies have set the optimum thermoregulation having its completion by the early adulthood as we observe the highest thermal variables shown by young men. With the increasing age, however, the average indices of the thermal imaging pictures prove to decline significantly. The growth in thermal asymmetry in groups of elderly men and young men up to 0.07 °C and 0.09 °C, respectively, requires further study and analysis. Interestingly, all the subjects exhibited higher temperatures in the examined body areas as compared to people living in more favorable climatic conditions owing, apparently, to the formation of region-specified characteristics of the Northerners' thermal features.

Thus, we can conclude that isolation adaptation is the type achieved by a decrease in the weighted average temperature with appropriate heat transfer, an elevated total body fat content, a lowered energy and gas exchange, though being within the standard ranges (oxygen consumption rate and oxygen utilization factor), owing to vasoconstriction of the circulatory system caused by activation of the sympathetic link of autonomic nervous system in the elderly men. In this case, age-associated isolation adaptation develops with the skin surface thermoregulation deficit which is aimed at optimizing the internal temperature of the body that is the core.

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THR RELATIONSHIP OF PARAMETERS OF THE PITUITARY-GONADAL AXIS AND DOPAMINE WITH METEOROLOGICAL FACTORS IN HEALTHY MEN LIVING IN THE SUBARCTIC

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Aim: to evaluate the influence of circannual dynamics of meteorological factors of the temperate continental climate on the levels of sex hormones and dopamine as well as antisperm antibodies in men living in subarctic environmental conditions.

Materials and methods. The concentrations of follicle stimulating hormone, luteinizing hormone, prolactin, progesterone, dopamine, cortisol, total and free testosterone, estradiol, sex hormone-binding globulin, dehydroepiandrosterone sulphate, antisperm antibodies were determined in the blood by the enzyme immunoassay on a quarterly basis (December, March, June, September) for one year in 20 healthy men of Arkhangelsk. The relationships between the hormonal data and the climatic data were assessed by using the Spearman correlation coefficient.

Results. Seasonal fluctuations in the levels of estradiol and antisperm antibodies are comparable to changes in the daylight hours, fluctuations in atmospheric pressure, temperature and relative humidity. Seasonal changes in luteinizing hormone levels are associated with the fluctuations in atmospheric air pressure. Daylight affect annual dopamine dynamics, which also correlated with atmospheric pressure and relative air humidity. Total and free testosterone levels in men are relatively constant throughout the year and do not appear to be influenced by the weather factors.

Conclusion. An increase in day length and air temperature is associated with an increase in estradiol and dopamine levels and a decrease in antisperm antibodies values. We believe that the seasonality of estradiol and antisperm antibodies is a daylight effect mediated by changes in the melatonin levels, just as dopamine seasonality is mediated by changes in vitamin D levels.

Keywords: sex hormones; circannual rhythm; dopamine; estradiol.

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Introduction. The change of the seasons of the year causes an adaptive restructuring of the body in the inhabitants of high latitudes. The physiological characteristics of the body allow most healthy people to adapt to the climate of the northern regions of the Russian Federation without noticeable disorders, and only a decrease in adaptive reserves can lead to various pathological conditions. At the same time, large-scale studies covering a number of populations in different climatic zones [5, 11, 16, 23] have shown a relationship between meteorological factors and the dynamics of sex hormones.

Several studies in recent years have received conflicting results in assessing the environmentally dependent rhythmicity of sex hormone secretion in men.

The contradictions can be explained by the different geographic location of each study, which affects the length of the seasons, temperature fluctuations, and day/night cycles. In Israel, the highest level of testosterone in men was observed in the summer-autumn season, when the weather is hot without rain, and the lowest - in the winter period with moderately cold and rainy weather [23]. In Italy, peak of testosterone levels in summer correlated with longer daylight duration and higher temperature, LH levels presented 2 peaks of secretion in autumn and spring, independently from environmental parameters, and FSH levels did not show any seasonal distribution [16]. In South Korea, on the contrary, testosterone showed a negative relationship with the length of daylight hours and air temperature, and its maximum levels in the serum of men were recorded in winter [11]. In a cross-sectional study of men living in northern Norway, the lowest testosterone levels were observed in the months with the highest temperatures and longest daylight hours [22]. At the same time, other authors considered cold as one of the stress factors that regulate sexual behavior and testosterone levels in men. The seasonality of testosterone levels in men living in the east of Turkey has been shown, with lower levels in winter [4]. In a longitudinal study of Norwegian men, the concentration of free testosterone during early winter depended on the study area: in Tromsø (69°4′ N) it reached a maximum, and in Oslo (60° N) it reached a minimum [20]. In men living in southern California, there was no seasonal variation in testosterone levels, no association between testosterone and mean air temperature, or testosterone and the hours of sunshine [28]. No seasonal rhythms of testosterone levels were found in 13 Belgian men [3]. Estradiol peaked in May in both a cross-sectional study of Norwegian men [21] and a longitudinal study of Finnish men [2].

Ultraviolet (UV) light is one of several environmental stimuli that can influence the circadian rhythm, playing an important regulatory role in reproduction [23]. Chronic UV light exposure led in male and female mice, to increased pituitary and gonadal hormone levels and to increased sexual responsiveness and attractiveness [27]. Arkhangelsk is located at 64°32′ N, where the difference in length of daylight between mid-summer and mid-winter is more than 18 hours, which allows you to test the hypothesis that seasonal fluctuations in daylight affect the level of sex hormones in the body of men living in high latitudes.

Thus, scientists in many countries recognize that seasonal variations in photoperiod or temperature can affect human reproductive biology. The prevailing part of the ongoing researches are focused on collecting biological material not from the same sample of subjects in dynamics (longitudinal studies), but from different people over a period equivalent to a year (cross-sectional study), for example, as in the works carried out in Israel [23] and Norway [21], which cannot give an objective picture of the true dynamics of the levels of sex hormones and dopamine, so it was preferable to use a longitudinal research of the same participants throughout the year.

Material and methods. The concentrations of follitropin (FSH), lutropin (LH), prolactin, progesterone, dopamine, cortisol, total and free testosterone, estradiol, dehydroepiandrosterone sulphate (DHEA-S), sex hormone-binding globulin, antisperm antibodies (ASAB) were determined in the blood by enzyme immunoassay on a quarterly basis (December, March, June, September) for one year in 20 healthy men from Arkhangelsk (mean age was 33.3±5.3 years). Blood samples were taken between 08:00 and 10:00. Indicators of the cardiovascular system, including heart rate (HR) diastolic (dBP) and systolic Blood Pressure (sBP), were analyzed. This study did not analyze the relationship between blood pressure and hormone levels and geomagnetic activity, since no geomagnetic storms were recorded during the study period according to the site https://www.spaceweatherlive.com: the magnetosphere was calm and no magnetic storms were recorded on the days of the survey of volunteers.

The candidates were recruited through social media platforms. Participants had to be aged 25-45 years and had no history of endocrine disorders. Ten of the men have children, and none of those surveyed had been treated for infertility. Subjects with any factor affecting the hypothalamic-pituitary-gonadal axis were excluded. The study was conducted in accordance with the ethical principles stated in Declaration of Helsinki of 1964 (revised in 2013) and was approved by the Ethics Committee of N. Laverov Federal Center for Integrated Arctic Research of the Ural Branch of the Russian Academy of Sciences (protocol No. 2 dated 04.11.2016, Arkhangelsk).

Climatic factors were assessed by 7 indicators, including length of daylight hours and monthly averages and actual data at 9 a.m. on temperature, humidity, atmospheric air pressure. Climatic data were obtained from the weather archive at https://rp5.ru/Archive_weather_in_Arkhangelsk.

Statistical processing was performed using STATISTICA v.10.0. Normality of the data was confirmed by a Shapiro-Wilk test. A nonparametric analysis of variance of Friedman's repeated measures was performed, followed by pairwise comparison using the Wilcoxon rank sum test using a Bonferroni correction, with p values less than 0.05 is considered significant. The relationships between the hormonal data and climatic data were determined by using Spearman's rank correlation test (ρ).

Results and Discussion. The men included in our study showed statistically significant changes in the levels of dopamine, LH, estradiol, and ASAB in different periods of the year. According to our results, no seasonal rhythms were found in the content of total and free testosterone. Similar results were demonstrated in a study of the seasonal fluctuations of certain hormones, including testosterone, in men living in the southwestern United States [11].

Despite the available literature data on the seasonal dynamics of blood pressure [17, 18], the examined men showed no changes in the levels of sBP, dBP, and heart rate (table).

Dopamine levels showed the largest individual seasonal fluctuations, the average difference between the maximum and minimum levels of the hormone was 64.4 ± 23.4%. 65% of men are characterized by the minimum levels of dopamine in the fall, and 60% - the maximum levels in the summer. The levels of estradiol and LH varied on average between the periods of maximum and minimum by 58 ± 18.7 and 45 ± 17.3%, respectively. In winter, the minimum levels of estradiol and LH were established in 45 and 47% of the examined persons, respectively. The maximum values of estradiol were recorded in 45% of the examined in the summer, and the maximum values of LH - in 45% of the persons in the spring.

LH showed a negative correlation with mean monthly atmospheric pressure (ρ=-0.27; p=0.015).

Estradiol showed a positive correlation with the average monthly air temperature (ρ=0.34; p=0.001), with the actual air temperature at the time of blood donation (ρ=0.34; p=0.001), with relative air humidity (ρ=0.23; p=0.04), with length of daylight (ρ=0.24; p=0.033); negative correlation with average monthly atmospheric pressure (ρ=-0.26; p=0.019), average monthly atmospheric air humidity (ρ=-0.25; p=0.023).

Dopamine showed a positive cor-

Levels of SBP, DBP, heart rate in men from Arkhangelsk depending on the photoperiod of the year (results are presented as a median and 10/90 percentiles)

Measure	March	June	September	December	p-level
sBP	127.0 (113.0; 140.5)	121.5 (115.0; 135.0)	122.0 (112.0; 155.5)	127.0 (103.0; 145.0)	p>0.05
dBP	81.0 (65.5; 93.5)	77.5 (70.5; 93.0)	75.0 (64.0; 100.5)	75.0 (69.0; 92.0)	p>0.05
heart rate	66.0 (58.5; 84.0)	66.0 (56.5; 80.5)	71.5 (56.5; 80.0)	71.0 (57.0; 88.0)	p>0.05

relation with length of daylight (ρ=0.28; p=0.012); negative correlation with average monthly atmospheric pressure (ρ=-0.40; p=0.0003), average monthly atmospheric air humidity (ρ=-0.23; p=0.039).

ASAB showed a positive correlation with the average monthly atmospheric air humidity (ρ=0.25; p=0.023); negative correlation with length of daylight (ρ=-0.28; p=0.011), average monthly air temperature (ρ=-0.24; p=0.029), actual air temperature at the time of blood donation (ρ=-0.31; p=0.006).

Seasonal fluctuations in sex hormone levels have been observed in several cross-sectional studies of men around the world [31, 32]. Others, however, did not show such circumannual changes [10, 28]. It is likely that the circannual seasonality of sex hormones in human can't be, evolutionarily, strictly required. In the present study, there was no seasonal rhythm of the levels of total and free testosterone, cortisol, DHEA-S, FSH and progesterone.

Monthly mean barometric pressure was the only climate parameter tested that appears to be contributing to seasonal fluctuations in LH levels. Correlation analysis showed that lower atmospheric pressure is associated with higher levels of LH, estradiol and dopamine, lower atmospheric humidity is associated with higher levels of estradiol and dopamine, and the longer the daylight hours – the higher the levels of estradiol and dopamine.

Atmospheric pressure probably affects the levels of the pituitary hormone LH not directly, by proxy, due to changes in the level of melatonin. Low barometric pressure is synonymous with low light levels. Low levels of natural light can cause our body to produce more melatonin. Thus, in men from northern Finland, the melatonin peak in May was associated with a significant increase in serum LH level [2].

In inhabitants of the Arctic territories, a short length of daylight in winter causes an increase in melatonin secretion [20]. Melatonin could act as a naturally occurring antiestrogen as demonstrated on *in vivo* models of animal mammary tumors [8] as well as *in vitro* human breast cancer cells [12]. This melatonin hypothesis may explain the lower level of estradiol in men in the darkest season in Arkhangelsk. This is confirmed by the fact that estradiol has the positive correlation with the length of daylight.

A decrease in the level of estradiol, which has cardioprotective effects, in the winter period correlates with an increase in the complications of cardiovascular diseases, often associated with an increase in blood pressure due to cold vasoconstriction [1]. An increase in blood pressure values during the winter period has been observed in many studies, but most often in elderly people or with chronic diseases, such as arterial hypertension or type 2 diabetes [13, 19, 26]. However, in our study of clinically healthy men, no seasonal dynamics and correlation with climatic factors were found in the values of sBP, dBP, and heart rate. While other studies have demonstrated a significant increase in sBP and dBP in winter compared to summer [17, 18]. These differences may be due to seasonal fluctuations in sunlight exposure. Recent studies show that ultraviolet A (UVA) and ultraviolet B (UVB) have been linearly and inversely associated with sBP. Due to the mobilization of reserve forms in the skin, ultraviolet radiation increases the availability of nitric oxide, the tonic production of which is associated with vasorelaxation and antiatherogenic and antiplatelet effects, thereby contributing to a decrease in blood pressure [6]. In addition, large observational studies have shown that low vitamin D levels are a risk factor for hypertension [7].

The absence of significant seasonal differences in blood pressure may be due to different approaches to the analysis of the seasonality of data, namely, 24-hour monitoring of sBP and dBP levels in work of Goyal A. et al. [17] and, on the contrary, a single measurement of blood pressure before blood sampling in our study.

At the same time, such an indicator of the activity of the sympathoadrenal system as dopamine showed significant seasonal changes and relationships with such climatic indicators as atmospheric pressure, humidity, and daylight hours, but not with air temperature.

The seasonality of the dynamics of dopamine levels with a maximum in summer has been established, which can be associated with both climatic factor (high intensity of ultraviolet radiation) and the connected increase in vitamin D synthesis [30]. Vitamin D modulates the hypothalamic-pituitary-adrenal system by regulating adrenaline, norepinephrine and dopamine production through vitamin D receptors in the adrenal cortex, and also increases expression of the tyrosine hydroxylase gene in adrenal medullary cells [25, 29, 33, 34]. Most vitamin D in the body is obtained by skin synthesis (80–100%), and the body's ability to synthesize vitamin D depends on the amount of sunlight that the skin receives [24]. If a person does not take a vitamin supplement, which was typical for the studied volunteers according to their personal data, then sun exposure is the most important source of vitamin D. In high northern latitudes (above 40°N), even with sufficient sun exposure, skin production vitamin D is low or absent in winter, which increases the need for food ration [9]. Because very few foods naturally contain vitamin D in amounts to meet this increased demand in winter, this leads to marked seasonal fluctuations in vitamin D levels and, as a result, to dynamic levels of biogenic amines.

Along with the suppression of reproductive function, short photoperiod is also reported to influence immune functions [35]. In wild animals, an increased level of melatonin in winter suppresses reproductive function, and stimulates immunity [15]. Apparently, elevated levels of melatonin in winter are associated with an increase in autoimmunity, which is demonstrated by significantly higher levels of ASAB in the studied men in winter compared to summer (p = 0.01).

Conclusion. Thus, while the effects of seasonal variations in length of daylight at high latitudes may be mitigated by the amount of artificial lighting in today's society, we were able to demonstrate significant seasonality in the levels of several hormones that affect human reproductive biology. Among the considered climatic factors, atmospheric pressure has the largest number of negative correlations with the levels of the studied hormones, which demonstrate a slight decrease in the autumn-winter period, characterized by increased atmospheric pressure and low air temperature. In general, the

weather conditions in the winter months in Russia require much more effort from the human body to maintain normal life than in the summer months. Such climatic factors of the study area as the length of daylight and air temperature are statistically interrelated, they cannot be considered in isolation from each other, since an increase in daylight hours corresponds to an increase in air temperature. An increase in day length and air temperature is associated with an increase in estradiol and dopamine levels and a decrease in ASAB values. We believe that the seasonality of estradiol and ASAB is a daylight effect mediated by changes in melatonin levels, just as dopamine seasonality is mediated by changes in vitamin D levels.

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SCIENTIFIC REVIEWS AND LECTURES

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MODERN CONCEPTS OF APOPTOSIS

The review is devoted to the generalization of modern knowledge about such a type of cell death as apoptosis. To date, it is known that apoptosis is not just a programmed cell death that ensures the elimination of old cells with minimal damage to surrounding tissues, but it is also a complex mechanism that can promote survival and proliferation, as well as induce and suppress the inflammatory process. Drugs that cause enhanced apoptosis are being actively studied for the treatment of oncological diseases. The mechanisms of cell death selection are still being studied, but it is already known that weak effects on the macroorganism promote the activation of apoptosis and autophagy, while stronger effects promote necrosis. Low levels of apoptosis contribute to the accumulation of damaged cells, aging, and genome instability. Reduced accumulation of senescent cells improves homeostasis and lifespan.

Keywords: apoptosis, apoptotic bodies, caspases.

The review is devoted to the generalization of modern knowledge about such a type of cell death as apoptosis. To date, it is known that apoptosis is not just a programmed cell death that ensures the elimination of old cells with minimal damage to surrounding tissues, but it is also a complex mechanism that can promote survival and proliferation, as well as induce and suppress the inflammatory process. Drugs that cause enhanced apoptosis are being actively studied for the treatment of oncological diseases. Another function of apoptotic bodies has been revealed - the delivery of nutrients to cells. The mechanisms of cell death selection are still being studied, but it is already known that weak effects on the macroorganism promote the activation of apoptosis and autophagy, while stronger effects promote necrosis.

Depending on the cell type, a decrease in temperature can lead to both stimulation and inhibition of the apoptosis process. For most cells, induction of apoptosis is obviously a typical response to cooling and subsequent return to physiological temperature. At the same time, blockade of opiate receptors during prolonged cold exposure (-4 C 4 hours / 7 days) reduces the percentage of apoptosis of lymphocytes.

It has been proven that exposure to heavy metals inhibits cell death through apoptosis and initiates the activation of necrosis. Although apoptosis is a defensive response to pathogen entry, some microorganisms, such as Staphylococcus aureus, can inhibit apoptosis, and some viruses have learned to model apoptosis in such a way as to support replication.

Various substances and preparations can exert their influence on the intensity of cell death. The ability of β-glucans to stimulate apoptotic pathways or proteins involved in apoptosis opens up a new area in cancer therapy. With unregulated apoptosis, the appearance of diseases associated with premature aging is likely. All molecular mechanisms of aging can regulate programmed cell death through apoptosis. Low levels of apoptosis promote accumulation of damaged cells, aging, and genomic instability, but in response to injury/damage, low levels of apoptosis prevent tissue destruction and promote cell survival, proliferation, damage repair, and regeneration. Reduced accumulation of senescent cells improves homeostasis and lifespan.

Introduction. Apoptosis is a genetically programmed cell death that occurs regularly to maintain a homeostatic balance between the rate of cell formation and cell death. Disruption of this balance can contribute to abnormal cell growth or proliferation, as well as cancer and autoimmune pathologies. It is believed that apoptosis is crucial in terms of embryo development throughout the growth of the organism, promoting tissue renewal, as well as getting rid of inflammatory cells [35,22]. Apoptosis is characterized by morphological changes in the cell structure, as well as a number of enzyme-dependent biochemical processes. As a result of apoptosis, cells are removed from the body with minimal damage to surrounding tissues [31,5].

The complexity of apoptosis has been the focus of a number of studies that have accumulated a vast amount of knowledge that has led not only to a better understanding of the fundamental process, but also to the creation of effective treatments for diseases. Drugs and therapeutic measures based on the current understanding of apoptosis have been used for a long time. Small molecule apoptosis inducers have been clinically used to kill abnormal cells and hence treat diseases such as cancer. Biological agents with improved apoptotic efficiency and selectivity, such as recombinant proteins and antibodies, are under active investigation. Apoptosis also produces membrane-bound vesicles resulting from the disassembly of apoptotic cells, now known as apoptotic bodies (ApoBDs). These small sealed sacs containing information as well as substances from dying cells were previously considered as a container for the disposal of a dead cell, until it was found that they were able to deliver useful materials to healthy recipient cells (for example, autoantigens) [37].

To detect signs of apoptosis in the study of cellular metabolism, there are several methods that are often used to detect DNA fragmentation as one of the most specific results of apoptosis. To date, three routine assays have been developed, differing in their principles for detecting DNA fragmentation. DNA ladder analysis reveals a characteristic "DNA ladder" pattern formed during internucleosomal DNA cleavage. The Nick-End Labeling Terminal Deoxynucleotide Labeling (TUNEL) assay detects DNA strand breaks using a terminal deoxynucleotidyl transferase that catalyzes the addition of modified deoxynucleotides to DNA strand breaks. The Comet assay can be used to detect nuclear decay resulting in single-/double-stranded DNA breaks [33].

The mechanisms that determine the choice of the path of cell death are not completely clear, but the stronger the impact, the stronger the response in

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the form of cell necrosis, a powerful inflammatory and immune response of the macroorganism. Weak effects cause an intensification of autophagy and cell apoptosis without obvious inflammatory and immune responses. The death of macroorganism cells (humans, animals), due to external or internal causes, causes an immune response to damage. At the same time, microbial effects are always dosed by the concentration and viability of the pathogen, its soluble products, and localization of the lesion [3].

The interplay between autophagy and apoptosis affects several pathologies, including multiple rheumatic diseases. Because mitochondria are important regulators in maintaining cartilage homeostasis, mitochondrial turnover through mitochondrial biogenesis and mitochondrial degradation may play an important role in the pathogenesis of osteoarthritis (OA). Scientists discuss the role of mitochondrial dysfunction in the pathogenesis of OA, identifying the peroxisome proliferator-activated receptor-gamma coactivator 1-alpha (PGC1α) as a potent regulator. Dysregulation of the balance between autophagy and apoptosis may be involved in the pathogenesis of rheumatoid arthritis, systemic lupus erythematosus, and Sjögren's syndrome. Indeed, it may regulate immune cell survival, peptide citrullination, self antigen presentation, and B and T cell maturation. Notably, several currently used disease-modifying antirheumatic drugs (DMARDs), including glucocorticoids, hydroxychloroquine, rapamycin, anti-TNFα, and Jak inhibitors, may act via autophagy/apoptosis pathways [25].

It is increasingly recognized that apoptosis is more than just cell elimination. It plays an important role in cellular communication with the microenvironment. These interactions with surrounding cells can have different and sometimes opposite results. Apoptotic cells can promote survival, proliferation, and inflammation, but can also prevent inflammation [32].

Thus, the modulation of programmed cell death has great therapeutic potential in a wide range of diseases, including infectious, neurodegenerative, autoinflammatory and metabolic diseases, and cancer. However, the manipulation of cell death and inflammation for therapeutic intervention is a delicate process, highly specific to the context of the disease of interest, making the selection of the appropriate target molecule critical [8, 12, 16].

1. Effect of cooling on apoptosis. Many works are devoted to the study of the mechanisms of cell death under various conditions. For example, the authors of [9] studied the effect of apoptosis on cell damage when the body temperature drops below 37°C. It was found that both proapoptotic and antiapoptotic processes are triggered during hypothermic exposure of cells. With a slight cooling, caspases take part in the processes of apoptosis, initiating the breakdown of anti-apoptotic proteins of the Bcl-2 family and proteolysis of the DNase inhibitor responsible for DNA fragmentation. It was found that RNA-binding protein (cold-inducible RNA-binding protein), which can inhibit the process of apoptosis caused by oxidative stress, is of great importance for the mechanisms of cold adaptation. Depending on the cell type, a decrease in temperature can lead to both stimulation and inhibition of the apoptosis process. For most cells, induction of apoptosis is obviously a typical response to cooling and subsequent return to physiological temperature. However, in some types of cell cultures, as well as in cells of cold-adapted animals, at a temperature below physiological, the content of proapoptotic factors does not increase, but, on the contrary, apoptosis is inhibited or the cell cycle stops in the “hibernation” (G0) phase. Studies have shown that apoptosis induced by hypothermia and subsequent warming of cells develops along an internal (mitochondrial) rather than external (receptor-dependent) pathway. The background for this is a higher content of IL-10 in the blood at low concentrations of IL-1. One of the causes of cell damage during hypothermic exposure may be oxidative stress, which is the result of an increase in the amount of hydroxyl radicals and other reactive oxygen species [9, 13].

Endogenous opioid peptides released during stress are involved in the regulation of lymphocyte apoptosis. It has been shown that blockade of opiate receptors during prolonged cold exposure (-4 C 4 hours / 7 days) reduces the percentage of lymphocyte apoptosis, while acute cold stress, regardless of the blockade of opiate receptors, enhances apoptosis of CD8 cells [13].

Stress is one of the factors contributing to the development and aggravating the course of diseases. According to the results of studies conducted on outbred rats, which were placed in a refrigerator, immunocompetent peripheral blood cells change their activity under the influence of cold exposure. Under cold stress in male and female mice, the absolute and relative content of T-regulatory lymphocytes in the peripheral blood decreased and the relative content of activated T-helpers increased, and the volume fraction of clear centers of lymphoid nodules in the spleen decreased. A number of differences were also revealed between males and females of the control groups: in the peripheral blood, males had a higher number of platelets and an absolute number of T-regulatory lymphocytes and a lower content of cytotoxic T-lymphocytes. [15]. It has been established that nonspecific immune responses provided by monocytes and neutrophils are reduced. However, on the 14th day there is an increase in their number, which may be due to short-term stimulation of leukocytopoiesis. The activity of cells of a specific immune response - lymphocytes - remains elevated in all groups of animals, increasing to the maximum on the 7th and 30th days of the experiment. It has also been shown that cold exposure is accompanied by a decrease in the number of platelets, which we consider as their appropriate response to the effect of cold. Animal hypothermia activates the processes of erythropoiesis and, accordingly, leads to increased hematocrit values. These changes in the cellular composition of blood cells are manifested in the characteristics of the reaction of erythrocytes, platelets and leukocytes during cold exposure to animals, are natural and are confirmed in this study [4]. The thymus is the central organ of immunogenesis, on the functioning of which the protective reactions of the body depend. Various stress factors can have an effect on the immune organs. One of these factors is exposure to low temperatures. It was found that as a result of cold stress on the 7th, 21st and 30th days, there is a redistribution of the volume and cellular composition, various structural and functional zones, indicating a decrease in the functional activity of the thymus, an increase in the death of lymphocytes by apoptosis, a decrease in mitotic activity and the accumulation of macrophages. Morphofunctional data of the 14th day of the experiment indicate the development of compensatory and adaptive changes in the thymus to cold exposure, manifested as activation of cell division in the cortical substance and the cortico-medullary zone [1].

While moderate exercise is good for the human body and its immune system, exhausting excess exercise in cold conditions can be detrimental. When studying the effect of high physical activity when exposed to low temperatures (100-kilometer ultramarathon at temperatures from -1 °C to +1 °C) on individual immunological, biochemical and hematological parameters. Under these conditions,

there is an increase in the number of immature and mature neutrophils, as well as monocytes, while the number of lymphocytes and eosinophils did not change. The level of IgG increased, but the content of IgA and IgM remained unchanged. The number of platelets increased, while erythrocytes, hematocrit and hemoglobin did not change. The levels of lactate dehydrogenase (LDH) and creatine kinase (CK) increased, but alanine aminotransferase (ALT) did not change. The greatest change was noted in the increase in the number of immature neutrophils (1019.2%) and CPK levels (1077.6%). Thus, it has been shown that running a 100-kilometer ultramarathon in cold conditions leads to changes in several immunological, biochemical and hematological parameters, which indicates a serious burden on the body associated with increased susceptibility to the development of infections [28]. We studied indicators characterizing the state of cellular components of adaptive and innate immunity in practically healthy test volunteers aged 27 to 34 years. In peripheral blood, the content of the absolute and relative number of lymphocytes with the phenotype CD3+, CD3+CD4+, CD3+CD8+, CD19+, CD3-CD16+CD56+, CD3+CD16+CD56+, CD3+CD25+, CD45RA+, CD4+CD45RA+, as well as monocytes and granulocytes expressing signal pattern-recognizing receptors of the Toll-like (TLR) family TLR2, TLR4, TLR6 on their membrane. It has been shown that a single exposure at -70 °C has a significant effect on the cellular factors of the human immune system. Under the influence of ultralow temperatures, multidirectional changes are observed in the adaptive and innate components of human immunity, which are a reflection of a complex adaptive process caused by a stress response to a short stay in an air cryosauna at ultralow temperature [7].

A variety of factors, including metals, can affect the intensity of the reaction of programmed cell death. On the example of the child population living under the combined influence of chemical technogenic and extreme climatic factors of the Far North, an imbalance in the immune status was established, which is expressed in the excessive expression of membrane (HLA-DR+, CD95+, TNFR) and intracellular (p53, bax) indicators with the formation of a program cell death along the path of necrosis (in contrast to the comparison group, which was exposed to only natural extreme factors), which characterizes the state of immunodeficiency and a high risk of viral infections and their complications [2]. Apoptosis under conditions of exposure to strontium has been studied. We examined the children's population consuming drinking water with a high content of strontium (Sr2+) (n = 49). Exposure to strontium in vitro was characterized by a significant decrease in the level of expression of regulatory factors of apoptosis of the membrane marker CD95 and intracellular transcription protein p53 by 1.56 times and 1.68 times, respectively. At the same time, there was a significant decrease of 4.68 times in the number of AnnV-FITC+-PI cells, as well as a statistically significant increase of 1.35 times in the percentage of AnnV-FITC+PI+ cells. In addition, in all samples, the number of AnnV-FITC+PI lymphocytes was below the physiological norm and control values, and the number of samples where the content of AnnV-FITC+PI+ lymphocytes exceeded the established standards and control values was 30.8%. Thus, it has been experimentally proven that strontium at a concentration corresponding to the MPC for water bodies inhibits cell death via apoptosis with a high degree of reliability, switching to cell death by necrosis according to the criterion of phosphatidylserine content, detected in the test with annexin V. The obtained data revealed the ability of strontium to have a significant impact on the regulation and maintenance of cellular homeostasis, influencing the intensity of the apoptosis process by shifting the balance towards the implementation of cell death through necrosis and reducing the expression of regulatory factors. The results of the study can be used to identify and substantiate marker indicators of immune response disorders in assessing the environmental impact of strontium on public health in a specific factor environment [6].

2. Infectious diseases and apoptosis. The effect of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) on the number of peripheral blood lymphocytes in most patients was studied. The results showed that the percentage of lymphocytes, CD4, and CD8+ T cells were reduced in patients with COVID-19 compared to the control group. In terms of clinical severity, lymphocyte counts, CD4+, CD8+ T cells, and NK cells were also reduced in severe cases compared to mild cases. The data also indicated an increase in mononuclear cell apoptosis in patients with COVID-19, which was more pronounced in severe clinical cases. The frequency of immune cells is a useful indicator for predicting the severity and prognosis of patients with COVID-19. These results could help explain the immunopathogenesis of SARS-CoV-2 and provide new biomarkers, therapeutic strategies, and vaccine candidates [21]. Although apoptosis is considered to be an innate cellular response to invading infectious pathogens, influenza A viruses have evolved to encode viral proteins that modulate host cellular apoptosis in such a way as to support efficient virus replication and propagation [19]. Recent trends in sepsis research indicate that the greatest mortality occurs during a prolonged immunosuppressive state, when patients die from secondary infections within weeks or months due to post-sepsis "immune paralysis". Alteration of immune cells caused by uncontrolled apoptosis is considered the main cause of significant immunosuppression. In particular, lymphocyte apoptosis increases the risk of secondary infections and adverse outcomes [3]. In most parvovirus infections, cell death enhances the spread of the virus and causes tissue damage, often resulting in disease. Cell cycle arrest also causes cytopathic effects in infected cells and is sometimes a prerequisite for apoptotic cell death. The mechanisms of cell death caused by parvovirus infections differ depending on the strain infecting the parvovirus and the cell lines involved. Apoptosis, however, is a common form of cell death caused by parvoviruses [17].

Well-studied intracellular bacterial pathogens such as Salmonella, Yersinia, and Listeria increase their chances of survival by disrupting programmed host cell death (PCD), an internal cellular response that kills cells under certain stressors. Although Staphylococcus aureus is considered a destructive extracellular organism, there is growing evidence that S. aureus is an experienced intracellular pathogen. WITH . aureus is able to enter, multiply, and persist in host cells to avoid bactericidal immune disorders (eg, opsonization and circulating antibodies), antibiotic treatment, and detection by surface receptors. Although staphylococcal toxins cause a wide range of biological consequences leading to apoptosis, they can either inhibit apoptosis to ensure the survival of infected host cells [28]. The molecular mechanism of bacterial effectors includes secreted proteins that bind to the signaling pathways of apoptotic cells and inhibit them [21, 27].

3. Effect of various substances on apoptosis. Various drugs also affect the intensity of apoptosis and the ability to proliferate. One study evaluated the effects of psychotropic drugs (lithium and valproic acid) in patients with bipolar disorder. According to the results of the study, T-lymphocytes of patients with bipolar disorder, especially those who re-

ceived lithium, have a reduced ability to proliferate compared to healthy people. In vitro studies have shown that valproic acid reduces the number of cell divisions and the percentage of proliferating cells regardless of health status, but mainly at very high doses, while lithium does not significantly affect the ability to proliferate T-lymphocytes of patients. The lymphocytes of patients with BD are also more prone to apoptosis compared to healthy people, which is associated with a high expression of the proapoptotic protein. Lithium in vitro protected the lymphocytes of patients from apoptosis in proportion to the dose used. Mood stabilizers used to prevent relapses of the disease have an anti-apoptotic effect on T-lymphocytes of patients with BD, but they are not able to improve their ability to proliferate [32].

The immunomodulatory effect of Nigella sativa (NS) fatty oil has been revealed. Studies have shown that unrefined, cold-pressed black cumin seed oil inhibits lymphocyte proliferation and induces lymphocyte apoptosis in a dose-dependent manner. In this study, we examined the immunomodulatory properties of essential oil obtained from NS seeds by hydrodistillation and its two main components: thymoquinone (TQ) and p-cymene. In summary, NS essential oil significantly inhibits CD4+ and CD8+ T-lymphocyte proliferation, induces cell death in a dose-dependent manner, and reduces the expression of CD28 and CD25 antigens required for lymphocyte activation. TQ inhibited T-lymphocyte proliferation and induced cell death, especially at high concentrations. Meanwhile, p-cymene did not affect the proliferation of lymphocytes. However, its high concentration induced cell necrosis. Thus, Nigella sativa essential oil has potent immunomodulatory properties that are at least partially related to the TQ component [23].

One of the substances that have an impact on the cellular immune response are β-glucans. They are polysaccharides usually obtained from the cell wall of bacteria, fungi, yeast and the aleurone layer of cereals. β-glucans are polymers based on β-1,3 glucose in a linear structure, but differ in the length of their main branches, bonds and branching patterns, resulting in high and low molecular weight β-glucans. They are well known cellular response modifiers with immunomodulatory, nutraceutical and health benefits, including antitumor and proapoptotic properties. β-glucan extracts have shown positive effects in controlling tumor cell proliferation and activating the immune system. The immunomodulatory effect of β-glucans enhances the body's antitumor defense against cancer. In line with the above, many studies have shown that treatment with β-glucan leads to the induction of apoptotic death of cancer cells. The ability of β-glucans to stimulate apoptotic pathways or proteins involved in apoptosis opens up a new area in cancer therapy. β-glucan may be a potential therapeutic agent for cancer treatment [24]. It has been proven that propolis and its components exhibit proapoptotic activity, inducing both mechanisms of cell death [26].

Extracellular calcium plays an important role in the processes of programmed cell death. It was found that the incubation of lymphocytes in Hank's solution, which does not contain calcium ions, and in Hank's solution with excess calcium (13 mmol/l) induces significant changes in metabolic processes and the state of the surface of lymphocytes compared with those for cells in the presence of calcium at a normal concentration (1 .3 mmol/l). These disorders initiate the process of lymphocyte death under conditions of calcium deficiency and excess, predominantly by the mechanism of apoptosis (mitochondrial pathway) and, to a lesser extent, necrosis [11].

Autophagy and apoptosis are two fundamental pathophysiological mechanisms of cell fate regulation. However, the signaling pathways of these processes are largely interrelated. The interaction of autophagy and apoptosis involves signal transduction pathways that are highly dependent on Ca 2+ . Indeed, the functions of Ca 2+ as a second messenger are critical to the coordination of fundamental physiological functions, including cell survival and growth, neuronal development, and/or maintenance of cellular functions. Coordination between Ca 2+ proteins/pumps/channels and Ca 2+ storage in various organelles is critical to maintaining cytosolic Ca 2+ levels that maintain the spatial resolution required for cellular homeostasis. Ca 2+ homeostasis is regulated by store-operated Ca 2+ entry (SOCE), which is activated by depletion of Ca 2+ from internal ER stores. Ca 2+ has been shown to control opposite functions, such as autophagy, which promote cell survival; on the other hand, Ca 2+ also regulates programmed cell death processes, including apoptosis. It has recently become apparent that a complex network of lipid-lipid and lipid-protein interactions contributes to the activation of various signaling pathways that regulate cellular homeostasis. Thus, specific plasma membrane microdomains called lipid rafts regulate various signaling pathways involved in specific cellular programs, including proliferation, apoptosis, differentiation, stress response, and autophagy, thus determining cell fate. However, lipid rafts are present not only in the plasma membrane, but also in the membranes of intracellular organelles, including the ER, the Golgi apparatus, endosomes, and lysosomes. These sites can catalyze key reactions that have a significant impact on the regulation of intracellular transport and sorting, cholesterol homeostasis, and cell fate. Mitochondrial associated membranes (MAMs) have been classified as critical "hubs" in the regulation of apoptosis, autophagy, and tumor growth. The recent discovery of lipid rafts as physical and functional platforms in MAMs has contributed to the elucidation of the mechanisms underlying the early stages of the autophagic process. In particular, it appears that microdomain proteins like ER lipid rafts, i.e. proteins 1 (ERLIN1) and 2 (ERLIN2) associated with the ER lipid raft, can regulate cell survival and death. The putative role of ERLIN in the degradation of the calcium channel (inositol-1,4,5-triphosphate receptor) may explain their role in the mechanism of autophagy, having a significant impact on the pathogenesis of several human diseases.

4. Apoptosis and aging. Researchers cannot yet predict how long a person can live. Life expectancy has risen steadily over the past century, but the quality of life may not always have gone hand in hand with it. Future generations will face the challenges of increasing life expectancy along with the emergence of new age-related diseases. A better understanding of the aging process is critical to improving, if not preventing, these predicted new diseases of old age. One of the mechanisms responsible for healthy aging is the efficient maintenance of physiological, biochemical and immunological functions. To do this, the body needs to create new cells to replace old ones and cause the old and damaged cells to disappear. Apoptosis is involved in all these processes. However, if apoptosis is not regulated, diseases associated with premature aging are likely to occur. All molecular mechanisms of aging can regulate programmed cell death through apoptosis. In mitotic and post-mitotic cells, low levels of apoptosis promote accumulation of damaged cells, aging, and genomic instability, but in response to injury/damage, low levels of apoptosis prevent tissue destruction and promote cell survival, proliferation, damage repair, and regeneration. In mitotic and post-mitotic cells, physiological levels of apoptosis have several advantages

for the aging process. In fact, eliminating dysfunctional cells and reducing the accumulation of senescent cells improves homeostasis and lifespan. A moderate increase in apoptosis in mitotic highly proliferative cells improves the rate of cell renewal, while in postmitotic cells, apoptosis promotes cell loss and tissue dysfunction [18,20].

The cellular theory of aging states that human aging is the result of cellular aging, in which an increasing proportion of cells reach senescence. Aging is an irreversible growth arrest that occurs in response to damaging stimuli such as DNA damage, telomere shortening, telomere dysfunction, and oncogenic stress, resulting in the suppression of potentially dysfunctional, transformed, or senescent cells. Cellular senescence is characterized by irreversible arrest of the cell cycle, flattened and enlarged morphology, resistance to apoptosis, changes in gene expression and chromatin structure, expression of aging-associated β-galactosidase (SA-β-gal), and the acquisition of an aging-associated secretory phenotype [16, 18].

Conclusion. So, the programmed cell death, or apoptosis, is the most important process of maintaining the homeostatic constancy of the body. Therefore, apoptosis is believed to be critical in terms of embryonic development throughout the growth of the organism, promoting tissue renewal and disposal. from inflammatory cells. Apoptosis begins with the triggering of various intra- and intercellular signals and stimulations that involve a number of extrinsic or intrinsic pathways of apoptosis. Understanding these complex pathways offers new approaches to the clinical management of deadly human diseases.

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THE ROLE OF THE BDNF (RS6265) AND CNTF (RS1800169) GENE POLYMORPHIC VARIANTS IN THE DEVELOPMENT AND PROGRESSION OF NEUROLOGICAL DISORDERS

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In recent decades the contribution of polymorphic variants of genes encoding neuroinflammation and neuroprotection proteins in pathology of the nervous system has been actively studied. Numerous studies have demonstrated the most important structural and functional role of single nucleotide variants in the BDNF and CNTF genes in the brain. This review provides evidence of the association of polymorphic loci of the BDNF and CNTF genes with a number of neurological disorders. It was found that an increased risk of developing depressive disorders, Alzheimer's and Parkinson's diseases are characteristic of the BDNF rs6265 polymorphism carriers. Moreover, the above mutation affects the length of stay in rehabilitation and the duration of remission in patients with substance dependence. A number of authors provide contradictory information about the relationship of the BDNF rs6265 genetic variant with changes in neurotrophin levels in schizophrenia. The BDNF rs6265 A/A genotype carriers in Caucasian and Asian populations showed an increased risk of the disease. The CNTF rs1800169 genetic variant does not affect the risk of schizophrenia in general but may play a more prominent role in its clinical manifestations together with other CNTF gene mutations. Some studies have shown an association of the rs1800169 polymorphism in the CNTF gene with schizophrenia spectrum disorders. A number of studies have confirmed a negative association between the rs1800169 polymorphism in the CNTF gene and Alzheimer's disease. Further studies are needed to clarify the role of the BDNF and CNTF genetic variants in pathology of the nervous system and to find an effective treatment.

Keywords: brain-derived neurotrophic factor, ciliary neurotrophic factor, the BDNF gene, the CNTF gene, SNP markers, genetic predisposition, neurological disorders.

Introduction. Nowadays neurological disorders remain one of the leading causes of disability and premature death and contribute significantly to the global burden of disease [5]. The development of molecular genetic methods has led to an increasing number of association studies of polymorphic variants of genes encoding proteins of neuroinflammation and neuroprotection which include brain-derived neurotrophic factor and ciliary neurotrophic factor.

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family and expressed in the central and peripheral nervous system, vascular endothelium, leukocytes, platelets, megakaryocytes, and muscles. Its functions are carried out through signaling pathways activated by TrkB (tropomyosin receptor kinase B) and p75NTR (p75 neurotrophin receptor) receptors. BDNF is found to be essential for the proper functioning of the nervous system and implicated in the pathogenesis of neural diseases. This neurotrophin modulates the work of dopaminergic neurons, myelination of nerve fibers, stimulates axon growth, synaptogenesis, and participates in the regulation of neuroplastic processes (long-term potentialization and depression of synaptic transmission). In the developing nervous system it promotes synaptic pruning, and also performs a neuroprotective function when being exposed to damaging factors. In addition, BDNF participates in the processes of oncogenesis, angiogenesis, gluconeogenesis and others [3, 25].

Ciliary neurotrophic factor (CNTF) belongs to the cytokines of the interleukin-6 family and performs a number of functions by binding to the high-affinity receptor complex CNTFRα/GP130/LIFRβ (CNTF receptor α/glycoprotein 130 receptor β/leukemia inhibitor factor receptor β). This cytokine supports self-renewal and differentiation of neural stem cells, stimulates neurite growth in sensory neurons, inhibits apoptosis of spinal ganglion neurons, accelerates regeneration of motor neurons and skeletal muscles, and participates in mechanisms of cardioprotection. CNTF plays an important role in the pathogenesis and treatment of psychiatric disorders, affective behavior, neurodegenerative diseases, spinal cord injury, and retinal degeneration. In addition, it is essential for energy metabolism: it activates in the hypothalamus pathogenetic pathways related to leptin metabolism. CNTF expression has been confirmed in astrocytes, Schwann cells, retinal pigment epithelium, and Müller cells [12].

The aim of this review is to update, systematize and summarize the data of scientific publications on the role of single nucleotide variants of the BDNF and CNTF genes in the pathogenesis of neurological disorders. To achieve this goal we have searched for English and Russian articles (up to 2023 inclusive) in Pubmed, Google Scholar, and eLibrary databases and have used the descriptive method.

The rs6265 polymorphism of the BDNF gene. The human BDNF gene is located on the short arm of chromosome 11 (locus 11p14.1) and includes

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12 exons. Recently more than 25000 of its polymorphic variants have been described, the most studied among them is the rs6265 polymorphism, associated with an increased risk of nervous system pathologies [4]. Transition of G to A in position 196 leads to the replacement of valine by methionine in codon 66. As a result of the mutation in the pro-domain, transport and secretion of neurotrophin are impaired [33]. Correlations between the rs6265 polymorphism of the BDNF gene, morphological changes in the brain and the results of cognitive tests have been found. Carriers of the BDNF rs6265 minor allele showed a decrease in the thickness of the hippocampal cortex, frontal lobe, caudate nucleus and amygdala, as well as in the volume of gray matter of the prefrontal cortex and hippocampus. This polymorphism is associated with decreased synaptic plasticity (mainly long-term potentialization and depression), impaired regulation of the hypothalamic-pituitary-adrenal system, worse working, episodic, spatial memory, executive functions, information processing speed, motor learning ability, and task performance [4, 31]. The frequency of the BDNF rs6265 A allele in the Russian population (14.5%) correlates with the distribution of similar data in European (19.4%) and Latin American (14.8%) countries [20].

M. Svetel et al. showed that the presence of the minor allele of the rs6265 locus of the BDNF gene did not affect the clinical characteristics of patients with Parkinson's disease (sex, family history, age of onset, severity of the disease course, cognitive function, severity of motor symptoms) [38], but contributed to an increase in the number of non-motor disorders [6]. Carriers of the BDNF rs6265 minor allele had worse scores on the United Parkinson's Disease Rating Scale (UPDRS) during levodopa monotherapy compared to those with the G/G genotype carriers [7]. Moreover, patients expressing the A allele of the rs6265 locus of the BDNF gene have a higher risk of developing levodopa-induced dyskinesia in early treatment compared to G/G genotype carriers [8]. Identification of genetic variants, such as the rs6265 polymorphism, allows predicting the nature of the disease and selecting effective treatment methods.

H. Ji et al. found no statistically significant relationship between the rs6265 polymorphism of the BDNF gene and Alzheimer's disease, but its prevalence is slightly varied in different populations [14]. It has been shown that the risk of developing the disease is higher in female with A allele of this gene, and predominantly in Caucasian women [27]. Patients with the BDNF rs6265 minor allele and dominantly inherited form of the disease associated with mutations of the APP, PSEN1, PSEN2 genes showed faster memory decline (4x), decrease in hippocampal volume (16x) and increase in Tau and pTau181 concentration in the liquor (6x) compared to the BDNF rs6265 G/G genotype carriers, but the rate of β-amyloid accumulation in the cerebral cortex or β-amyloid 42 in the cerebrospinal fluid did not change [28]. The BDNF rs6265 gene A allele carriers at the preclinical stage of the disease have a high risk of the onset and progression of mild cognitive impairment or dementia within 12 years' period [29]. The BDNF rs6265 polymorphism should be considered as a potential factor influencing treatment and prevention outcomes of the disease. BDNF may be a female-specific risk gene for the development of Alzheimer's disease.

Currently, there are conflicting data regarding the relationship between the rs6265 polymorphic variant of the BDNF gene and changes in neurotrophin concentrations in schizophrenia [42]. No significant effect of genotypes of the rs65265 locus of the BDNF gene on serum protein levels was observed, but several studies reported a significant decrease [18] or conversely an increase [36] in its concentration in patients compared to the control group. It was found that carrying the A/A genotype of the BDNF rs6265 gene increases the risk of developing schizophrenia in European and Asian populations [15].

The probability of depression is significantly increased in individuals with the rs6265 polymorphism of the BDNF gene [30]. In addition, a modulating effect of such environmental factors as chronic stress, childhood abuse, and brain trauma on the association of the rs6265 locus of the BDNF gene with the risk of developing depressive disorder has been noted [2]. Lower neurotrophin concentrations were found in postmortem brain tissue samples from suicide victims, carriers of the BDNF rs6265 A allele, compared to healthy individuals [43]. In patients with psychoactive substance dependence, the rs6265 polymorphism of the BDNF gene through the processes of neuroplasticity can influence the duration of being in rehabilitation and the duration of remission [16, 35]. For example, in pharmacogenetic placebo-controlled studies of the efficacy of pregabalin for the treatment of alcohol dependence, it was found that the BDNF rs6265 A allele carriers dropped out of the treatment program earlier [16]. The BDNF rs6265 G/G genotype individuals who use only psychostimulants or with combined dependence on psychostimulants and cannabioids are able to stay in the rehabilitation program for a long time and have the longest remissions [35]. Besides, the rs6265 functional polymorphism of the BDNF gene has been shown to be a protective marker for family predisposition to addictive disorders [17].

While studying the rs6265 polymorphism of the BDNF gene, the G/A and A/A genotypes associated with a decrease in neuropsychic stability and professional reliability prevailed in the group of subjects systematically exposed to noise, vibration, barometric pressure variations, and other extreme factors [19]. The lowest risk of mental performance deterioration and the longest service record in the conditions of the Arctic zone are characteristic of military personnel with the BDNF rs6265 G/G genotype [23, 24]. Another study showed that 20% of flight personnel had a heterozygous G/A variant (rs6265) of the BDNF gene associated with low levels of neurotrophin and increased susceptibility to the development of psychoemotional stress [21]. This molecular genetic marker is necessary for the system of monitoring the mental health of persons in extreme professions and improving their professional training programs.

The rs1800169 polymorphism of the CNTF gene. The human CNTF gene is localized on the long arm of chromosome 11 in the 11q12.1 region, contains two exons and more than 2000 single nucleotide variants. Some studies have shown that the rs1800169 polymorphism of the CNTF gene is associated with an increased risk of neurologic diseases [11, 37]. Transition of G to A in position 6 of the acceptor splice site leads to CCAG insertion in the second exon, a shift of the reading frame and premature appearance of the stop codon in position 63. As a result of this mutation, the aberrant mRNA encodes a truncated protein consisting of 62 amino acids, which degrades rapidly after translation [39]. The frequency of occurrence of the CNTF rs1800169 minor allele in the Russian population is 18.9% and correlates with the data obtained when studying the population of European (14.5%), Asian (13.2%), and Latin American (11.5%) countries [20].

A few studies demonstrated a positive association of the carrier frequency of the CNTF rs1800169 polymorphism with the prevalence of schizophrenia spectrum disorders [40, 41]. R.C. Pierce

and A.A. Bari suggest that CNTF may enhance psychostimulant-induced behavioral sensitization that can form aspects of paranoid psychosis and drug craving [34]. The CNTF protein dysfunction in the developing brain is probably involved in delusional, schizoaffective, and bipolar disorders rather than in the development of schizophrenia [26].

P.-Y. Lin and G. Tsai did not confirm the association of this mutation with the risk of developing schizophrenia in a group of patients with a family history of psychiatric disorders; however, a significant association was found in a group of patients with no history of psychiatric disorders in their pedigrees compared to the control group [26]. A study by J. Nishiyama et al. did not provide evidence for the association of the CNTF rs1800169 polymorphic variant with the disorder as well as personality traits in the Japanese population [32]. J. Benkovits et al. also found no association of the CNTF rs1800169 polymorphism with the disorder in Hungarian patients compared to controls [1]. In a placebo-controlled study it was shown that the therapeutic effect of iloperidone is enhanced in patients with the CNTF rs1800169 G/G genotype [22]. The results suggest that the rs1800169 polymorphism of the CNTF gene does not affect the risk of schizophrenia in general, but it may play a more prominent role in its clinical manifestations. In addition, other mutations of this gene may also be involved in the pathogenesis of the disease.

R. Giess et al. found the CNTF rs1800169 A/A genotype in 2.4% of 288 individuals with multiple sclerosis and noted that carriers of this genotype are characterized by early onset of the disease with predominant motor disorders [10]. V. Hoffmann et al. found no significant correlation between the CNTF rs1800169 polymorphism and the age of patients at the onset of the disease, its course and severity [13]. This mutation is not a risk factor for the development of multiple sclerosis. The need in the CNTF protein for myelogenesis or cell survival can be replaced by excessive functional activity of other neurotrophic factors.

A negative association between the rs1800169 polymorphism of the CNTF gene and Alzheimer's disease has been confirmed in a number of studies [9, 11]. E. Grunblatt et al. reported that the disease developed in 17% of patients with mutant genotypes (A/A or G/A) and 25.2% of patients with normal genotype (G/G) of the rs1800169 locus of the CNTF gene. The presence of the CNTF rs1800169 G/G genotype presumably increases the risk of its occurrence [11].

According to N.N. Strambovskaya, patients with chronic cerebral ischemia have an increased frequency of the CNTF rs1800169 minor allele depending on the severity of the disease. The CNTF rs1800169 G/A genotype prevails in patients with chronic cerebrovascular insufficiency and increases the risk of its development 2.3 times. Weakening of the trophic action of CNTF probably leads to premature degeneration of nervous tissue, impaired integrity of the blood-brain barrier, and early onset of chronic vascular disease [37].

Conclusion. Lately a significant number of publications have been devoted to the identification of associations of polymorphic variants of the BDNF (rs6265) and CNTF (rs1800169) genes with the risk of development and features of the course of neurological disorders, as well as the effectiveness of drug therapy, with some data being contradictory. Sex, age, ethnicity, epigenetic processes and the presence of comorbid pathology affect the interpretation of the results. The issues of epistatic gene interaction require more detailed consideration. The problem of correlation between the contribution of genetic and environmental determinants remains unresolved and is still relevant. Further studies in this direction will help to clarify the role of single nucleotide variants of the BDNF and CNTF genes in various aspects of pathogenesis of nervous diseases and analyze genotypic and phenotypic heterogeneity in populations.

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POINT OF VIEW

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BACKGROUND FOR STUDYING CIRCULATORY SYSTEM DISEASES USING BIOIMPEDANCE ANALYSIS IN RESIDENTS OF YAKUTIA

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The scientific review highlights the results of studies of the features of the circulatory system diseases (CSD) and human body parameters among the inhabitants of Yakutia and confirms the existence of a research backlog for studying the association of CSD regional characteristics with the body composition, determined by bioimpedance analysis. The relevance of continuing to study the regional characteristics of CSD is confirmed by the data of Rosstat, indicating the predominance of working-age population CSD mortality in Yakutia over the national indicator.

Keywords. North, Arctic, Siberia, metabolic syndrome.

Introduction. Circulatory system diseases (CSD) occupy a dominant position in the structure of the total deaths in Russia. In addition, in recent decades, their “rejuvenation” has been noted [14]. CSD are also the leading cause of death worldwide [50]. CSD mortality rates in Russia have significant regional variability [27].

The results of Russian and foreign studies demonstrate a strong connection between CSD risk factors and indicators of anthropometry and body composition [5,23,40]. A bioimpedance analysis has become a simple and useful diagnostic tool for assessing the human body composition. Bioimpedance analysis uses the difference in electrical resistance between the fat and lean components to assess the development of lean (non-fat) mass, total fat and visceral body fat [46].

In Yakutia, the regional features of CSD have been studied for quite a long time [37]. Medical anthropometric research in Yakutia also has a long history [1]. However, bioimpedance analysis for assessing the Yakutia inhabitants’ body composition has been used relatively recently [11]. There are no scientific works exploring in the Yakutia population links between the CSD features and assessed by bioimpedancemetry the body composition.

Therefore, the purpose of this review article is to substantiate the existence of a research backlog for studying the association of CSD features with determined by bioimpedance analysis the body composition among the Yakutia inhabitants. It was decided to highlight the Yakutia CSD mortality in comparison with the all-Russian mortality for confirming the relevance of continuing regional studies of CSD and to identify existing studies on the CSD features and anthropometric parameters among the Yakutia inhabitants.

Materials and methods. For a comparative analysis of the situation related to the CSD in Yakutia and in the Russian Federation the latest open data from the Federal State Statistics Service (Rosstat) [32] were used, access to which is in the state information resource "EMISS" [9]. Information about studies on the features of CSD and anthropometric parameters of the Yakutia inhabitants was extracted from the eLIBRARY.RU electronic library, a Russian information and analytical portal in the field of science, technology, medicine, and education, containing scientific publications [35].

Results and discussion. Rosstat data is presented in the compiled by the authors of this article Table 1. The comparison shows that in Yakutia the CSD mortality including mortality from ischemic heart diseases, heart attack, cerebrovascular diseases, and acute cerebrovascular accident is lower than the national average. However, in such a seemingly more favorable condition, the CSD mortality of the working-age population in Yakutia prevails over the all-Russian similar indicator. The most contribution in the mortality both in Yakutia and in the all-Russia is made by ischemic heart diseases, which is also confirmed by third-party studies [3].

Yakutia has a heterogeneous ethnic structure of the population. According to the 2010 census, representatives of more than 120 nationalities live in Yakutia. The most numerous in the total population of the Republic are Yakuts - 48.7% and Russians - 36.9%. The share of Evenks, Evens, Dolgans, Yukaghirs and Chukchis is 4.2% [20].

A large number of medical scientific works devoted to the Yakutia inhabitants including the study of various aspects of CSD as well as anthropometric studies are aimed at identifying ethnic differences. Most often, patients are grouped into "indigenous people" (or "indigenous peoples, residents, ethnic groups") and "non-indigenous people".

The indigenous peoples of Yakutia are Yakuts, Evenks, Evens, Dolgans, Yukaghirs and Chukchis. There is no unified definition of the term “indigenous peoples”. The UN highlights the fundamental criterion of a person's belonging to indigenous peoples - a person's own awareness of himself as a representative of an indigenous people. Indigenous people have a historical hereditary connection with a certain territory and a strong connection with their lands; have their own languages, traditions, beliefs and knowledge systems [47].

Studies of CSD among the Yakutia inhabitants, which were carried out during the last decade of the last century and the first decade of this century, found that atherosclerosis is less common among the indigenous population [37]. Among patients with ischemic heart diseases, non-indigenous residents of Yakutia compared with indigenous (Yakuts) were more often observed atherogenic changes in the lipid profile, increased BMI, central obesity, and arterial hypertension. But regardless of ethnicity arterial hypertension and central obesity indicated the risk of developing ischemic heart diseases [22, 28]. The differences were that an increased level of cholesterol among the Yakuts and an increased level of triglycerides and smoking among non-indigenous people were additional risk fac-

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tors for ischemic heart diseases [25]. In a comparative study that included a 40-year follow-up (1963-2004) it was found an increase in the prevalence of coronary artery atherosclerosis for both indigenous and non-indigenous populations [2], and clinical observations showed an equal prevalence of heart attack among them [36].

As a result of CSD studies conducted in the north of Yakutia - in the Arctic zone, it was found that among the indigenous peoples of the northern regions of Yakutia arterial hypertension and abdominal obesity are significantly widespread and related to each other [29]. Also the relationships between arterial hypertension and the atherogenic fraction high cholesterol [4] and between the level of systolic blood pressure and BMI [21] were identified.

A comparative study conducted among the indigenous inhabitants of the Arctic and southern regions of Yakutia, selected in a one-stage population study by random sampling, showed that the atherogenic coefficient calculated from a blood test exceeded the normal level in the residents all regions without statistically significant differences. Elevated levels of total cholesterol were less common in the northern regions indigenous inhabitants than in the inhabitants of the southern regions. It was noted that in a direction from the north to the south the indigenous people have an increase in blood triglycerides, cholesterol, and atherogenic lipid fractions [30].

In studies conducted in other northern regions of Russia were found that non-indigenous residents of the Yamalo-Nenets Autonomous Okrug are characterized by a higher prevalence and severity of ischemic heart diseases and arterial hypertension compared with residents of the south of the Tyumen region [12]. Residents of the temperate climate zone of the Tyumen region were less likely to have heart attack and chronic heart failure compared to residents of the Yamalo-Nenets Autonomous Okrug, who had heart attack and chronic heart failure at a younger age [31].

In general, taking into account the data of epidemiology, etiology, clinical picture, diagnosis and treatment of complications, the prevalence of CSD is increasing among the population of all northern territories around the world [45]. Researchers come to the conclusion that living in the North is associated with a significant stress of adaptation, which leads to a restructuring of metabolism, primarily carbohydrate and lipid metabolism, and contributes to the development of the metabolic syndrome that affects the occurrence, course, and outcome of CSD [24].

According to clinical guidelines developed on behalf of the Ministry of Health of Russia and approved by the Russian Medical Society for Arterial Hypertension and the specialized commission on cardiology the characteristics of the metabolic syndrome are as follows: an increase in the mass of visceral fat, a decrease in the sensitivity of peripheral tissues to insulin and hyperinsulinemia, which cause the disorders of carbohydrate, lipid, purine

CSD mortality in the Russian Federation and the Sakha (Yakutia) Republic

Indicator	Methodology for calculating the indicator	Year of indicator *			Web link:
			RF	S(Y)R	
CSD mortality	The ratio of the number of deaths from CSD to the average annual population, per 100 thousand people	2019/2021	573.2/640.3	357.1/405.5	https://www.fedstat.ru/indicator/55382
CSD mortality of the working-age population	The ratio of the number of deaths from CSD to the average annual population at the corresponding age, per 100 thousand people of the corresponding age	2019	141.76	154.34	https://www.fedstat.ru/indicator/57315
Heart attack mortality	The ratio of the number of deaths from a given cause of death (ICD-10 codes - I21-I22) during the calendar year to the average annual population according to the current estimate, per 100 thousand people	2021	38.27	23	https://www.fedstat.ru/indicator/59776
Ischemic heart disease mortality	The ratio of the number of deaths from a given cause of death to the average annual population, per 100 thousand people	2021	348.13	171. 82	https://www.fedstat.ru/indicator/62025
Cerebrovascular diseases mortality	The ratio of the number of deaths from a given cause of death to the average annual population, per 100 thousand people	2021	190.73	90.87	https://www.fedstat.ru/indicator/62026
Acute cerebrovascular accident mortality	The ratio of the number of deaths from a given cause of death (ICD-10 codes - I60-I64) during the calendar year to the average annual population according to the current estimate, per 100 thousand people	2021	90.25	53.8	https://www.fedstat.ru/indicator/59777

Note: * The year of collection of the indicator is shown according to the latest available open data in the EMISS.

metabolism and arterial hypertension [26].

According to the National Heart, Lung, and Blood Institute (USA) definition, patients were considered to have metabolic syndrome when they have abdominal obesity and three or more of the following: high blood pressure, sugar, triglycerides and low blood levels of high-density lipoprotein cholesterol [44].

Polish professional medical societies - Hypertension Society, Obesity Treatment Society, Lipid Association, etc. proposed that the definition of metabolic syndrome should include the presence of obesity and two of the following three criteria: high blood pressure, impaired glucose metabolism, and elevated levels of low-density lipoprotein (non-HDL) cholesterol (atherogenic dyslipidemia) [43].

The International Diabetes Federation (IDF) defines the metabolic syndrome as a group of the following factors: diabetes and prediabetes, abdominal obesity, high cholesterol and high blood pressure [42].

In all definitions abdominal obesity is noted as a characteristic of the metabolic syndrome. In the medical literature abdominal obesity is often referred to as visceral and central obesity [34]. Its diagnosis is a problem, since uniform methods and criteria have not been developed for it. The risks of CSD determine not only the degree of abdominal fat development. It is very likely that the CSD is associated with the peculiarities of the combination of metabolically conditionally neutral subcutaneous fat and metabolically aggressive abdominal fat that is from the metabolic phenotypes of obesity [39].

The external reflection of the human phenotype is his body type [17]. Body features are determined genetically and are formed by the external environment [10].

The results of recent anthropometric studies indicate that indigenous people (women, Yakuts) have smaller body dimensions compared to non-indigenous women, but the increase in BMI, overweight and obesity with age does not depend on ethnicity [7]. The study of age and territorial differences among the indigenous women of the northern and central regions of Yakutia showed that the “northern women” of 75-89 years old have the maximum values of body weight, BMI, the occurrence of overweight and obesity [6]. Another study, already devoted to men, indigenous people, revealed that the greatest development of the fat and muscle components of the body was observed in the age group of 36-60 years [8,18]. Diagnosis and assessment of the obesity degree is a problem associated with the lack of uniform methods and criteria. The most commonly used assessment of obesity is based on the calculation of body mass index (BMI), but it is difficult to use it to assess the development of adipose tissue outside the development of muscle and, thus, to differentiate the distribution of fat in the body. It is known that an increase in BMI in obese individuals may not be associated with an increase in internal fat and an increase in the severity of metabolic disorders [5]. Anthropometric indicators such as waist-to-hip ratio [26] and waist circumference [48] are used to reflect the distribution of central fat. As an indicator of central obesity, it is also proposed to take into account the ratio of waist circumference and height [49].

Among the Yakut population a waist circumference is most often used as an indicator of abdominal obesity [16,29,33]. But the waist circumference reflects not only the amount of visceral fat in the abdominal region, but also the thickness of the subcutaneous adipose tissue of the lumbar region and the anterior abdominal wall.

A significant relationship has been established between the component of the body composition calculated using bioimpedance analysis - fat mass, lean mass, visceral fat and muscle mass with ischemic heart diseases and metabolic syndrome factors in domestic and foreign studies, [23,38]. Studies conducted among the inhabitants of Yakutia a considerable time ago without the use of bioimpedancemetry showed that, in general, the functional reserves of the human cardiovascular system are interconnected with the body composition [15]. This connection is also confirmed in other scientific works [13,19,46].

To identify obesity, it is necessary to establish standard anthropometric indicators taking into account ethnic specifics [41]. Currently scientific work with bioimpedance analysis is underway to standardize anthropometric indicators so far only in young Yakuts in Yakutia [11].

Conclusion. The relevance of continuing to study the regional characteristics of CSD is confirmed by Rosstat data indicating the predominance of CSD mortality in the working-age population in Yakutia over the all-Russian similar indicator. Scientific articles review covering the results of studies on regional features of CSD and the human body parameters confirms the presence of a research backlog for studying the relationship (association) of CSD features with the body composition determined by bioimpedance analysis in Yakutia residents.

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HELICOBACTER PYLORI INFECTION AND NON-ALCOHOLIC FATTY LIVER DISEASE IN THE WORKING POPULATION OF SOUTH YAKUTIA: ASSOCIATION RESEARCH

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A one-time study of the working population of non-indigenous nationality in the southern zone of Yakutia was carried out. A total of 78 people were analyzed, including 31 men, Me age 45.0 [35.0-54.0] years, and 47 women, Me - 43.5 [36.0-53.0] years. A high incidence of Helicobacter pylori infection was found, regardless of comorbidity. The association of HP infection with body mass index, waist circumference, lipid spectrum disorders and metabolic syndrome according to IDF 2005 criteria was not obtained (p>0.05). In the course of the study, the relationship between NAFLD and infection with Helicobacter pylori infection was not obtained (p>0.05).

Keywords: Helicobacter pylori infection, obesity, non-alcoholic fatty liver disease, non-indigenous population, Yakutia.

There is a steady increase in the spread of non-alcoholic fatty liver disease (NAFLD) in the world, along with obesity and metabolic syndrome. Metabolic disorders in addition to metabolic syndrome, insulin resistance, type 2 diabetes mellitus includes NAFLD. The development of both metabolic syndrome and NAFLD is often based on insulin resistance, leading to disorders of lipid, carbohydrate and fat metabolism, the release of free fatty acids and the accumulation of fat and an inflammatory reaction in the liver. It has been proven that NAFLD is an independent predictor of the development of malignant tumors, including hepatocellular carcinoma [4; 10; 13]. In patients, one of the risk factors for NAFLD is increased permeability of the intestinal wall caused by excessive bacterial growth and the action of endotoxins (lipopolysaccharides). The influence of *Helicobacter pylori* (HP) on the development and course of gastritis, peptic ulcer and stomach cancer is evident, but in recent years there has been an assumption about its systemic effect on many different organs, special attention is paid to the contribution to the development of metabolic syndrome and NAFLD. But the evidence is few and mostly contradictory. Some researchers have proved that HP–infected individuals had a more unfavorable metabolic profile, increased body mass index (BMI), blood pressure (BP), triglyceride level (TG) and the highest prevalence of NAFLD [7; 8; 11; 16]. Other studies have not revealed a connection between them [12; 14; 15]. Previously, studies were conducted on the non-indigenous population of South Yakutia, where the highest frequency of pathology of the digestive, cardiovascular and endocrine systems was revealed [3]. Given the high frequency of detection of digestive diseases in this contingent, as well as the few studies of the effect of *Helicobacter pylori* on the development of NAFLD, the relevance of our study is beyond doubt.

The aim of the study: identify the association of HP infection with the development of non-alcoholic fatty liver disease in the working population of non-indigenous nationality living in south Yakutia.

Materials and methods of research. A pilot cross-sectional study of 78 industrial and social workers in the southern zone of Yakutia was conducted. All were representatives of non-indigenous nationality, including 31 men, whose median (Me) age was 45.0 [35.0-54.0] years, and 47 women, Me age 43.5 [36.0-53.0] years.

All study participants were surveyed, their complaints and anamnesis were taken, anthropometric study with the

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determination of height, body weight, measurement of waist circumference (WC) and hips, measurement of blood pressure (BP) were conducted, their blood was taken from the ulnar vein in the morning on an empty stomach with an 8-12-hour interval after the last meal, a clinical examination by a physician was also conducted. The voluntary consent of the participants to the study was a prerequisite, according to the Protocol of the local Ethics Committee of the YSC CMP.

In order to detect abdominal obesity, a measurement was performed from a standing position, patients wearing only underwear. The measurement point is the midpoint of the distance between the top of the iliac crest and the lower lateral edge of the ribs. It does not necessarily have to be at the navel level. At > 94 cm in men and > 80 cm in women, it can be assumed that the patient has abdominal type of obesity (IDF criteria 2005, RSC 2009).

Blood pressure was measured twice with an automatic tonometer "OMRON M2 Basic" (Japan) in a sitting position with the calculation of average blood pressure with a limit of permissible measurement error of ± 3 mm Hg (ESH/ESC, 2013). Arterial hypertension (AH) was assumed at a blood pressure level≥140/90 mmHg or if the patient was taking antihypertensive drugs during the examination period (ACC/AHA Guideline, 2017).

Laboratory analyses were carried out by the enzymatic method on an automatic biochemical analyzer "Labio" using "Analyticon" reagents (Germany). Laboratory research methods included: determination of the lipid spectrum (total cholesterol (TC), low-density lipoprotein cholesterol (LDL cholesterol), high-density lipoprotein cholesterol (HDL cholesterol), TG) and glucose. The enzyme immunoassay included the determination of total antibodies to the *CagA Helicobacter pylori* antigen in blood serum on the Uniplan enzyme immunoassay analyzer using "Vector-Best" reagents.

The diagnosis of "Non-alcoholic fatty liver disease" was made on the basis of an ultrasound examination of the liver on an empty stomach and the conclusion of a general practitioner. The exceptions were alcoholic liver damage, chronic viral hepatitis and cirrhosis.

Metabolic syndrome (MS) was assessed according to the criteria of the IDF (International Diabetes Federation) 2005: the presence of the main component - abdominal obesity (in men from ≥94 cm and in women from ≥ 80 cm) and two other criteria from the listed options: blood pressure≥140/90 mmHg, fasting glucose > 5.8 mmol/l or type 2 diabetes mellitus, TG>1.7 mmol/l (>150 mg/dl), HDL cholesterol in men <1.0 mmol/l (<40mg/dl), in women <1.1 mol/l (<50mg/dl).

Statistical analysis was carried out using the SPSS STATISTICS software package (version 26.0). Qualitative variables are described by absolute and relative frequencies (%), quantitative variables are described using the mean and standard error of the mean, median (Me) and interquartile range (Q1-Q3). The share comparison of the groups was carried out using the nonparametric Spearman criterion χ2. The odds ratio (OR) and 95% confidence interval (95% CI) were calculated. The correlation analysis was carried out using the Spearman coefficient. The statistical significance of the differences (p) was less than 5%.

The work was carried out under the research project of the YSC CMP "Regional peculiarities of biochemical, immunological and morphological indicators in the indigenous and non-indigenous population of the Republic of Sakha (Yakutia) in normal conditions and pathology" (FGWU-2022-0014) and the research of the Academy of Sciences of the Republic of Sakha (Yakutia) "Assessment of radiation exposure of the population of the Aldansky district due to natural sources of radiation and recommendations for carrying out protective measures to reduce it."

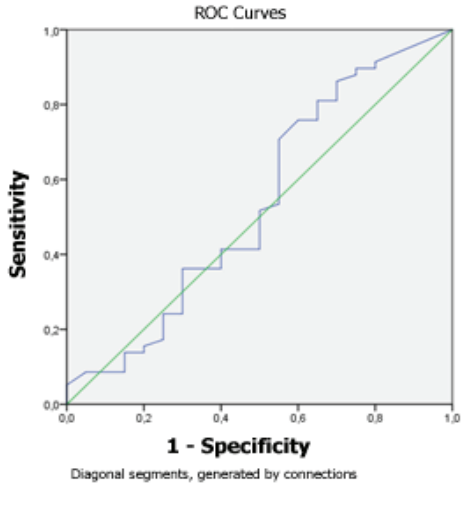
Research results and discussion. The conducted study for the presence of antibodies to the Helicobacter Pylori antigen in the non-indigenous population of South Yakutia revealed that more than half of the examined individuals (n=60 or 76.9%) had positive antibody tests, 24 out of 31 men (77.4%) and 36 women (76.6% of all women) without a significant difference (p>0.05).

We analyzed the relationship of HP infection with anthropometric parameters (BMI, WC). For this purpose, two groups were identified: HP-positive (or main) group (60 people) and HP-negative (control) group (18 people). The average titer of antibodies to Helicobacter Pylori was 4.39±0.31 cu in the main group, 0.35±0.22 cu in the control group with a significant difference (p=0.000).

As a result of statistical analysis, it was found that 35% of the main group (n=21) were overweight, obesity was found in 20 people, or 33.3%. In the group without infection, overweight was registered in 16.7% (n=3), obesity in 61.1% (n=11). We did not obtain statistically significant differences between the groups in the frequency of occurrence of overweight and obesity by BMI (χ^2=3.76, p=0.052). A comparative analysis of the frequency of occurrence of AO revealed a high frequency regardless of the presence of infection. Thus, in the main group, 65.5% (n=40) had AO, in the control group 82.3% (n=14), there were no significant differences (p=0.370). A correlation analysis of HP infection with anthropometric data was further carried out, resulting in a negative correlation with BMI (r=-0.204,

The correlation of *Helicobacter pylori* with the lipid spectrum

Parameter	TG	TC	HDL cholesterol	LDLcholesterol
r	- 0.174	- 0.077	0.073	- 0.050
p	0.127	0.503	0.525	0.664



Model: NAFLD- no and NAFLD- yes
Variables: HP antibody titer
NAFLD prognosis 1 – positive;
0- negative
N=78

ROC-titer curve of antibodies to *Helicobacter pylori* in relation to the development of NAFLD

p=0.073) and WC (r=-0.088, p=0.444).

Thus, the conjugacy of HP infection with BMI was not obtained (p0,05). The high incidence of obesity is due to the general prevalence in the population.

To identify the association of HP infection with systolic blood pressure, a correlation analysis was performed, during which a negative insignificant correlation was obtained (r= -0.062, p=0.589).

The relationship between the presence of *Helicobacter pylori* and MS according to the criteria of the IDF 2005 was also not revealed. Thus, 21 people or 35% of the HP-positive group (OR 1,122 [95% CI: 0.863-1.459]) and 8 people (47%) of the HP-negative group (OR 0.680 [95% CI: 0.295-1.564]) had MS (χ^2=0.820, p=0.365).

The correlation of HP infection with blood biochemical parameters, in particular with the lipid spectrum, was analyzed (Table 1). The table clearly shows the absence of a significant correlation of infection with lipid indicators with some negative association.

Thus, the association of HP infection with lipid metabolism disorders, blood pressure, MS in the non-indigenous population of South Yakutia has not been revealed.

Of all the examined persons, 25.6% (n=20) were diagnosed with NAFLD, of which 10 were men (32.3%) and 10 were women 21.3% (χ2=1.18, p=0.277). Our data on the frequency of NAFLD detection are consistent with many studies of scientists [1; 6; 9]. The analysis of the relationship of NAFLD with the biochemical parameters of blood, in particular with the lipid spectrum, was carried out. The association was revealed only with an increased level of TG, the odds ratio was 3.079 ([95%CI: 1.078-8.792], p=0.032). With other indicators of lipid metabolism: increased levels of TC, LDL cholesterol, reduced HDL conjugacy was not obtained (OR 1.310 [95%CI: 0.472-3.633], p=0.604; OR 0.741 [95%CI: 0.233-2.351], p=0.424; OR 0.500 [95%CI: 0.119-2.109], p=0.424).

AO (100%) was found in all patients with NAFLD, thereby confirming the close association of obesity with the development of NAFLD, confirming its higher prevalence among patients with NAFLD, 75-93% [2; 5]. Taking into account the overall picture of the scale of the obesity epidemic in the world, the "control" also revealed a high incidence of AO, detected in 34 people, which was 58.6% (χ2=11.95, p=0.000). An analysis of the conjugacy with the level of systolic blood pressure was also carried out, during which no statistically significant correlation with NAFLD was obtained (r=0.201, p=0.078).

The incidence of NAFLD in the HP-positive group was 23.3% (n=14), in the HP-negative 33.3% (n=6), with no significant difference (OR 0.55 [95%CI: 0.17-1.87], p=0.302).

For the prognostic significance of *Helicobacter pylori* in relation to the risk of developing NAFLD, we additionally performed an ROC analysis (Fig.1).

To determine the significance, an ROC curve was plotted on a square diagram for each biomarker, a threshold cut-off point was determined with maximum specificity and sensitivity of the test when using it, as well as the area under the ROC curve - AUC (AreaUnderCurve). We selected a threshold point value of 4,135 cu with a sensitivity of 43.1% and a specificity of 50.0%. The area under the ROC curve – AUC of the antibody titer was 53.8% (0.538), (95% CI 0.378-0.698), p=0.081. According to the analysis, we can conclude a negative diagnostic significance of the antibody titer index for *Helicobacter pylori* in the development of NAFLD.

Conclusion. Thus, in our pilot study, the relationship of NAFLD with helicobacter infection was not obtained, possibly due to a small sample size. Nevertheless, we have confirmed the research results of a number of foreign authors [12; 14; 15]. A high incidence of *Helicobacter pylori* infection was revealed in the working population of non-indigenous nationality of South Yakutia, regardless of concomitant pathology. Mandatory eradication of HP infection with subsequent serological examination or fecal analysis for the *Helicobacter pylori* antigen is recommended for all patients of the positive group, taking into account its direct impact on the development of pathology of the gastroduodenal system, in particular gastric ulcer and duodenal ulcer, as well as stomach cancer. Patients with NAFLD are recommended to regularly conduct ultrasound examination of the liver, pathogenetic treatment, commit to weight loss, follow a balanced hypocaloric nutrition and comply with the principles of a healthy lifestyle.

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THE LEVEL OF ENDOGENOUS INTOXICATION IN OVERWEIGHT INDIVIDUALS

According to the data obtained, there is an increase in the levels of low and medium molecular weight substances and oligopeptides in plasma, erythrocytes, and urine, depending on body weight. In obese individuals, there was a significant increase in the concentrations of low and medium molecular weight substances and oligopeptides compared to those with normal body weight in all biological samples studied, indicating endogenous intoxication in the body of obese individuals.

Keywords: low and medium molecular weight substances, oligopeptides, obesity, overweight, endogenous intoxication.

Introduction. At present, the problem of obesity is urgent both worldwide and in Russia [5]. Modern man is exposed to several factors leading to obesity (abundance and caloric content of food, hypodynamia, psycho-emotional-stresses, bad habits, and ecology).

Obesity leads to serious complications in human health. People suffering from obesity are more prone to the development of cancer [6, 13], cardiovascular [9], autoimmune diseases [11]. Obesity is a risk factor for severe outcomes in COVID-19 disease [8]. In addition, obesity is the cause of leading a person to disability and death [5].

Obesity is a complex, multifactorial disease, the development of which, along with genetic predisposition, is greatly influenced by external factors that lead to metabolic disorders in the human body. Disturbance of metabolic processes leads to endogenous intoxication (EI) development. According to many authors, EI is a nonspecific process in diseases of different etiology, pathogenesis, and severity [1]. In patients with chronic EI, there is often a shift in homeostasis, leading to decreased body resistance [10, 12].

In connection with those mentioned above, detection EI in risk groups for correction of this condition is an urgent task. In scientific and clinical studies, the determination of low and medium molecular weight substances (LMMWSs) as markers of EI is widespread.

There have been no studies on the level of LMMWSs as markers of EI in overweight and obese residents of Yakutia.

Materials and methods. The present work was carried out in the Yakut Science Centre of Complex Medical Problems under the research work: "Regional characteristics of biochemical and immunological parameters in the indigenous and native population of the Republic of Sakha (Yakutia) in norm and pathology". The material was collected during medical and biological expeditions during the health examination of the population of Yakutia in the spring period from 2015 to 2019. It was mandatory to obtain informed consent of the respondents for the study (according to the protocol of the Ethical Committee of Yakut Science Centre of Complex Medical Problems №49 dated 25.03.2018).

Fifty people aged 31 to 50 years were examined. The body mass index (BMI) = m (kg) / h^2 (m), where m-body weight, h-height, was calculated for each individual. According to the BMI, the examined persons were divided into three groups: the first group included 15 people with normal body weight (BMI from 18 to 24.9), the second group included 20 people with overweight (BMI from 25 to 29.9) and the third group included 15 obese people (BMI from 30 and above). The study material was blood from the ulnar vein on an empty stomach.

The content of LMMWSs estimated the level of EI according to the method of M.Y. Malakhova [7]. The determination of oligopeptides (OPs) was evaluated using the Lowry protein assay. In plasma, blood erythrocytes, and urine we calculated the coefficients of the complex assessment of endotoxemia: K1 - ratio of LMMWSs concentration in plasma to LMMWSs concentration in erythrocytes; K2 - ratio of LMMWSs concentration in urine to the sum of LMMWSs concentrations in blood plasma and erythrocytes; K3 - ratio of oligopeptide (OPs) concentration in urine to the sum of OPs concentrations in blood plasma and erythrocytes.

Biochemical parameters were studied in serum. The concentrations of cholesterol, triglycerides, high-density lipoproteins, glucose, alanine aminotransferase and aspartate aminotransferase were determined on an automatic biochemical analyzer Chem Well 2902.

The obtained data was performed using IBM SPSS Statistics 19 program. In this article, quantitative indicators are presented in the format Mean ± SD, where Mean is the arithmetic mean, and SD is the error of the arithmetic mean.

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Table 1

LMMWSs and OPs content in plasma, urine and erythrocytes

BMI	LMMWSs in plasma (CU)	LMMWSs in erythrocytes (CU)	LMMWSs in urine (CU)	OPs in plasma (g/L)	OPs in erythrocytes (g/L)	OPs in urine (g/L)
Normal body weight	16,714±0,039	26,180±0,086	23,928±0,017	0,060±0,009	0,094±0,002	0,621±0,001
Overweight	17,875±0,096	29,344±0,081	26,971±0,053	0,071±0,002	0,105±0,007	0,733±0,009
Obesity	20,213±0,069*	31,105±0,076*	30,260±0,015*	0,083±0,005*	0,110±0,006*	0,854±0,009*

Note: * - level of statistical significance $p<0.05$, CU – conventional units.

The normality of the distribution of quantitative data was checked using the Kolmogorov-Smirnov test. As a result, the data in all the studied samples for the studied indicators differed from the normal distribution. In this regard, the Mann-Whitney U test was chosen to compare the studied groups. The significance level for accepting the null hypothesis was accepted at $p<0.05$.

Results and discussion. The spectrogram of erythrocytes, when analyzing the mean values of the supernatant after precipitation of erythrocyte mass with trichloroacetic acid, had the form of a hyperbola with maximum extinction at a wavelength of 258 nm, which is mainly due to the presence of LMMWSs containing nucleotides. The level of LMMWSs in erythrocytes of blood of obese individuals was significantly higher by 1.2 times compared to individuals with normal body weight.

The spectrogram of blood plasma had the form of an ascending curve with absorption maximum at 282 nm. The content of LMMWSs in blood plasma of obese individuals was significantly higher by 1.2 times.

The urine spectrogram had absorption maxima in the ranges of 238 and 270 nm, corresponding to the presence of urea, uric acid, and creatinine. In obese individuals, the concentration of LMMWSs in urine was significantly 1.3 times higher ($p<0.05$) (Table 1).

Determination of OPs allowed us to assess the proteolytic process activity in blood quantitatively. A significant increase of OPs values in plasma, erythrocytes, and urine was observed in obese individuals 1.3 times; 1.2 times; 1.4 times, respectively, compared to those with normal body weight.

In obesity, there is a metabolic disorder, which leads to the accumulation of toxic substances of endogenous and exogenous nature in the body, as evidenced by the accumulation of LMMWSs and OPs in blood and urine. The accumulation of LMMWSs and OPs in biological samples of obese patients is evidence of EI in the body. In EI, many studies cite the relationship between the increase in the concentration of LMMWSs and the deterioration of the patient's general condition. For example, Margity et al. (2021) found that maximum concentrations of LMMWSs and OPs were observed in severe and minimum concentrations in mild varicella. The levels of LMMWSs and OPs in patients with complications were higher than in patients without complications [3]. Prokofieva et al (2022) showed that the volume of ischemic myocardium, affects the severity of endogenous intoxication in patients with myocardial infarction [4]. The danger of chronic course of EI in obese patients is associated with decreased body resistance and the possibility of developing many serious diseases (cardiovascular, oncological, and autoimmune diseases). In our study, there is a direct correlation between the increase in BMI and the content of LMMWSs ($r=0.74$; $p=0.01$).

In obesity, the biochemical parameters of the blood changes towards an increase in blood glucose due to a decrease in tissue resistance to insulin. In obesity, the pancreas intensively synthesizes insulin, while the same gland compensatory secretes glucagon. Glucagon damages the nephron tubule. As a result, the kidney's barrier function is reduced, and proteins are intensively excreted with urine [2], as evidenced by our calculated coefficient of endotoxemia - K3 tending to increase with increasing body weight. This coefficient characterizes the process of kidney elimination of OPs (Table 2).

Obesity-induced hypertrophy of adipose tissue leads to activation of lipolysis and an increase in blood levels of triglycerides, free fatty acids, and cholesterol, from which low and intermediate density lipoproteins are formed in the liver. Our results confirm this (Table 3).

Table 2

EI coefficients

EI coefficients	Normal body weight	Overweight	Obesity
K1	0.634±0.084	0.601±0.040	0.649±0.059
K2	0.691±0.019	0.665±0.087	0.667±0.027
K3	4.137±0.036	4.296±0.049	4.475±0.015*

Table 3

Blood biochemical parameters

Biochemical parameters	Normal body weight	Overweight	Obesity
Cholesterol	5.324±0.120	5.721±0.254	5.833±0.096*
Triglycerides	0.765±0.026	0.915±0.042*	1.427±0.033*
Low-density lipoproteins	0.356±0.097	0.414±0.016	0.656±0.012*
Glucose	4.750±0.031	5.156±0.081	5.281±0.074
Alanine aminotransferase	14.236±0.112	18.238±0.994	21.002±0.295*
Aspartate aminotransferase	22.761±0.301	22.310±0.135	25.332±0.514

Thus, in obese individuals, compared to those with normal body weight, there are significantly high values of biochemical parameters of cholesterol, triglycerides, low-density lipoproteins, alanine aminotransferase activity in the blood, which is evidence of metabolic disorders. Comparison of LMMWSs and OPs in all studied biological samples (erythrocytes, plasma, urine) in obese individuals compared to those with normal body weight showed significantly high values of these indicators, which is evidence of EI in obese individuals. Correction of EI in the body of obese individuals may prevent the development of serious diseases such as type 2 diabetes mellitus, cardiovascular, oncological, and autoimmune diseases.

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CLINICAL CASE

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ACUTE TOXIC HEPATITIS ON THE BACKGROUND OF TYPE 1 DIABETES MELLITUS IN AN ADOLESCENT

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The article presents the interesting clinical case of acute toxic hepatitis in a child suffering from diabetes mellitus of the 1st type against the background of taking analgesic drugs.

Keywords: toxic hepatitis, acute hepatitis, type 1 diabetes mellitus, examination, diagnosis.

Introduction. Toxic hepatitis is a liver damage caused by exposure to chemicals and hepatotropic poisons, which leads to liver inflammation and necrosis of hepatocytes. The prevalence of toxic hepatitis is 2 cases per 100 thousand population [5]. There is enough information about toxic hepatitis in modern scientific literature, the forms, clinic, diagnosis and therapy, as well as predisposing factors and methods of prevention of this condition in adults are described [3]. Clinical cases of toxic hepatitis in children are rarely described. The available literature describes rare cases of toxic hepatitis against the background of using antipyretics [2,4].

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In April 2022, WHO reported cases of severe acute hepatitis of unclear etiology in children. By the end of May 2022, 650 cases were known to have occurred in children in 35 countries worldwide. In ≈10% of cases hepatitis was complicated by formation of liver failure, at least 11 lethal outcomes were registered [1]. In this connection, description of clinical examples of toxic hepatitis becomes very relevant for problem clarification.

There are almost no data on clinical examples of toxic liver damage in children against the background of type 1 diabetes mellitus. The presented clinical example is a vivid case of how a child with a severe underlying disease like type 1 diabetes mellitus suffered severe acute liver damage on the background of long-term use of analgesic, anti-inflammatory drugs.

Clinical Example: A 14-year-old child consulted the admission and diagnostic department of the Republican Hospital No.1-NCM with the following complaints: general weakness, back and leg pains, unstable sugar level, palpitations, shortness of breath. He was admitted to the Department of Pediatric Endocrinology and Gastroenterology, Pediatric Center, Republican Hospital No.1-NCM.

Anamnesis of life: A child from the 13th pregnancy, which proceeded smoothly. P-4, natural childbirth, at 40 weeks. Weight at birth was 3530 grams, length was 52 cm. He was applied to the breast for 1 day. He sucked actively. Apgar score was 8/9. He was discharged on the 5th day. He was breast-fed till the age of 4 months, complementary feeding was from the age of 6 months. Psychomotor development was up to 1 year of age. Preventive vaccinations were given according to schedule. Transferred diseases: SARS, chicken pox. He had Coronavirus infection in February 2022. He had no injuries or surgeries. Heredity was aggravated by diabetes, sister had diabetes mellitus type 1.

Allergologic history was normal. Epidemiological history: he denied any contacts with infectious patients.

Anamnesis of the disease: Diabetes mellitus debut since the age of 13. He was admitted in the state of ketoacidosis. Examination. Clinical diagnosis: Diabetes mellitus, type 1. First detected. Ketoacidosis. Received treatment and recommended replacement therapy: Tresiba (prolonged-acting insulin) at 22 hours, 18 units, fiasp (short-acting insulin) 10-12 units 3 times a day. The child was issued disability.

In September 2022 the patient was admitted to the Pediatric Endocrinology and Gastroenterology Department of Pediatric Center of Hospital No.1 - NCM. Taking into account the patient's stable condition, replacement therapy was not changed.

At the beginning of December 2022 the child felt unwell. Biochemical blood tests were performed in the district clinic and increased blood transaminases levels were revealed: ALT was 1232.2 units/l, AST was 740.9 units/l. The child was urgently admitted to the Admission and Diagnostic Department of the Pediatric Center of the Republican Hospital No.1-NCM. While collecting anamnesis from the child it was found out that the child took Naiz, Ibuprofen, Ketorol for headache not constantly for 6 months.

Objective status: Condition was satisfactory. Height was 166 cm. Weight was 45 kg. BMI was 16.3. Body temperature was 36.4, respiratory rate was 20 per 1 minute, heart rate was 87 per 1 minute, blood pressure was 120/70 mm Hg. Consciousness was clear, active. The build was correct, reduced in nutrition. The skin was clean, normal coloring, no strictures. The pharynx was calm. Thyroid gland was not enlarged, painless. There was no tremor. Breathing in the lungs was vesicular, no rales. Heart tones were

clear and rhythmic. The abdomen was soft, moderately painful in the epigastric region. The liver was not enlarged and painless on palpation. Physiological excretions were normal, according to his words. Sexual development was of the male type, pubertal. Tanner III-IV.

In the general blood test of December 09, 2022: hemoglobin (HGB) - 122 g/L (RI: 108-145g/L); red blood cells (RBC) -3.7x10^{12}/L (RI: 3.9-5.29x10^{12}/L); platelets (PLT) - 320 10^9/L (RI: 175 - 345x10^9/l); white blood cells (WBC) -4.58x10^9/l (RI: 3.84 - 9.84x10^9/l); lymphocytes (LYMF) - 52.6% (RI 16. 4-52. 7%); monocytes - 9.0x10^9/l (RI: 4.4 - 12.3x10^9/l); stab neutrophils - 5% (RI: 1-5%); segmented neutrophils - 34. 0% (RI: 32.5 - 74%); eosinophils - 5% (RI: 0-5%), monocytes (MONO) 9% (RI: 4.4 -12.3%); determination of ESR by the Panchenkov method was 34 mm/h (RI: 1-15 mm/h). Conclusion: thrombocytosis and increased ESR were observed.

Biochemical blood analysis of February 09, 2022: total protein 61 gp (60-80 g/l); albumin 38.4 gp (38-54 g/l), alanine aminotransferase (ALT) 603. 5 U/L (RI:0 to 27 U/L), aspartate aminotransferase (AST) 720.6 U/L (RI: 0-29 U/L); alkaline phosphatase 212.7 U/L (RI: 0-75 U/L); total bilirubin 61.1 mol/L (RI:3.4-17.1 µmol/L), total cholesterol 6.22 mmol/L (RI: 1. 2-5.2 mmol/l) Creatinine 54.5 µmol/l (27 to 62 mmol/l), high-density lipoproteins (HDL) 1.51 mmol/l (RI: 0.96-1.91), lactate dehydrogenase (LDH) 502 sl/l (RI: 0-250 sl/l), glucose 6.98 mmol/l (RI: 3.3-5.6 mmol/l). Conclusion: increased ALT and AST, glucose level, total cholesterol, LDH.

Study of the hormonal profile dated December 09, 2022: Thyrotropic hormone (TSH) - 1.2 mIU/L (RI: 0.4 - 4.00 mIU/L), free thyroxine (T4) - 10 nmol/L (RI: 9.00-22. 00 nmol/L), thyroperoxidase antibodies (AT to TPO) - 2.4 units/mL (RI:00-30.00 units/mL), triiodothyronine (T3) 3.58 pmol/L (RI: 2.6-5.7 pmol/L).

Glycemic profile:

December 08-09, 2022 Blood sugar at 10 pm - 13.38 mmol/L, at 3 am - 7.58mmol/L, at 07 am - 5.15 mmol/L.

December 14-15, 2022 Blood sugar at 7 am - 9.59 mmol/l, at 11 am - 13.50 mmol/l, at 4 pm - 22.77 mmol/l, at 8 pm - 19.63 mmol/l, at 3 am - 3.32 mmol/l, at 07 am - 3.33 mmol/l.

December 18-19, 2022 Blood sugar at 7 am - 6.63 mmol/l, at 11 am - 7.11 mmol/l, at 4 pm - 11.30 mmol/l, at 8 pm - 3.23 mmol/l, at 3 am - 10.08 mmol/l, at 7 am - 9.77 mmol/l.

December 22-23, 2022 Blood sugar at 7 am - 5.5 mmol/l, 11 am - 6.08 mmol/l, 4 pm - 14.42 mmol/l, 03 am - 5.82 mmol/l, 07 am - 4.88 mmol/l.

PCR for hepatitis on December 12, 2022: HBV DNA was negative. HCV RNA was negative.

Abdominal ultrasound on December 8, 2023: The liver was enlarged, the left lobe was 73 mm thick, the right lobe was 173 mm thick. Craniocaudal dimension was195 mm. The contour was smooth. The structure was homogeneous, grooming was increased, symptom of "fading ultrasound". The spleen was enlarged, size: 121*37mm, S-57cm2. The contour was smooth. Additive lobule d 12mm. The structure was homogeneous, medium echogenicity. Spleen vein was not enlarged.

Intrahepatic bile ducts were not dilated. Vascular pattern was preserved. The hepatic veins were moderately dilated to 7 mm. The portal vein was 14 mm dilated. Gallbladder was located typically. The shape was oval. The wall was thin, the lumen was clear. The gallbladder was 89 x 30 mm in size, it was enlarged. The common bile duct was not enlarged. The pancreas was not enlarged. The contour was smooth. Dimensions of the pancreatic head were 18 mm, body was 8 mm, tail was 17 mm. The structure was homogeneous, with medium echogenicity.

The kidneys were located typically. The right kidney measures were 120 by 35mm. The contour was smooth. Parenchyma was 14 mm thick. Cortico-medullary differentiation preserved. Bowel-lacrimal system was not enlarged. The left kidney measured 118 by 41 mm. The contour was smooth. Parenchyma was additional 14mm. Cortico-medullary differentiation was preserved. Bowel-lobule system was not enlarged. Additionally: Bladder was empty. Conclusion: Hepatosplenomegaly, diffuse changes of liver parenchyma. Free fluid in the abdominal cavity.

Ultrasound examination of the thyroid gland on December 9, 2022: Thyroid gland V=4.44 cm3, length was 3.28 cm, thickness was 1.12 cm, width was 1.29 cm. The contour was smooth. Echostructure was homogeneous. Echocosity was medium. The isthmus was 0.21 cm. Regional lymph nodes were not enlarged.

Esophagogastroduodenoscopy on December 21, 2022: Esophageal mucosa was pink, shiny, moderately hyperemic in the lower third. The dentate line was clear. Cardia occluded. There was a small amount of secretory fluid in the gastric cavity. The gastric folds were of normal shape and height. The gastric mucosa in the antral part was stained hyperemic, in the descending part of the duodenum it was stagnant. Conclusions: Erythematous gastroduodenopathy expressed. Nonerosive reflux esophagitis.

Electrocardiogram dated December 13, 2022: Sinus rhythm, pronounced tachycardia. Heart rate was 105 beats per minute, electric axis of the heart was normal.

Electroneuromyography dated December 19, 2022: Syndrome of impaired absorption conduction along the median, ulnar, peroneal, and tibial nerves on both sides not detected.

Consultation of gastroenterologist, dated December 16, 2023: Toxic liver damage, proceeding according to the type of acute hepatitis. Without cholestasis.

Determination of infections by immunofluorescence analysis from December 16, 2022: Ebstein Barr virus - early immunoglobulin G (EA) antigens not detected; Ebstein Barr virus - nuclear immunoglobulin G (NA) antigen not detected.

On the basis of the complaints, medical history, clinical examination, biochemical tests (increased transaminases, alkaline phosphatase, total bilirubin), ultrasound examination of the liver (hepatosplenomegaly, diffuse changes in the liver parenchyma, free fluid in the abdomen), the clinical diagnosis was: Toxic liver injury, with the type of acute hepatitis (K 72.1). Diabetes mellitus type 1. Without complications (E 10.9).

Disintoxication and hepatoprotective therapy with positive effect was carried out in the department. Treatment was prescribed: ward regimen. Table #9, replacement therapy: 48-50 units/day (1 U/kg/day) - Degludek (Tresiba), 22 hours - 18 units, Insulin Aspart-Nicotinamide (Fiasp): 8 hours-10 units, 12 hours-10 units, 18 hours-10 units.

Infusion therapy with disintoxication purpose: plasmafusol 500 ml; physiological solution 0.9% - 300 ml intravenous drip.

Recommendations: diet table 9 with restriction of easily digestible carbohydrates with calculation of BE - the daily requirement - 18-20 BE/day. Blood glucose control in the morning on an empty stomach, before and 2 hours after each meal, before going to bed with a portable glucose meter or continuous blood glucose monitoring system (CMMS) and keeping a self-monitoring diary.

Insulin therapy in the basal-bolus regimen - insulin Degludek (Tresiba) and insulin aspart-nicotinamide (Fiasp) in accordance with the federal clinical guidelines: Breakfast 6XU - Fiasp 10IU, for glucose above 14 mmol/L, for glucose above 18 mmol/L. Lunch 6XU - Fiasp

10IU, for glucose above 14 mmol/L, for glucose above 18 mmol/L. Dinner 6XU - Fiasp 10IU, for glucose above 14 mmol/L, for glucose above 18 mmol/L. Tresiba 10 p.m. 18 IU.

Control of glucose and ketones in urine at glucose above 15 mmol/l and for intercurrent diseases, increase insulin dose if necessary.

In case of hypoglycemia (blood sugar below 3.9 mmol/l), take easily digestible carbohydrates for 1 CGU (a glass of juice, 4 pieces of raffinate), then after 15 minutes measure blood glucose, if the blood glucose is low again repeat carbohydrate intake.

Change of injection sites is recommended to avoid lipodystrophy formation.

Monitor glycated hemoglobin HbA 1c, blood chemistry, morning urine for microalbuminuria every 3 months.

With hepatoprotective purpose - Ursosan 500 mg once a day - a course of 1 month.

Control of biochemical blood tests (ALT, AST, LDH, creatinine, urea, total protein, gamma-glutamyl transpeptidase, bilirubin total, free, albumin) after 1 month. In case of negative dynamics of the biochemical blood test, hospitalization in the Department of Pediatric Endocrinology and Gastroenterology of the Pediatric Center of RB №1-NCM is recommended.

Conclusion: An interesting clinical case from the Department of Pediatric Endocrinology and Gastroenterology of Pediatric Center of the Republican Hospital No.1-NCM is described in the article. Cases of toxic hepatitis in children have been described in the literature. However, there are practically no articles describing cases of toxic hepatitis in children against the background of diabetes mellitus type 1. The article will be of interest to pediatricians.

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ADRENAL AND THYROID DYSFUNCTION IN NON-TRAUMATIC SUBARACHNOID HEMORRHAGE REQUIRING THERAPEUTIC NORMOTHERMIA. CLINICAL OBSERVATION

Subarachnoid hemorrhage (SAH) is a life-threatening variant of hemorrhagic stroke. Therapeutic normothermia to reduce the risk of adverse outcomes. At the same time, the initially existing decompensation of body systems, followed by induced normothermia, gives impetus to the development of a number of pathophysiological processes in the human body. **Objective.** Timely detection and adequate correction of endocrinopathy of critical illness in patients in the most acute period of SAH against the background of therapeutic normothermia with a target temperature regime of 36 °C. **Materials and methods.** A 57-year-old patient with a diagnosis of non-traumatic subarachnoid hemorrhage, saccular aneurysm of the anterior communicating artery and anterior cerebral artery, Hunt Hess III. **Results.** The development of critically ill adrenal dysfunction considered based on the need for vasopressors. Thyroid dysfunction caused by a critical illness considered with a decrease in the level of TSH and free T3 in blood plasma against the background of therapeutic normothermia, the development of intestinal dysfunction and sinus bradycardia. **Conclusions.** Against the background of the introduction of hydrocortisone at an initial dose of 300 mg, the administration of norepinephrine discontinued on the third day. When levothyroxine sodium 300 mcg/day added to therapy, intestinal dysfunction resolved, normal resting heart rate achieved.

Keywords: critical illness, hypothyroidism, thyrotropin, thyroid hormones, thyroxine, triiodothyronine, extracorporeal membrane oxygenation, targeted temperature management, cortisol, adrenocorticotropic hormone.

Introduction. Subarachnoid hemorrhage (SAH) is a life-threatening variant of hemorrhagic stroke [1]. In the acute period of SAH, among other methods of treatment, aggressive correction of hyperthermia and maintenance of normothermia using specialized TTM body temperature control systems are justified [15]. Prophylactic normothermia is recommended for patients with a poor prognosis of SAH (Hunt-Hess 111-V) [6]. TTM, which has actively used over the

past decades in the conditions of intensive care units and intensive care units for the purpose of neuroprotection and correction of intracranial pressure, has made it possible to reduce the risk of adverse outcomes in critical illness. At the same time, the initially existing decompensation of body systems, followed by induced normo/hypothermia, gives impetus to the development of a number of pathophysiological processes in the human body. There have been no works on the diagnosis and treatment of endocrinopathies of critical illness in TTM.

The presented clinical observation is to demonstrate the importance of timely detection and adequate correction of endocrinopathy of critical illness in patients in the most acute period of SAH against the background of therapeutic normothermia with a target temperature regime of 36°C. The observation period for the patient was three days.

Materials and methods. This article describes a clinical observation of a 57-year-old patient with a diagnosis of non-traumatic subarachnoid hemorrhage, saccular aneurysm of the anterior communicating artery and anterior cerebral artery (AComm-ACA). Hunt Hess III, requiring therapeutic normothermia. When prescribing hydrocortisone and levothyroxine sodium, the patient was in a serious condition with drug-induced depressed consciousness. Based on p. 9.1 art. 20 of the Federal law № 323-FZ from 21.11.2011 as medical intervention was necessary for emergency indications to eliminate the threat to the life of the patient, and the patient's condition did not allow the patient to express his will, the decision to prescribe drugs was made by the council without the consent of the patient. **Results.** *Patient Z., 57 years old*, transferred from the primary hospital on the second day of illness with a diagnosis of non-traumatic subarachnoid hemorrhage, saccular aneurysm of the anterior communicating artery and anterior cerebral artery (AComm-ACA). Hunt Hess III" to A.I. Burnasyan Federal Medical Biophysical Center FMBA. *Associated pathology.* Hypertonic disease. Obesity 1 degree according to WHO (BMI 33,2 kg/m2). *At the time of admission to the clinic,* the patient's condition was of moderate severity, the level of consciousness on the Glasgow coma scale was 14 points (stunning): disoriented in space and time, mild dysarthria. Body mass index 31,9 kg/m2. Temperature 36,9 °C. Breathing spontaneously. O2 saturation 98%. Heart sounds are muffled, rhythmic, heart rate (HR) 75 beats / min, blood pressure (BP) 170/80 mm Hg. art. The result of the electrocardiogram (ECG): sinus rhythm. There was no stool for three days. *Surgery on the day of admission* - microsurgical clipping of the AComm-ACA aneurysm using neurophysiological monitoring. *In the early postoperative period.* Consciousness is drug-depressed, - 5 points on the Richmond Agitation Sedation Scale (RASS), sedation (propofol). Body temperature 36,7 °C. Continued artificial ventilation of the lungs. Hemodynamics is unstable, supported by the introduction of norepinephrine at a dose of 1-1,5 mg/kg/min. For the purpose of invasive blood pressure monitoring, a radial artery catheterization performed. Invasive BP was 110/73 mmHg. Despite hemodynamically significant hypotension, heart rate of 67-71 beats per minute, a nasogastric tube placed. There was no reset on the probe. On auscultation, peristalsis is sluggish. There was no stool for three days. Urinary function preserved, a urethral catheter installed. *Instrumental studies:* CT scan of the brain with the introduction of a contrast agent. Signs of subarachnoid hemorrhage. *Laboratory data:* potassium 3,1 mmol/l, sodium -137 mmol/l. Due to the development of cerebral vasospasm and secondary ischemia, coma 1 (GCS 9 points), fever up to 40 °C, *external cooling* was performed for three days in the automatic control mode of the BLANKETROL II system (CSZ, USA). Central body temperature monitored by inserting a 400 series esophageal probe. The target core temperature was 36°C. Sedation with morphine, sodium thiopental/propofol, and rocuronium performed for episodes of muscle tremors. Once, with a decongestant purpose, an infusion of 3% hypertonic solution carried out. Thus, (day 4 from the moment of critical illness development) in a patient after surgery (aneurysm clipping), the severity of the condition is due to multiple organ dysfunction with a predominance of cerebral dysfunction (cerebral vasospasm, secondary ischemia), respiratory and cardiovascular dysfunctions. *On* second day (D2) *and D3 days of observation*, according to laboratory data c-reactive protein - 284 - 212 mg/l, potassium 4,3 - 3,9 mmol/l, sodium 140 - 149 mmol/l, glucose 11,4 - 9,8 - 7,3 mmol/l In connection with the appearance of signs of infection - an increase in markers of inflammation, fever at D2, antibiotic therapy (cefoperazone-sulbactam and amikacin) was added to therapy. *Diagnosis and correction of adrenal and thyroid dysfunctions. Reference ACTH values are (4.7-48.8 pg/ml). The reference value of total cortisol in plasma (*171–536 *nmol/l). Reference values of the level of TSH in plasma (0.4 - 4.0 mU / l). Reference values of the level of free T4 (12–22 pmol/l) and free T3 (3.1 - 6.8 pmol / l).* On the day of admission (D0), plasma levels of cortisol and adrenocorticotropic hormone (ACTH) were: cortisol (1435 nmol/l) and ACTH (3 pg/dl). The level of TSH in blood plasma was 0,2 mU/l, St. T4 17 pmol/l, St. T3 2,1 pmol/l. On the first and third days against the background of therapeutic normothermia: the level of cortisol on C1 (1655 nmol/l), C3 (1514 nmol/l); ACTH level at C1 (3 pg/dl), C3 (6.7 pg/dl). TSH level at D1 (0.01 mU/l), D3 (0,01 mU/l); the level of free T4 on D1 (18.8 pmol / l), on D3 (20 pmol / l), the level of FT3 on D1 (2,1 pmol/l), on D3 (1,9). Due to the need for noradrenaline more than 0,25 mcg/kg/min in the early postoperative period (D0), hydrocortisone was added to therapy at an initial dose of 300 mg (100 mg IV bolus, then 50 mg every 6 hours). On D1 of therapeutic normothermia, intestinal dysfunction persisted (on the 4th day of illness). Against the background of trial enteral nutrition, there was no discharge through the nasogastric tube; there was no stool for 4 days. Prokinetics prescribed: erythromycin at a dose of 200 mg 3 times a day, i.v., and metoclopramide at a dose of 10 mg, 4 times a day, i.v. [9, 11]. A cholinesterase inhibitor, neostigmine methyl sulfate, prozerin, 1.5 mg 3 times a day, also used. Against the background of hydrocortisone, the dose of norepinephrine at D1 decreased to 1–0,7 μg/kg/min, and sinus bradycardia developed (heart rate 48–52 beats/min). According to the results of the electrocardiogram: sinus bradycardia, left ventricular hypertrophy with insufficiency of coronary blood supply in the anterolateral and apical region (systolic load). The level of lactate is 1,3 mmol / l (the norm is up to 1,6 mmol / l). Troponin level up to 0,11 (norm up to 0,023). Echocardiogram result: ejection fraction 62%, violation of local and global myocardial contractility not detected. Considering: the duration of the critical illness caused by non-traumatic SAH; development of the adrenal dysfunction caused by a critical illness (CIRCI); persisting intestinal dysfunction, low levels of TSH and FT3, the development of sinus bradycardia, it was decided to consider this clinical and laboratory picture as thyroid dysfunction caused by a critical illness (TDCCI). Levothyroxine sodium (L-T4) added to therapy. Replacement therapy of L-T4 was carried out at the rate of 3 mcg/kg/day (150 mcg. 2 times a day) - the starting dose, the next day - 100 mcg. 2 times a day. The drug administered through a nasogastric tube into an empty stomach in crushed

form, diluted in saline in a volume of 20 ml, at 6 am and 6 pm. The probe clamped for 2 hours. During the period of therapeutic normothermia, heart rate, assimilation of enteral nutrition were assessed daily, levels of TSH, free T4, free T3, glucose control (with insulin correction for hyperglycemia over 14,5 mmol / l) and electrolyte control. Criteria for reducing the dose of levothyroxine sodium: with the development of fever (body temperature above 38,3, outside therapeutic normothermia), the dose of levothyroxine sodium was reduced by 50% of the initial dose; with an increase in heart rate above 60 beats per minute, the dose of levothyroxine sodium was reduced by 25-50 mcg once a day. At D5, the patient had large stools. At D9, the dose of L-T4 was reduced to 100 mcg/day; on D11 up to 75 mcg / day, on D13 the abolition of L-T4. In the period after the completion of therapeutic normothermia, the administration of norepinephrine discontinued at D3, after which the dose of hydrocortisone reduced by 50 mg per day. With the introduction of hydrocortisone at a dose of 50 mg per day, the patient transferred to the tablet form of hydrocortisone. At D13, hydrocortisone was completed. The patient transferred to the specialized department on day D13. According to the FOUR scale (Full Outline of UnResponsiveness) at the time of discharge: E4, M3, B4, R4 - 15, which corresponded to moderate stunning. The patient's quality of life on the Glasgow Outcome Scale was 4 points.

Discussion. Multiple protective neuroimmunoendocrine reactions that develop when critical illness occurs, lead to the formation of a systemic inflammatory response syndrome. At the first stage of critical illness development, the hypothalamic-pituitary-adrenal system is activated by pro-inflammatory cytokines and afferent pathways of the vagus nerve [5, 9, 12]. If the vital functions of the body not restored within a few days, the critical illness passes from the acute to the subacute phase. The development and formation of the subacute phase of critical illness is based on the increasing systemic level of pro-inflammatory cytokines, hypoxic-ischemic brain damage, pathological permeability of capillary endothelial cells that form the blood-brain barrier and, as a result, the penetration of pro-inflammatory mediators and other neurotoxic molecules into the brain [4]. *The high level of cortisol in the blood plasma that we observed on the second, third and fourth days and the increasing level of inflammatory markers from the third day of critical illness development reflected just the transition of the acute to the subacute phase. It is during this period that adrenal dysfunction develops, caused by a critical illness* [16]. The high concentration of cortisol in the blood plasma can explained by several factors. A decrease in the level of thyroid hormones against the background of a low or low-normal TSH level in the subacute and chronic phases of critical illness may be a consequence of depletion of the hypothalamic centers that regulate pituitary activity [13]. *In the results obtained by us, we also observed a sharp decrease in the level of TSH and free T3 in blood plasma, but against the background of therapeutic normothermia.* Before considering adrenal/thyroid dysfunction in terms of critical illness, it is necessary to state why endocrinopathy considered in terms of dysfunction and not insufficiency. “Dysfunction” is a dysfunction of a system, organ or tissue of the body, expressed as an inadequacy of the response to stimuli [7], and it is the term “dysfunction” of the adrenal glands/thyroid gland that most fully characterizes the ambiguity of both laboratory and clinical results in the diagnosis of CICRI/TDCCI. When the term “adrenal insufficiency” is used, a violation in the HPA system itself implied, without affecting cortico-resistance in critical illness. *To date, there are no unambiguous criteria for diagnosing adrenal dysfunction in critical illness* [14]. The syndrome of euthyroid pathology in the acute phase of critical illness considered as an adaptive response of the hypothalamus-pituitary-thyroid gland-target tissue system and does not require correction by substitution therapy [3]. However, this recommendation should not considered as the only correct solution. A similar denial of hydrocortisone use observed in patients with septic shock until 2016 year, when the issue of "critically ill adrenal dysfunction" went from "does not need to be treated" to "needed to be treated." In conditions of damage to the diencephalic zone, the drug of choice is T4 at a dose of 2–3 μg/kg/day. The feasibility of combining T4 with T3 as initial therapy is controversial [10, 17]. We used in our clinical observation, due to the lack of an intravenous form of levothyroxine sodium, the oral form of L-T4. In addition, we did not use triiodothyronine due to its absence on the territory of the Russian Federation. Before starting treatment for critically ill thyroid dysfunction (TDCCI), the presence/absence of clinical evidence of critically ill adrenal dysfunction (CIRCI) should assessed. If CIRCI detected, hydrocortisone prescribed, and L-T4 prescribed on the second day after the start of hydrocortisone treatment.

According to the results of our study, the development of CIRCI and TDCCI during the use of therapeutic normothermia observed in a patient due to several reasons. First, the development of critical illness caused by CNS damage is an independent factor in the impairment of the hypothalamic-pituitary-adrenal system. At the same time, the duration of the critical illness leads to the depletion of the hypothalamic-pituitary-thyroid system and can no longer considered as a "syndrome of euthyroid pathology". Secondly, the purpose of using TTM is to suppress the activity of metabolic processes in the brain, which in turn also inhibits the activity of the hypothalamic region [2]. Thirdly, long-term use of sedative, narcotic and narcotic drugs to control body temperature, as well as to overcome muscle tremors, especially at target values of 36^0 C, also suppress the hypothalamic-pituitary system. The use of propofol, benzodiazepines and barbiturates increases the sensitivity of gamma-aminobutyric acid receptors (GABA receptors) to the GABA mediator and leads to inhibition of the activity of the cerebral cortex [11], which in turn can cause inhibition of the activity of the hypothalamus-pituitary-thyroid gland/adrenals system [9]. The use of selective α2-adrenergic receptor agonists (dexmedetomdine) suppresses the activity of the locus coeruleus of the brainstem [11]. In addition to the level of consciousness, the noradrenergic system also regulates the functional activity of the hypothalamus during a stress response [8]. Since opiate receptors are located not only in the pathways of pain, but also in the hypothalamus, hippocampus and amygdala, these areas are also subject to the inhibitory effects of drugs. Thus, drugs for general anesthesia, sedation and analgesia, routinely used during TTM and during ECMO, lead to inhibition of the function of the diencephalon.

Conclusion. Thus, the high level of cortisol observed on the day of admission against the background of vascular insufficiency was caused by corticoresistance and is not a criterion for making a decision on the appointment of hydrocortisone. The need for norepinephrine is a key factor in the decision to prescribe hydrocortisone. The development of the clinical picture of hypothyroidism (bradycardia, intestinal dysfunction) against the background of low TSH and free T3 during therapeutic normothermia was considered as TDCCI. L-T4 therapy made it possible to achieve normocardia, and combination with prokinetics and antiparetic therapy, resolution of intestinal dysfunction.

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CLINICAL CASE OF MEGALOBLASTIC ANEMIA IN A TEENAGER WITH NEW CORONAVIRUS INFECTION

This article presents a clinical case of newly diagnosed megaloblastic anemia in a 15-year-old teenager girl from the Republic of Sakha (Yakutia), in combination with intercurrent disease COVID-19. The new coronavirus infection occurs rapidly on the background of of suppressed hematopoiesis, with the development of complications in the form of community-acquired bilateral severe polysegmental pneumonia and bilateral exudative pleurisy, which required observation and treatment in a hospital. The performed standard complex therapy for megaloblastic anemia and pneumonia caused by SARS-CoV-2 made it possible to achieve clinical and laboratory improvement in the patient and restore the function of the red bone marrow.

Keywords: megaloblastic anemia, cobalamin deficiency, folic acid deficiency, COVID-19, new coronavirus infection, pneumonia.

Introduction. Megaloblastic anemia (MA) encompasses a heterogeneous group of macrocytic anemias characterized by the presence of large red blood cell precursors called megaloblasts in the bone marrow [2]. Megaloblastic anemia is ubiquitous, regardless of gender and age.

This condition is due to impaired DNA synthesis, which inhibits nuclear division. Cytoplasmic maturation, mainly dependent on RNA and protein synthesis, is less impaired. This leads to an asynchronous maturation between the nucleus and cytoplasm of erythroblasts, explaining the large size of the megaloblasts [2].

The main diagnostic criteria for megaloblastic anemia are the detection of macrocytic, hyporegenerative anemia, thrombocytopenia, a decrease in the level of vitamin B12 and folic acid in the blood serum, Jolly bodies and Cabot rings, granulocytic hypersegmentation can be detected in a peripheral blood smear.

The main cause of megaloblastic anemia is a deficiency of vitamin B12 (cyanocobalamin) and folic acid. Folic acid is found in foods such as green vegetables, fruits, meat, and liver. The daily requirement for children is: for newborns - 65 mcg, for children up to a year - 85 mcg, from 1 to 3 years - up to 300 mcg, from 4 to 8 years - up to 400 mcg, from 13 years - 18 years - 400-500 mcg [2]. The main sources of cobalamin/vitamin B12 are meat, fish, eggs and dairy products. The daily requirement for children is: 0.3 to 1.4 mcg/day [2]. Vitamin B12 deficiency may be due to the refusal to consume animal products, which is currently an urgent problem among children and adolescents. The most dangerous is the development of vitamin B12 deficiency in newborns and infancy, since there is a high risk of developing irreversible complications, including delayed physical and psychomotor development, but this can be prevented by preventing vitamin B12 and folic acid deficiency during pregnancy [4].

Although safe and effective therapy is available and treatment of anemia is simple, diagnosis can be extremely difficult due to the many and varied clinical manifestations, often coexisting diseases, and the limitations of currently available diagnostic tests [3]. Megaloblastic anemia is most often detected during routine laboratory tests, since anemia develops gradually, and symptoms are present only in patients with severe anemia [2].

In this regard, we would like to share our own clinical observation.

Clinical case: patient A., 15 years old. entered the oncology department of the Pediatric Center of Republic Hospital №1 – National Center of Medicine, named after M.E. Nikolaev 17.01.22

From the history of life: a girl from the fourth pregnancy, which proceeded against the background of anemia of mild severity in the mother. Giving birth naturally at a period of 39 weeks. Birth weight is 3600 g, birth length is 54 cm. Apgar score 8/9 points. Breast feeding to the age of 12 months. The mental and physical development of girl age-appropriate. Vaccination of girl, According to the national vaccination schedule in Russia. Heredity: on the maternal side - diseases of the cardiovascular system, on the father's side - strokes. Earlier diseases: acute respiratory infections (ARI), COVID-19. Allergic anamnesis: to the vaccine "Grippol" in the form of a rash. There were no surgeries, no injuries.

Anamnesis of the disease: since the autumn of 2022, the girl began to be disturbed by epistaxis, which became more often in winter, her parents noticed paleness of cutaneous integuments, the child complains about fatigue.

At the beginning of December 2021, the girl got sick with the ARI: the body temperature rises to C39.3C, a sore throat and general weakness. The parents consulted a doctor, was prescribed treatment. On the background of the therapy, there was an improving the health.

At the beginning of January 2022 the body temperature rises to 39-40C, dry cough. Parents lowered the temperature with paracetamol. On January 7, 22, was called district pediatrician at home, were recommended taking antibiotics, a swab was taken from the pharynx and nose for the diagnosis of COVID-19. From 08.01.2022 Polymerase chain reaction - the result is positive. 01/10/22 was prescribed treatment: azithromycin. On January 13, 22 her health deteriorated, increased cough and shortness. The girl was hospitalized in an emergency at infectious department of Central District Hospital.

Based on the investigation results: total blood count: there was a pronounced three-growth aplasia, ESR 79 mm/h, MCV 112 fl, MCH 39.1 pg, hemoglobin 27 g/l, thrombocytopenia 29*10*9/l, leukopenia 0.9* 10*9/l, biochemical blood test: increased transaminases, inflammatory reaction, total protein - 54.4 g/l; total bilirubin - 166.2 μmol/l; ALT- 277 U/l; AST-153 U/l; LDH 1605.34 U/l, CRP - 74 g / l. According to the results of the coagulogram - hypocoagulation, PTI 58.6%; INR - 1.7; APTT - 42 sec. CT scan of the chest - Conclusion: Polysegmental pneumonia, high probability of covid-19, CT2 (48%).

The child received the following therapy: the antibacterial drug Ceftriaxone at a daily dose of 2 g, 4 procedures of hemotransfusion with washed erythrocytes, two transfusions of fresh frozen plasma.

On January 17, 22, a PCR test of COVID-19 was taken: the result was negative. The child was delivered by emergency aircraft to the The Pediatric Center of National Centre of Medicine The Republic of Sakha (Yakutia), the Medical Commission held a meeting. The decision of the council: for emergency indications she was moved into intensive care on. after stabilization of state, the patient was transferred to the pediatric oncology department.

An objective examination on admission: Height-163cm. Weight-58.5kg. Objectively, the condition is grave, caused by anemic syndrome. The child is conscious. Body temperature is 36.0C. The state of health is reduced, sluggish. Appetite is selective (eats more white meat, snacks, sweets, starchy foods). Stature is correct, normosthenic. The skin is very pale, with an icteric tint, dry, hemorrhagic rash on the arms and legs and at the injection sites on the elbow bend. Visible mucous membranes are pale, clean. Pharynx unchanged from the tonsils. Nasal bleeding from the left nasal passage has stopped by wicking. Regular peripheral lymph nodes aren't enlarged. Breathing is weakened with the medium and sonorous fine bubbling rales. Respiratory rate – 28/min. Saturation - 95%. Heart sounds are rhythmic, clear. Heart rate – 85/min. BP 119/66 mm Hg. Abdomen is soft and painless in palpation. Liver and spleen are regular in size. Fecal retention for 2 days. Urination is regular and painless. Urine is deep yellow.

Paraclinically: total blood count: leukocytes - 1.7 thousand / μl, erythrocytes - 1.7 million / μl, MCV 96.9 fl, MCH 34.8 pg, Hb - 53 g / l, platelets - 12 thousand / μl, myelocytes - 1%, reticulocytes-1, ESR - 68 mm/h Biochemical blood test: total protein - 43.8 g/l; albumin - 27.7 g/l; total bilirubin - 34.8 μmol / l; direct bilirubin - 12.7 μmol/l; ALT - 67.5 U / l; AST - 43.9 U/ l; sugar - 5.08 mmol/l; LDH - 928.8 U / l; alkaline phosphatase - 29.6 U / l. Coagulogram: PTI - 13.6 sec; INR - 1.2; APTT - 39.7 sec; fibrinogen - 5.99 g/l. Blood serum from 01/18/22: folic acid - 0.6 ng / ml (3.10-20.50); vitamin B12 - 8.8 pmol / l (20.60 - 196.70).

A bone marrow puncture was performed from 4 points (anterior and posterior iliac crests). Myelogram: Blast cells 0.4%; 0%; 0.2%; 0%. Puncture of varying degrees of cellularity with increased proliferation of erythroid germ with impaired maturation with signs of megaloblastoidity. The morphological picture of megaloblastic anemia.

CT scan of the chest organs: CT-signs of bilateral polysegmental pleuropneumonia, average risk of covid-19, CT-2. Bilateral pleural effusion. Ultrasound of the pleural cavities: Free fluid in the pleural cavities: on the right, the approximate volume is 64 cm3, on the left, the approximate volume is 78 cm3.

In order to identify the pathology of the gastrointestinal tract, the patient was prescribed an examination:

Urea1 breath tests for Helicobacter pillory - the result is negative;

Esophagogastroduodenoscopy - Superficial gastritis, duodenitis. A biopsy was taken.

Biopsy results: Fragments of the gastric mucosa without pathology. HP has not been identified.

Fragments of the mucosa of the duodenum, the ratio of the lengths of the villi and crypts ~2-2.5:1. Enterocytes are high. The number of MELs is 10 per 100 enterocytes. Own plate with infiltration by lymphocytes, plasmocytes, single granulocytes.

Ultrasound of abdominal cavity organs: Hepatosplenomegaly, sediment in the gallbladder, diffuse changes in the liver parenchyma are visualized;

Feces on helminth eggs: negative.

Results of laboratory and instrumental studies confirms the clinical diagnosis of the patient.: Megaloblastic anemia, severe severity (cobalamin and folic acid deficiency). Comorbidities: Community-acquired bilateral polysegmental pneumonia, viral-bacterial, severe. Respiratory failure 3 stage. Bilateral exudative pleurisy. Status after COVID19 dated 08.01.22. CT2.

Treatment was carried out: antibacterial, antifungal and symptomatic therapy. With a pathogenetic purpose, it was prescribed: Cyanocobalamin (Vitamin B12) at a dosage of 400 mcg x 1 time per day in / in drip, folic acid 5 mg, 1 tab x 3 times a day [1]. Hemotransfusion therapy was carried out with blood components: washed erythrocytes according to individual selection, hardware thrombus suspension.

At discharge: total blood count: leukocytes - 4.95 thousand / μl, erythrocytes - 3.47 million / μl, MCV 98.6 fl, MCH 31 pg, HGB - 102 g / l, thrombus - 334 thousand / μl, reticulocytes-15. Biochemical blood test dated 01/31/22: total protein - 64.8 g/l; total bilirubin - 20.1 μmol/l; ALT - 74.8 U / l; AST- 42.9 U / l. According to the results of CT of the chest organs in comparison with the CT scan of 01/18/2022, positive dynamics, CT-1, lesion volume 15%, regression of effusion in both pleural cavities.

Against the background of the therapy, there was an improvement in the condition and normalization of blood counts. Clinically, the girl became more active, her general condition improved, pallor and yellowness of the skin decreased, due to the improvement in her condition, on the 15th day the girl was discharged, with recommendations: dispensary observation for 2 years with a pediatrician at the place of residence; include in the diet foods rich in folate, vitamin B12, vegetables, fruits, gray-grinded cereals, meat products; continue drug treatment with vitamin B12 preparations at a dosage of 400 mcg s / c once a week for 2 months; then 2 times a month for 6 months and folic acid 5 mg, 1 tab x 3 times a day, 1 month; control of the KLA and a biochemical blood test 1 time in 2 weeks - 1 month, then 1 time per month.

Conclusion. In the case described by us, anemia was hyperchromic, hyporegenerative, macrocytic, in combination with an increase in transaminase activity, there was a pronounced three-line aplasia, a decrease in the level of vitamin B12 and folic acid in the blood serum, erythroid proliferation with impaired maturation with signs of megaloblastoidity, aplastic anemia was excluded, acute leukemia. Based on the medical history of the girl, the probable cause of the disease was established: inadequate, poor nutrition, with the exception of animal products. Against the background of the girl's habitual diet and lifestyle, the anemic syndrome developed slowly. The deterioration of the condition was gradual, the first signs of anemia in the girl were still in the fall of 2022, but did not attract the attention of her parents, and only an infectious intercurrent disease forced her to see a doctor. In connection with the identified megaloblastic anemia in a child, it is necessary to conduct a differential diagnosis with other types of anemia, such as aplastic anemia, autoimmune pancytopenia, Marchiafava-Micheli disease. The combination in the clinical picture of severe anemic syndrome, abdominal pain and dyspeptic disorders, icterus of the skin and sclera requires a targeted diagnostic search to exclude oncopathology of the abdominal organs. In all cases of vitamin B12 deficiency, broad tapeworm invasion should be excluded. It is important to find out the features of previous operations on the patient's digestive organs (gastrectomy, resection of the stomach, anastomosis with the presence of a blind loop of the intestine).

Early diagnosis of megaloblastic anemia, timely initiation of treatment and adequate follow-up after the elimination of anemia provide a favorable outcome of the disease and avoid the development of irreversible complications.

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DENGUE FEVER IN THE REPUBLIC OF SAKHA (YAKUTIA): A CASE OF IMPORTED INFECTION

In this article, on the example of clinical observation, the course of imported dengue fever from the Kingdom of Thailand is presented. The disease proceeded with symptoms of lymphadenopathy, rash, hyperemia of the pharynx with symptoms of enanthema and mild intoxication syndrome. It is necessary to differentiate dengue fever from other infectious pathologies that occur with exanthema syndrome, enanthema, and enlarged lymph nodes. An epidemiological history also plays a significant role in the diagnostic search; if tourists who have returned from endemic countries have a fever, it is necessary to conduct an examination for Dengue fever.

Keywords: dengue fever, imported infection, fever, exanthema, lymphadenopathy, Yakutia.

Introduction. After the COVID-19 pandemic, the tourist flow towards Southeast Asia began to gain momentum again. As a result, along with this, the likelihood of the occurrence of various imported infections on the territory of the Russian Federation began to increase, incl. and dengue fever, an acute viral disease caused by an RNA virus of the Flaviviridae family and transmitted by mosquitoes of the genus Aedes [9]. Many cases of dengue infection are asymptomatic or with mild symptoms, but in some cases this infection can cause a more severe course of the disease and even lead to death [2, 5].

It is important to differentiate dengue fever from other infectious pathologies that occur with exanthema syndrome, enanthema, and enlarged lymph nodes. An epidemiological history also plays a significant role in the diagnostic search; if tourists who have returned from endemic countries have a fever, it is necessary to conduct an examination for dengue fever.

Research objective: Clinical analysis of dengue fever in the Republic of Sakha (Yakutia).

Materials and methods: the medical documents of patient V., a 31-year-old man who was hospitalized in the infectious diseases department of the State Budgetary Institution of the Republic of Sakha (Yakutia) "Yakutsk Republican Clinical Hospital" from February 23 to March 3, 2023, were studied. The considered example of dengue infection is the second case on the territory of the Republic of Sakha (Yakutia) [4].

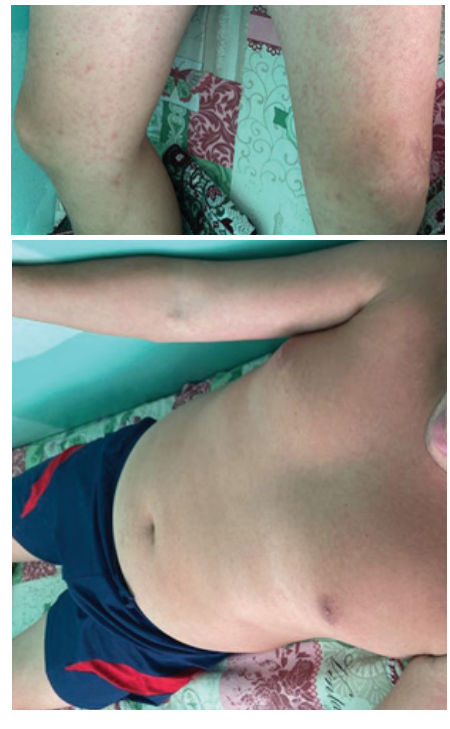
Results: The patient was admitted to the Infectious Diseases Department on February 23, 2023 with complaints of a macular-popular, in some places confluent rash all over the body, slight skin itching and occasional fever up to +37 °C. (photo 1). There was an increase in the parotid, occipital and submandibular lymph nodes, soreness of the behind the ear lymph nodes.

Photo 1. Rash in patient V. at the time of admission (1st day of illness)

From the anamnesis: From January 17, 2023, the patient was with his wife and 3-year-old daughter on vacation in the Kingdom of Thailand (Phuket), lived in a hotel. Denied contact with infectious patient, drank bottled water, fruits, vegetables were thoroughly washed with boiled water. In the first days of rest, while swimming, he was stung by a jellyfish, after which, he noted a slight burning sensation and redness of the skin, near the wrist and on the back in the region of the left shoulder blade, which itself disappeared for 2-3 days. No insect bites were noted. On February 7, 2023, he flew with his family to Moscow, and 2 weeks later (February 22) - to Yakutsk.

On February 23, 2023, he noticed a rash on his chest in the morning and took one tablet of an antihistamine. The rash persisted; body temperature was normal. By the evening of the same day, the parotid, submandibular, and occipital lymph nodes increased. An ambulance was called. The patient, in view of the presence of a rash, lymphadenopathy, as well as the current unfavorable situation with measles, was taken to the infectious diseases department of the «Yakutsk Republican Clinical Hospital» with a preliminary diagnosis of «measles», «mumps».

Upon admission, the condition was noted as moderate. Body temperature



Rash in patient B. at the time of admission (1st day of the disease)

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+36.4 °C. The pharynx and tonsils are hyperemic, loose, clean, without raids. Tongue coated white, moist. Small punctate rash on the soft palate. There was a whitish spot in the area of the buccal mucosa on the right. Breathing through the nose is free, there is no discharge. The voice is sonorous. Breathing is vesicular, carried out through all lung fields, no wheezing. Respiratory rate 18 per minute, SpO2 98%. Heart sounds are muffled, rhythmic. Pulse 84 beats per minute, blood pressure 110/60 mm. rt. Art. The abdomen is soft and painless. Symptom of irritation of the peritoneum is negative. Liver on the edge of the costal arch. The symptom of tapping is negative on both sides. Diuresis is adequate, urine is light. Urination free, painless. The bowel movement is regular, formed. There is no peripheral edema.

According to paraclinical research methods, the report notes: leukopenia (3.98x10* 9 / l), monocytopenia (0.40x10* 9 / l), C-reactive protein is normal, alanine aminotransferase and aspartate aminotransferase are increased (77.3 units / l and 36.6 u/l). A preliminary diagnosis of "Measles" or "Infectious enanthema" was made. The patient was placed in a boxed ward, diet "table number 15", detoxification therapy, mouth rinses with antiseptics, antihistamines were prescribed. During the treatment, the rash disappeared on the 4th day, the body temperature did not increase from the moment of admission.

In the analyzes in dynamics from February 27, 2023, it was revealed: - in the general blood test: leukocytes 6.97 x 109 / l (4.00-10.00) monocytes 8.40% (3.00-12.00) monocytes 0.59 x 109/l (0.12-1.20), basophils ↑ 1.70% (0.00-1.00), basophils ↑ 0.12 x 109/l (0.00 - 0.10), platelets ↑ 315.00 x 109/l (100.00-300.00), thrombocrit ↓ 0.22% (1.08-2.82), ESR ↓ 2.0 mm/h (3.0-10.0);

- in a biochemical study: total cholesterol ↑ 5.3 mmol/l, gammaglutamyl transpeptidase ↑ 81.0 U/l, alanine aminotransferase ↑ 51.4 U/l, aspartate aminotransferase 22.5 U/l, C-reactive protein 1.5 mg /l;

- ELISA for measles anti-IgM - negative, anti-IgG (+), for rubella IgM - negative, anti-IgG (+), for Epstein-Barr virus IgM - negative, anti-IgG (+)

- according to ultrasound examination of the abdominal organs: Diffuse changes in the liver parenchyma. Diffuse changes in the parenchyma of the pancreas. Seal of the pelvicalyceal system of both kidneys.

On February 28, 2023, a consultation was held. Based on the patient's complaints, anamnesis, physical examination, instrumental and laboratory tests, the diagnosis was made: Allergic urticaria, of moderate severity, it was recommended to be tested for dengue fever, PCR for HIV.

Taking into account the above complaints, epidemiological history and clinical data, a serum sample for dengue, zika, chikungunya fever was taken and sent for a reference study to the FBSI SRC VB "Vector" of Rospotrebnadzor.

The reference study by immunochromatography dated March 3, 2023 established the presence of specific class G antibodies to the dengue virus and the final diagnosis was made: "Dengue fever, moderate severity."

IgG antibodies are usually detected in low titers at the end of the 1st week of illness, slowly increase in the future and can remain in the blood of the ill person for life.

Patient V. was discharged on the 8th day from the start of hospitalization with improvement.

The rash completely disappeared on the 4th day. In general, according to the Ministry of Tourism and Sports of Thailand, their country was visited by 435,000 tourists from Russia in 2022, and by the end of 2023, this indicator may increase more than two times [6]. Cases of dengue fever were registered in 2020-2022. in 35 subjects of the Russian Federation. During this period, 159 cases were detected, 93 of which (58.5%) were imported from Thailand, 11 from the Republic of Maldives, 8 from Vietnam. Dengue fever has also been reported in returnees from Africa (Egypt, Tunisia, Central African Republic, South Africa, Kenya, Seychelles, Republic of Chad), South Asia (India, Maldives, Sri Lanka), Southeast Asia (Indonesia, Cambodia, Philippine Islands), East Asia (China), North America (Mexico), West Indies (Cuba, Dominican Republic, Guadeloupe), Europe (Spain). The largest number of cases was detected in Moscow (in 2020 - 28 cases, in 2021 - 5, in 2022 - 16), the Novosibirsk region (in 2020 - 16 cases, in 2021 - 2, in 2022 - 3). During 2013-2019 there was a tendency to an increase in the total number of imported cases of dengue fever to the territory of Russia, and since 2020 - a decrease (Table). Obviously, this is due to the introduction of quarantine measures aimed at containing the COVID-19 pandemic [3].

The activity of mosquitoes that spread dengue occurs during the daytime hours, which is important for the prevention of infection with this infection. To reduce the risk of dengue infection, it is necessary to wear clothing that covers the body as much as possible. During daytime sleep, use mosquito nets, ideally treated with insect repellant. You should also use window screens, repellents containing DEET, picaridin or IR3535, coils and mosquito repellents.

So far, only one vaccine against dengue (Dengvaxia) has been registered and approved for use in some countries. It, however, only protects people who have previously been infected with the dengue virus. There are several other candidate vaccines against dengue currently under study [1, 7, 8].

Conclusion. In connection with the growth of tourism to countries with a trop-

Number of reported cases of dengue fever in Russia in 2013-2022

Year	Country where possible infection occurred								Total for a year
	Thailand	Vietnam	Indonesia	India	Philippines	Maldives	Sri Lanka	Other	
2013	127	9	12	6	4	1	0	11	169
2014	54	17	12	4	4	2	1	11	105
2015	65	23	19	3	3	4	3	16	136
2016	45	32	26	4	9	2	3	24	145
2017	100	34	6	10	6	3	4	33	196
2018	179	25	1	4	0	8	1	41	259
2019	260	64	16	10	8	16	14	27	415
2020	88	5	3	1	3	1	2	18	121
2021	0	1	1	1	0	2	2	2	9
2022	5	2	1	3	0	8	4	6	29
Total	923	212	97	46	37	47	34	189	1584
Distribution in %	58.4	13.3	6.1	2.9	2.3	3	2.1	11.9	100

ical and subtropical climate, where there is a high risk of infection with the dengue fever virus, it is necessary that doctors of narrow specialties, district therapists and pediatricians should be informed and familiarized with the theoretical basis of the clinic of this infection, since timely detection and provision of proper medical care contribute to a significant reduction in mortality rates from severe cases of dengue.

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A CLINICAL CASE OF NON-IMMUNE HYDROPS IN A PREMATURE NEWBORN WITH A CHROMOSOMAL ANOMALY

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The article presents the features of diagnosis and dynamic monitoring of a premature baby with non-immune dropsy on the background of a chromosomal anomaly (Down syndrome). The data of scientific literature on the epidemiology and etiology of this disease are analyzed, as well as the outcomes of the disease are considered.

Keywords: non-immune fetal hydrops, NIFH, premature newborn, chromosomal abnormalities, trisomy of chromosome 21, Down syndrome.

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Introduction. Non-immune fetal hydrops (NIFH) - is defined as an accumulation of extravascular fluid in two or more serous cavities and fetal tissues that occurs at various stages of pregnancy in the absence of circulating antibodies to erythrocyte membrane antigens. Fluid accumulation can be in the subcutaneous space (more than 5 mm), pericardial, pleural and abdominal cavities [1, 2, 3]. NIFH was first described in 1892 by J.W.Ballantyne [1].

The registered incidence of non-immune fetal hydrops is about 3 per 10,000 births, however, the incidence is significantly higher due to spontaneous or artificial termination of pregnancy in the first and second trimesters [2].

Non-immune fetal hydrops is based on various causes (more than 150 nosologies), which, according to the results of a large-scale study conducted by Bellini et al. (a systematic review that included an analysis of 5,437 cases of NIFH) were divided into 14 categories. Among them, chromosomal abnormalities occupy one of the leading places and are represented by trisomies, triploidies and monosomy X, regardless of the presence of concomitant fetal malformations [3].

Due to the widespread use of ultrasound, in most cases non-immune hydrops is diagnosed prenatally [4]. The criterion for diagnosis is an excessive accumulation of fluid in two or more areas of the body (chest, abdominal cavity, pericardium, skin). In most cases, NIFH is a fatal condition - mortality with NIFH, according to some data, reaches 90%. NIFH over time, as a rule, remains unchanged or gradually increases, however, cases of its spontaneous resolution are described. Information about the prognosis for fetuses with non-immune dropsy is extremely scarce due to high

Table 1

Complete blood count

	in 1 day	in 2 day	in 3 day	in 4 day	in 5 day	in 6 day	in 7 day	in 8 day	in 9 day	in 10 day	in 11 day
Hemoglobin. g/l	161	123	110	98	126	126	118	129	119	125	109
Red blood cells. x1012/l	3.92	3.02	2.75	2.4	3.56	3.69	3.48	3.74	3.74	4.05	3.57
Hematocrit. %	47.1	36.3	32.9	27.9	36.7	36.6	34.7	37.7	35.2	38.1	33.5
White blood cells. x109/l	50.5	20.12	7.74	7.7	7.59	9.18	6.8	5.1	3.97	5.79	6.05
Blasts. %	14	9	2	-	4	0	0	0	0	0	0
Myelocytes. %	5	2	2	-	5	2	1	1	0	0	0
Metamyelocytes. %	1	2	1	-	0	2	0	0	0	0	0
Stab neutrophils. %	5	2	1	-	1	0	0	1	0	0	2
Segmented neutrophils . %	27	66	67	-	65	58	55	39	14	23	27
Lymphocytes. %	42	15	18	-	19	30	38	52	78	59	59
Eosinophils . %	3	0	1	-	2	0	0	0	2	1	2
Basophils. %	0	0	0	-	0	1	1	1	0	1	0
Monocytes. %	3	4	8	-	4	7	5	6	6	16	10
Platelet count. x109/l	103	91	70	71	80	111	118	169	125	209	202
Reticulocytes. %	-	-	-	-	-	-	6.28	8.08	5.7	6.12	10.96

mortality. The mortality rate directly depends on the cause of NIFH. [5, 6]

Clinical case. Child R., male, from a 41-year-old mother, was born at a gestation period of 32/3 weeks. The woman has this sixth pregnancy, has a history of three artificial abortions, premature and urgent childbirth at 36 and 37 weeks, respectively (the children are healthy). The woman got registered in the women's consultation for this pregnancy at 17 weeks. From anamnesis: chronic nicotine intoxication, chronic gastritis, cholecystitis in remission, allergy (bronchospasm) to penicillin antibiotics, overweight (BMI 29.74). Pregnancy proceeded against the background of acute respiratory infection at 14 weeks with catarrhal phenomena without an increase in body temperature.

Due to the late referral of the woman to the women's consultation, biochemical and ultrasound screening of the first trimester was not carried out. The first ultrasound was performed at the time of 22.4 weeks, revealed placenta previa and marginal attachment of the umbilical cord to the placenta without signs of circulatory disorders.. Considering her age (41 years), she was consulted by a geneticist: the risk of birth defects is general population, the risk of chromosomal pathology is increased. She refused to perform an invasive diagnosis. At the gestation period of 32.3 weeks, according to ultrasound data, signs of non-immune fetal dropsy were revealed for the first time. During ultrasound in dynamics after 10 days, signs of non-immune hydrops (ascites, hydropericardium, hydrothorax) progress, polyhydramnios is diagnosed (amniotic fluid index - 361 mm). A survey was conducted on the TORCH complex, the results were negative.

Premature labor at 33 weeks, 2 days, naturally, the duration of the first period of labor is 5 hours 30 minutes, the second period is 14 minutes, prenatal discharge of amniotic fluid, anhydrous interval of 8 hours. A boy was born with a weight of 3050g, a length of 46cm, a head circumference of 31cm, a chest circumference of 30cm. Apgar score is 2/6 points, Silverman score is 10 points. At birth, the child's condition was regarded as extremely severe in terms of the severity of respiratory failure, edematous syndrome, and central nervous system depression. Primary resuscitation measures were carried out according to the methodical letter "Resuscitation and stabilization of newborns in the delivery room" [7]. When assessing the physical development of the child by the centile method, the body weight exceeded the 90th centile on the Fenton scale. The indicators of height and head circumference were within the average values (10-90 centile).

The child's condition remained extremely severe for three days, which was due to severe respiratory insufficiency, hemodynamic instability, pronounced edematous syndrome to the degree of anasarca. Spontaneous motor activity is reduced, diffuse hypotension, innate reflexes are suppressed. Breathing on the background of a ventilator is sharply weakened in both halves of the chest, auscultation is practically not heard. Heart tones are deaf rhythmic with a tendency to bradycardia (90-120 in 1 min). Hypotension. The abdomen is sharply enlarged in volume, tense. Palpation of the liver and spleen is difficult.

Particularly noteworthy were the phenotypic signs characteristic of Down syndrome: a flat profile of the face (in particular, flattening of the bridge of the nose), a flattened nape, additional skin folds around the back of the neck, epicanthic folds are present in the inner corners, ears are small and rounded, hands are short and wide, transverse palmar fold, fingers are short, macroglossia.

Respiratory ventilator support in PCV mode has been continued in the NICU

Table 2

Coagulogram in dynamics

Day of life	APTT	PTT	INR	TT	Fibrinogen g/l
1	38	14.9	1.29	27.3	0.36
3	59	14.6	1.17	78.8	0.32
6	49.2	13.4	1.17	17.2	1.3

Table 3

Dynamics of indicators of loss of body weight, rate of diuresis

Day of life	1	2	3	4	5	7	8	9	11	12	13	14	15
Body weight, g	3050	2960	2780	2575	2466	2339	2322	2250	2070	2040	2030	2071 (+41)	2103 (+32)
Loss of body weight, %	-	3	9	15	19	23	24	26	32	33	33	-	-
Rate of diuresis, ml/kg/h	1.7	2.9	3	2.0	2.5	3.7	5.2	4.4	5	5.3	2.6	3.8	3.7

Table 4

Biochemical parameters of blood plasma in dynamics

	in 1 day	in 3 day	in 4 day	in 6 day	in 8 day	in 13 day	in 25 day	37 day
Total protein, g/l	33.4	23.2	31.5	31.7	36.7	46.3	47.8	46.0
Albumin, g/l	22.2	-	-	23.1	27.1	32.3	-	-
Alaminoninotransferase (ALT), U/L	32.21	23.1	-	10.7	9.7	9.4	25.37	38.2
Asparto-aminotransferase (AST), U/L	79.75	-	-	12.0	13.9	15.9	74.28	114.6
Glucose, mmol/L	2.06	2.7	3.5	3.7	-	-	5.17	3.98
Urea, mmol/L	3.85	-	7.77	12.53	6.94	6.26	1.67	1.25
Creatinine, μmol/L	68.77	-	98.4	108.4	90.5	78.8	46.26	43.56
Total bilirubin, μmol/L	42.3	-	273.9	270.0	281.8	196.4	163.9	149.6
Bilirubin straight, μmol/L	9.8	-	28.0	48.6	58.3	79.3	71.9	62.0
Alkaline phosphatase, U/L	-	-	-	-	-	541.4	1051.2	1421.9
C-reactive protein(CRP), mg/l	2.51	1.9	-	8.8	6.3	1.4	3.13	-

ward. During the first hour of life, negative dynamics was noted due to the increase in signs of respiratory failure, an increase in the need for O2 subsidies to 60%, and replacement surfactant therapy was performed. Radiologically, signs of bilateral polysegmental pneumonia were revealed. According to echodopplercardiography: atrial communication 5.5 mm with left-right reset, functioning arterial duct 3.3 mm, bidirectional reset. The right parts of the heart are enlarged. Ejection fraction is 73%, there are no signs of effusion in the pericardial cavity. Ultrasound of pleural cavities - echographic signs of bilateral hydrothorax, the probability of compression atelectasis. (Fig. 1) Puncture and drainage of the right pleural cavity was performed, 22.0 ml of cloudy yellow liquid was obtained. The initial antibacterial therapy has been started. On the second day of life after the ultrasound examination of the pleural cavities, an increase in hydrothorax on both sides was revealed, pleural drains were installed by the surgeon. Hemorrhagic syndrome was noted. With a substitution purpose, therapy was performed: vikasol, transfusion of NIFH. On the third day of life, according to the control ultrasound, the presence of free fluid in the abdominal cavity in the volume of 160.0-180.0 ml was revealed. A pediatric surgeon drained the abdominal cavity, 179.0 ml of fluid was evacuated during the day. General clinical blood tests were performed in the NICU (Tables 1, 2). The features of the course of the first days of life were anemia, leukocytosis and thrombocytopenia. Antibacterial therapy with aminoglycosides in age-related dosage was enhanced, hypoproteinemia was corrected. In the future, positive dynamics was noted:the ventilation mode of the ventilator was changed to SIMV+PC, hemorrhagic syndrome was stopped, anemia was corrected by replacement hemotransfusion with erythrocyte mass, a significant decrease in edema with a significant weight loss relative to the initial body weight. According to the Echo-KG in dynamics: fenestrating defect of the atrial septum 6.5mm, reset of the left.

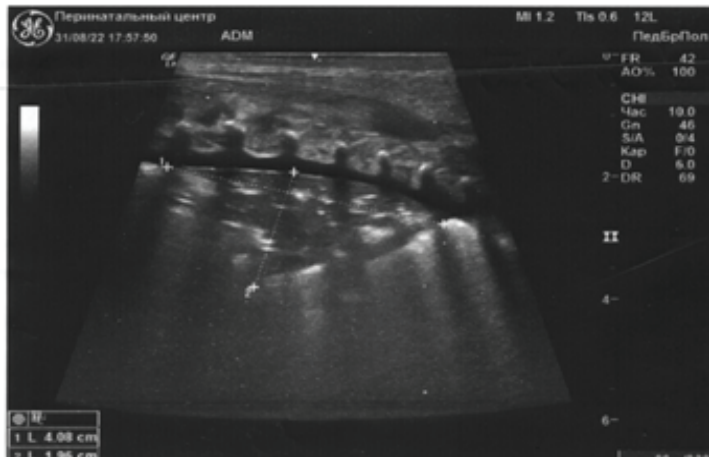

Fig. 1. The area of consolidation of lung tissue along the posterior surface of the left lung in the projection of the lower lobe

Enteral feeding was started from the first day with expressed native milk with a gradual expansion of enteral nutrition to the physiological norm. Consulted by a neurologist, geneticist. Karyotyping was performed – an abnormal karyotype was

Fig. 2. Abnormal karyotype: trisomy of chromosome 21 (Down syndrome)

revealed: trisomy on chromosome 21 (Fig. 2).

At the time of discharge, the condition is stable and satisfactory. Sucks actively from the chest, does not regurgitate. Lactation is sufficient. The skin is clean, pale pink, there is no swelling. In the lungs, the breathing is puerile, there are no wheezes. Heart tones of medium sonority, rhythmic. The abdomen is soft, accessible to deep palpation. Moderate hepatosplenomegaly persists: liver +3.0cm, spleen +1.0 cm. The chair is independent, regular. Urinates enough. Body weight on the day of discharge (38 days of life) – 2427g, postconceptual age 39 weeks. The prognosis for life is favorable.

Conclusion. In the described patient, taking into account the anamnesis data, the results of antenatal diagnosis and the condition at birth, the preliminary diagnosis was formulated as follows: "Intrauterine infection of unspecified etiology. Non-immune dropsy", due to the fact that infectious processes and, first of all, parvovirus infection are one of the causes of NIFH. However, a thorough diagnostic search, taking into account the identified characteristic phenotypic signs, made it possible to exclude the infectious genesis of NIFH and establish the correct clinical diagnosis and cause of non – immune dropsy in this case - trisomy on chromosome 21 (Down syndrome). The key feature of this clinical case is the complexity of differential diagnosis, as well as the high probability of death in such patients. A description of a clinical case of non-immune hydrops in a premature newborn with a chromosomal anomaly and the experience of managing a newborn child with this disease and a life-friendly outcome may provide.

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