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ORIGINAL RESEARCH

N. V. Zaitseva, M. A. Zemlyanova, Yu. V. Koldibekova, E.V. Peskova

STUDY OF NEGATIVE EFFECT INDICATORS IN CHILDREN UNDER THE INFLUENCE OF ADVERSE FACTORS OF THE SUBARCTIC CLIMATE

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Currently, considering the regional features of subarctic climatic conditions affecting the increased public morbidity is of importance. There it is necessary to carry out in-depth study devoted to changes in the indicator level reflecting the negative effects of target organs. This study aims at examining the biochemical and general clinical indicators of negative effects in children living under the adverse factors of the subarctic climate.

Materials and methods. The object of the study was the state of children's health. The exposure of adverse factors of the subarctic climate, the values of biochemical and general clinical indicators were assessed, information regards children's diseases was analyzed, statistical data processing was carried out using the Statistica 10 program and the dependence between the frequency of the disease and the impact of a complex of adverse climatic factors was parameterized.

Results and discussion. Under the influence of adverse factors of the subarctic climate, changes in the level of biochemical and general clinical indicators were found out in children 4-7 years old. This indicators prove the development of such negative effects as stress of thyroid function (an increase by 1.4 times in the level of TSH in blood serum), the formation of an inflammatory process (an increase of up to 1.7 times in the level of leukocytes and ESR in the blood), the risk of early vascular disorders (a decrease by 1.2 times in Apo A1 and an increase of up to 1.3 times in the level of Apo-B and Apo-B/ApoA1 in blood serum), deterioration of endogenous vasomotor activity in the myocardial tissues and neuro-endocrine regulation (a decrease by 1.2-2.5 times in the content of cortisol and serotonin, and, on the contrary, an increase in the level of adrenaline in the blood). 1.2-5.6-times increased frequency of functional disorders of the nervous, endocrine and circulatory systems proves the negative effects regards the target organs. This might happen due to the complex impact of adverse factors of the subarctic climate.

Conclusion. The found indicators of negative effects should be used to monitor the health state and improve the effectiveness of the development of medical and preventive measures for children living under the influence of adverse (extreme) factors of the subarctic climate.

Keywords: adverse factors of the subarctic climate, air temperature, air humidity, wind speed, target organs, negative effects, biochemical and general clinical indicators, child population.

Introduction. The climate is one of the priority environmental factors that determines the comfort of living conditions. Low temperature, high relative humidity and wind speed, huge atmospheric pressure swings typical for territories with a subarctic climate can have both direct and indirect long-term adverse effects on human health [3-5]. It is known that these adverse effects lead to the disorders in the regulatory homeostasis mechanisms, the development of maladaptive reactions, an increase in the rate of oxidation-reduction processes, stress on the mechanisms of immuno-hormonal regulation, blood circulation, and the bronchopulmonary system, and, as a result, an increased chronic morbidity in the population living under extreme exposure to adverse climatic factors [3-5, 9]. Considering the regional features of subarctic climatic conditions leading to an increased morbidity, in children in particular, as the most sensitive subpopulation to the effects of environmental factors, requires to perform in-depth study of changes in the indicator level showing negative effects of target organs.

In this regards, this study aims at examining the biochemical and general clinical indicators of negative effects in children living under the adverse factors of the subarctic climate. The found biomarkers of negative effects can be used to improve the monitoring system of the public health status and to develop effective measures for the prevention of non-communicable diseases associated with the impact of adverse (extreme) factors of the subarctic climate.

Materials and methods. The study object was the health state of children living under adverse factors of the subarctic climate. The comparison area is characterized by an extreme continental climate, which differs from the observation area by milder climatic conditions, namely, positive long-term temperatures, weak winds, and short warm summers. The observation and comparison areas have almost the same population endowment, socio-economic indicators, and minimal chemical pollution of atmospheric air.

General information in terms of climate factors (temperature and relative humidity, wind speed, atmospheric pressure) was obtained from meteorological observations from 01/01/2016 to 31/12/2018 provided by the Federal Service for Hydrometeorology and Environmental Monitoring of the studied areas and open sources of climate and geographical information. As a scenario for the exposure of climatic factors, their complex impact is taken for 11 months per year within 70 years. At that, annual leave outside the residential area was not considered. The most effective and informative indicator reflecting the complex impact of three climatic factors (air temperature and humidity, wind speed) is the normal equivalent-effective temperature (NEET). It was calculated using the formulas A. Missenard [16] and I. V. Buteva [1]. The values of NEET in the range from 12 to 24 °C are accepted as comfortable and sub-comfortable [2]. The atmospheric pressure exposure is calculated based on the value of daily atmospheric pressure differences in the ranges 0 – 3 hPa, 3.1 – 6 hPa, 6.1 – 17 hPa for the year season, which is the difference between the average atmospheric pressure

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values for the day between subsequent days. The climatic factor exposure was assessed by the specialists of the Risk Analysis Department (Lear D. N., Head of the Department, Candidate of Medical Science).

218 children aged 4-7 years living in the subarctic climate areas (observation group) were examined. The comparison group consisted of 109 children exposed to milder climatic factors. The examination of children was carried out in compliance with the ethical principles of the Helsinki Declaration (WMA Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects, 2013) and approved by the Committee on Biomedical Ethics of the FBSI "Federal Scientific Center for Medical and Preventive Health Risk Management Technologies" . During this process, we collected informed voluntary consent of a legal representative, because it is a must. The conducted studies did not infringe on the rights, did not endanger the well-being of children, and did not harm their health.

Laboratory tests included biochemical and general clinical indicators reflecting possible negative effects of target organs: the level of red blood cells, white blood cells, hemoglobin, erythrocyte sedimentation rate (ESR) of blood, the content of apolipoproteins A1 (Apo A1) and B100 (Apo-B) with the determination of their ratio, the level of thyroid-stimulating hormone (TSH) and thyroxine (T4), serum cortisol, catecholamines (epinephrine, dopamine, norepinephrine, serotonin) in the blood plasma. The obtained values of indicators in children of the observation group were compared to the indicators in the comparison group. The results of the conducted studies are presented in the form of the mean value (X), the standard error of mean (SEM) and the interquartile range (Q_{25}-Q_{75}).

Children's diseases were analyzed based on the results of a comprehensive objective medical examination meeting the criteria of the International Statistical Classification of Diseases and Related Health Problems. What is more, the ICD-10 was used to determine the number of the diseases during the examination. The medical examination was carried out by specialists of the mobile team of the Department of Hygiene of Children and Adolescents (Valina S.L., Head of the Department, Candidate of Medical Sciences). To identify priority diseases from critical organs and systems, the following criteria were applied: the level of morbidity in the observation group that differs significantly from the level of morbidity in the comparison group ($p < 0.05$). To establish the relationship between the disease frequency (according to the Federal Compulsory Medical Insurance Fund) and the impact of a complex climatic factors, the dependencies were parameterized using the linear regression analysis method by the formula:

$$y = b_0 + b_1 \cdot x_1 + b_2 \cdot x_2 + b_3 \cdot x_3 + b_4 \cdot x_4 + b_5 \cdot x_5 , \quad (1)$$

where y is the incidence of children population, sl./1000; x is a measure of the level of climate impact, °C, or hPa; b_0 is the parameter of the model intercept term; b_1 is the parameter characterizing the action of NEET; b_2 is the parameter characterizing the action of atmospheric pressure; b_3 is a parameter characterizing the effect of changes in NEET; b_4 is the parameter characterizing the effects of changes in atmospheric pressure; b_5 is the parameter characterizing differences in the incidence of children population between the areas of observation and comparison due to the other factors.

Modeling of cause-and-effect relationships was carried out by specialists of the Department of Mathematical Modeling of Systems and Processes (Kiryanov D.A., Head of the Department, Candidate of Technical Sciences). Statistical analysis of the data was carried out using the program Statistica 10. by nonparametric Mann-Whitney test. To assess the significance of the differences, the p -confidence criterion (≤0.05) was used [2].

Results and discussion. Our research showed that children living in the observation area are more exposed to adverse factors of the subarctic climate (a decrease of up to 4.3 times in the NEET values and an increase of up to 2.4 times in the daily changes in atmospheric pressure with a larger amplitude) relative to the comparison area.

Under exposure to the studied factors in the children of the observation group, a statistically significant increase of up to 1.7 times in the level of white blood cells and ESR of the blood relative to the indicators in the children of the comparison group was observed (Table. 1). It means the development of an inflammatory reaction of the target organs and systems, bronchopulmonary system in particular.

The effect of inflammation, including on the organs of the bronchopulmonary system, is characterized by damage and violation of the integrity of the bronchial mucosa, a decrease in the thickness of the alveolar-capillary membrane, the egestion of inflammatory mediators, and further progression of the process-, the development of morpho-functional changes in the respiratory parts of the lungs [7, 11]. In children of the observation group, a stress of thyroid function was observed. It was proved by a 1.4-times increase in the level of TSH in the blood serum relative to the same indicator in the comparison group (p=0.0001).

According to the data of annotated scientific sources, under the influence of low atmospheric temperatures, especially their differences, there is an increase in the level of thyroid hormones in the blood responsible for tolerance of low temperatures due to an increase in oxygen consumption and an increase in heat production [4, 6]. At the same time, steady stress of the thyroid function might lead to a violation of ventricular relaxation, the appearance of supraventricular arrhythmias, an increase in blood pressure and a further cascade of pathological processes. As a result, it may lead to vascular disorders and heart failure [15].

The assessment of indicators characterizing the risk of early development of vascular disorders in children of the observation group relative to the comparison group indicates a 1.2-times decrease in Apo A1 and an increase up to 1.3-times in the level of Apo-B and Apo-B/ApoA1 in the blood serum (p=0.0001). The found changes in lipoprotein levels are similar to the results of other studies devoted to the development of vascular disorders in children, which may result in the development of atherosclerotic changes in an older age group (younger than working age) when exposure to low temperatures keeps up [5].

It is known that all stages of the atherosclerosis development from early endothelial dysfunction to the formation of atherosclerotic plaques can cause myocardial hypoxia, pro-inflammatory cytokines leading to local arrhythmogenic activity. What is more, sympatho-adrenal regulation is also violated [10]. Therefore we should consider the change in the level of a number of hormones and neurotransmitters reflecting the dysregulation of the sympatho-adrenal system. Thus, in the children of the observation group relative to the comparison group, a decrease in the content of cortisol (1.2 times) and serotonin (2.5 times) (p = 0.0001-0.040) was found, with an increased level of adrenaline in the blood (p = 0, 0001). Low cortisol levels correlate with disorders in the central regulation of corticotropin-releasing factor production, which is carried out by the limbic structures of the brain associated with the production of neurotransmitters,

Table 1

The average value, the error of the average, the interquartile range of the studied indicators in the groups of examined children

Indicator	Observation group (n=218)		Comparison Group (n=109)		The significance of the differences p≤0.05
	$\bar{X}$±SEM	Q_{25}-Q_{75}	$\bar{X}$±SEM	Q_{25}-Q_{75}	
Blood					
Red blood cells, 10^{12}/dm^3	4.71±0.06	4.5-4.9	4.41±0.07	4.2-4.6	0.0001
Hemoglobin, g / dm^3	132.87±1.72	127-139	132.71±1.63	128-137	0.900
White blood cells, 10^9/dm^3	6.68±0.35	5.3-7.7	5.85±0.38	4.6-6.8	0.0001
ESR, mm / hour	7.53±0.65	5-10	4.35±0.38	3-5	0.0001
Blood serum					
TSH, mcm/cm^3	3.45±0.22	2.2-4.3	2.43±0.23	1.6-2.9	0.0001
T4 free, pmol/dm^3	12.37±0.29	10.92-13.62	13.76±0.32	12.71-14.73	0.0001
Apo-B/ApoA1, g/dm^3	0.57±0.027	0.5-0.62	0.45±0.035	0.32-0.53	0.0001
Apo A1, g/dm^3	1.42±0.03	1.35-1.50	1.69±0.09	1.35-2.02	0.0001
Apo-B, g/dm^3	0.82±0.03	0.72-0.89	0.72±0.04	0.63-0.76	0.0001
Cortisol, nmol/cm^3	241.59±18.71	160.5-318.9	281.85±31.89	178.6-362.4	0.040
Blood plasma					
Epinephrine, pg/cm^3	79.49±2.01	74.3-84.6	69.55±3.97	59.8-78.4	0.0001
Dopamine, pg/cm^3	58.44±2.9	50.7-65.9	59.35±3.91	52.4-66.8	0.710
Norepinephrine, pg/cm^3	383.99±19.29	328.4-422.7	384.23±24.22	341.8-455.0	0.990
Serotonin, ng/cm^3	99.18±13.57	66.5-133.1	250.06±29.05	188.4-290.9	0.0001

including serotonin [12]. The imbalance between the secretion of catecholamines and serotonin in the blood under the influence of adverse climatic factors is likely one of the violation of the body's protective and adaptive response. It results in a decrease in resistance to hyperthermia, hypoxia, as well as deterioration of endogenous vasomotor activity in myocardial tissues and impaired cardiomyocyte metabolism [6, 13]. In turn, the imbalance of neurotransmitters has both direct and indirect effects on the activity of the hypothalamic-pituitary-thyroid system [8].

The found changes in indicators characterizing the negative effects of the respiratory, endocrine, nervous and circulatory systems are confirmed by the increased degree of incidence of the mentioned target systems. Thus, when compared to the comparison group, children of the observation group have an increased incidence of diseases of the circulatory system (sinus node weakness syndrome) up to 5.6 times (p=0.0001-0.007), the nervous system (functional disorders) up to 2.6 times (p=0.031), the respiratory system (tonsillar hypertrophy) up to 1.7 times (p=0.010-0.024), the endocrine system (unspecified thyroid disease) up to 1.2 times (p=0.033) (Table 2).

The established frequency of unspecified thyroid diseases (5.9 %) and cardiomyopathy in children in the observation group in the absence of these diagnoses in the comparison group (p=0.010) should be addressed. The obtained data in terms of the morbidity structure of examined children correspond to the results of Russian and foreign studies considering the influence of unfavorable climatic conditions on the formation of diseases of the respiratory system, neuro-endocrine system and circulatory organs [3, 7, 9, 14, 17, 18].

The found trends correlate with a 1.2-2.9-times increase in the primary in-

Table 2

Comparative analysis of the morbidity structure in children of the studied groups, %

Disease class/ Nosology (ICD-10)	Degree of incidence, %		Statistical significance in groups (p≤0.05)
	Observation group (n=218)	Comparison group (n=109)	
Respiratory diseases (J00-J99), including:	59.2	44.9	0.010
- hypertrophy of the palatine tonsils (J35. 1)	24.8	14.7	0.024
Circulatory diseases (I00-I99), including:	18.8	5.5	0.0001
- unspecified cardiomyopathy (R01. 0)	5.9	0.0	0.010
- Sinus node weakness syndrome (I49. 5)	10.1	1.8	0.007
Diseases of the endocrine system (E00-E920), including:	53.7	44.95	0.033
- thyroid diseases, unspecified (E07)	5.9	0.0	0.010
Functional disorders of the central nervous system and autonomic vegetative nervous system, including: - Autonomic dysfunction syndrome (G90. 8); - astheno-neurotic syndrome (G93. 8)	11.9	4.6	0.031

cidence rate of the children in the observation area (according to the data on the number of people seeking medical care during the analyzed period) in terms of diseases of the endocrine, nervous and circulatory systems. The analysis of the relationship between the frequency of the disease and the complex impact of adverse climatic factors enabled us to determine a reliable dependence increase in eventual developing of diseases due to the complex influence of adverse factors of subarctic climate, including: nervous system (functional disorders) (R^2=0.12-0.80; 6.71≤b_0≥114.99; -0.001≤b_1≥-0.09; 0.01≤b_2≥-0.15; 0.006≤b_4≥=0.08; 1.31≤b_5≥ 2.29; p=0.0001)endocrine system (R^2=0.32; b_0=37.58; b_2=-0.048; b_4=0.17; b_5=2.22; p=0.0001), and the circulatory system (cardiac conduction disorder) (R^2=0.35; b_0=-4.24; b_2=0.008; b_4=0.09; b_5=2.65; p=0.0001).

Conclusion. Under the influence of adverse factors of the subarctic climate, children aged 4-7 years suffer from changes in the level of biochemical and general clinical indicators characterizing the development of negative effects in the form of stress of thyroid function, the formation of the inflammatory process, the risk of early vascular disorders, deterioration of endogenous vasomotor activity in myocardial tissues and neuro-endocrine regulation. 1.2-5.6-times increased frequency of functional disorders of the nervous, endocrine and circulatory systems proves the negative effects regards the target organs. This might happen due to the complex impact of adverse factors of the subarctic climate. The found indicators of negative effects should be used to monitor the health state and improve the effectiveness of the development of medical and preventive measures for children living under the influence of adverse (extreme) factors of the subarctic climate.

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A.A. Tappakhov, T.E. Popova, T.G. Govorova, N.A. Schneider, E.E. Vaiman, R.F. Nasyrova

ASSOCIATION OF SINGLE NUCLEOTIDE VARIANTS IN THE *DRD3* AND *LINGO1* GENES WITH THE DEVELOPMENT OF DRUG DYSKINESIAS IN PARKINSON'S DISEASE: RESULTS OF A PILOT STUDY

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Levodopa is the "gold" standard of pharmacotherapy for Parkinson's disease (PD). Levodopa has its own advantages, such as high efficiency at all stages of the disease, low incidence of side effects, availability, at the same time, long-term levodopa therapy is associated with the development of levodopa-induced dyskinesias (LID). Approximately one third of patients in the fifth year of illness already have LID; by the 10th year of illness, almost all patients have this disorder. LID can be associated with gene polymorphisms, the products of which are involved in the metabolism of levodopa. The aim was to study the association of single nucleotide variants (SNV) rs6280 (DRD3 gene) and rs9652490 (LINGO1 gene) with the development LID in PD. The study included 47 patients with PD, 21 (44.7%) men and 26 (55.3%) women. The average age was 69.0 ± 7.67 years. Patients with a mixed form of PD predominated (72.3%). The average duration of the disease was 5.94 ± 4.09 years. Results. Patients with PD in both groups (with and without LID) did not differ in age, gender and ethnicity, stage of disease, non-motor symptoms, and degree of cognitive impairment. At the same time, patients with LID showed frequent development of motor fluctuations, a longer duration of levodopa therapy, and a higher levodopa equivalent daily dose. Analysis of the effect of DRD3 (Ser9Gly, or rs6280) and *LINGO1 (rs9652490)* polymorphisms on the development of LID in PD was not found. Conclusion. The results of a pilot study indicate the absence of a predictive role of the carriage of SNV rs6280 (DRD3 gene) and rs9652490 (LINGO1 gene) on the development of LID in PD patients living in the Republic of Sakha (Yakutia). However, the authors do not exclude the influence of a small sample size on the results of the associative genetic study.

Keywords: Parkinson's disease, movement disorder, levodopa, levodopa-induced dyskinesia, side effect, pharmacogenetics, personalized medicine, single nucleotide variants, *DRD3, LINGO1.*

Introduction. Parkinson's disease (PD) is one of the most common neurodegenerative diseases in the world [1, 24]. Levodopa remains one of the effective drugs in PD pharmacotherapy [5, 19, 24]. Levodopa has advantages such as high efficacy at all stages of the disease, low incidence of side effects, availability, however long-term therapy is associated with the development of levodopa-induced dyskinesias (LID) – choreiform hyperkinesias that result from overstimulation of dopamine receptors [4, 14]. The development of LID is mainly based on the continuing death of nigrostiary neurons with a loss of their "buffer capacity" [3]. Approximately one third of patients already have LID by the fifth year of the disease, and by the 10th year of the disease all patients may have this complication [16].

It has been established that LID can be associated with gene polymorphisms, the products of which are involved in the levodopa metabolism. Thus, A allele of COMT gene (rs4680), AA genotype of MAO-B gene (rs1799836, A644G), T allele SLC6A3 gene (653 + 4065C>A, rs393795), allele 15 of DRD2 gene (CAnSTR) and other polymorphisms were associated with earlier development of LID in patients with PD [7, 9, 12, 22, 27]. At the same time, there is a limited number of studies on the effect of mutations in the DRD3 and LINGO1 genes on the development of LID.

The DRD3 gene encodes the D3 subtype of dopamine receptors, which are in the presynaptic membranes of nerve cells (autoreceptor) and in postsynaptic membranes. A large number of D3 receptors are localized in the limbic system, which is associated with cognitive, emotional and endocrine functions. Carriage of single nucleotide variants of the DRD3 gene can influence the formation of a therapeutic response to antiparkinsonian and antipsychotic drugs, the development of side effect and contribute to the development of alcohol, nicotine and heroin addiction [11, 30].

The aim was to study the association of single nucleotide variants (SNV) rs6280 (DRD3 gene) and rs9652490 (LINGO1 gene) with the development of LID in PD.

Material and methods. The object of the study was patients with PD, observed at the Center for Extrapyramidal Disorders and Botulinum Therapy of the Clinic of the M.K. Ammosov North-Eastern Federal University (Yakutsk). The research protocol was approved by the Local committee on biomedical ethics of the Yakutsk Scientific Center for Complex Medical Problems (Protocol No. 43, November 9, 2016).

The study included 47 patients with PD who underwent careful history and clinical selection using inclusion and exclusion criteria. Inclusion criteria: 1) clinically reliable diagnosis of PD according to the MDS criteria [15]; 2) the ability to complete the full scope of research; 3) the patient's consent to genetic analysis. Exclusion criteria: 1) cases of secondary and tertiary parkinsonism; 2) failure to

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Table 1

Nucleotide sequence of primers

Single nucleotide variants, gene, localization	Nucleotide sequence of primers
rs6280, DRD3, 3q13.31	Forward: GTAGGAGAGGGCATAGTAG Reverse: CTGTCTCCTCACAGGAAG
rs9652490, LINGO1, 15q24.3	Forward: AGGAGAAGAAAAGAGGTG Reverse: GGAGAATAGGAAGGAGAC

Table 2

Clinical and anamnestic comparison of patients with Parkinson's disease and with / without levodopa-induced dyskinesia

Parameter	PD – LID	PD + LID	p-level
Age, year	69.0 [64.0; 75.8]	68.0 [62.0; 75.0]	0.473
Men / women, abs	20 / 20	1 / 6	0.112
Yakut / Russian, abs.	25 / 15	4 / 3	0.55
Duration of illness, year	4.5 [2.0; 7.75]	6.0 [5.0; 12.0]	0.065
The stage of the disease on the Hoehn-Yahr scale	3.0 [2.0; 3.0]	3.0 [3.0; 3.0]	0.567
Number of NMSs by NMSQuest	10.5 [5.25; 13.75]	7.0 [4.0; 9.0]	0.119
MoCA, score	22.5 [16.0; 25.75]	24.0 [17.0; 27.0]	0.6
MMSE, score	27.5 [23.25; 30.0]	29.0 [24.0; 30.0]	0.465
FAB, score	17.0 [11.25; 18.0]	17.0 [13.0; 18.0]	0.654
3 part UPDRS, score	46.5 [29.25; 56.0]	27.0 [24.0; 53.0]	0.22
Motor fluctuation, %	17.5	57.1	0.04
Levodopa-therapy, %	82.5	100	0.57
Levodopa therapy experience, years	2.0 [1.0; 3.0]	4.0 [3.0; 6.0]	0.007
LEDD, mg per day	675.0 [500.0; 787.5]	1150.0 [862.0; 1187.0]	< 0.001

Abbreviations: PD¬ – LID ¬– PD patients without LID; PD + LID – PD patients with LID; NMS - non-motor symptoms; NMSQuest – non-motor symptoms questionnaire scale; MoCA – Montreal Cognitive Assessment; MMSE – Mini-Mental State Examination; FAB – Frontal Assessment Battery; UPDRS – Unified Parkinson's Disease Rating Scale; LEDD ¬– levodopa equivalent daily dose.

Table 3

Relationship of *DRD3* and *LINGO1* gene polymorphisms with levodopa-induced dyskinesias in Parkinson's disease, abs. (%)

Genotype	PD – LID	PD + LID	χ^2	p	OR (95 % CI)
Ser9Gly polymorphism *DRD3* gene					
CC *CT* *TT*	3 (7.5) 8 (20) 29 (72.5)	1 (14.3) 0 6 (85.7)	1.86	0.394	2.06 (0.18–23.2) Н/Д 2.28 (0.24–21.1)
rs9652490 polymorphism *LINGO1* gene					
CC *CT* *TT*	9 (22.5) 21 (52.5) 10 (25)	1 (14.3) 4 (57.1) 2 (28.6)	0.243	0.886	0.57 (0.06–5.41) 1.2 (0.24–6.1) 1.2 (0.2–7.18)

complete the full scope of the study; 3) refusal of the patient or legal representative from genetic research; 4) non-use of antiparkinsonian drugs.

There were 21 men (44.7%) and 26 women (55.3%). The average age of patients was 69.0 ± 7.67 years, the median age was 69.0 [64.0; 75.0] years. Most of the patients were of the Yakut ethnic group (29 people, 61.7%), 18 people (38.3%) were of the Russian ethnic group. Patients with a mixed form of PD predominated (72.3%). The average duration of the disease was 5.94 ± 4.09 years, the median duration of the disease was 5.0 [3.0; 9.0] years. The average stage of the disease according to the Hoehn-Yahr scale was 2.78 ± 0.87, the median was 3.0 [2.0; 3.0].

DNA-sorb-V reagent kit (Diagnost, Russia) was used for DNA isolation. DNA genotyping was performed on a CFX96 Real-Time PCR amplifier (Bio-Rad Laboratories, USA) using an amplification reagent kit (Testgen, Russia). The amplification program included the first denaturation at 95 ° C for 2 minutes, then 40 cycles at 94 ° C for 10 seconds and at 60 ° C, 62 ° C and 58 ° C for 20 seconds, the fluorescence signal was measured at the second stage. Table 1 shows the nucleotide sequence of the forward and reverse primers of the studied polymorphisms.

Statistical analysis was performed using SPSS Statistics 25. Descriptive statistics are given as median and 25th and 75th quantiles (Me [Q25; Q75]). Data analysis for two independent groups was carried out by the Mann – Whitney U-test. For the analysis of nominal data, we used four-field contingency tables using Pearson's χ2 test and Fisher's test. The ratio of the frequencies of genotypes and allelic variants of genes was checked for compliance with the Hardy-Weinberg equilibrium. The frequencies of genotypes and alleles of each polymorphism were calculated as a percentage of their total number with the calculation of rela-

tive odds (OR) and 95% confidence interval (CI). The critical level of statistical significance for the two groups was determined at $p \leq 0.05$.

Results. LID were detected in 7 patients with PD (14.9%). Comparative analysis of clinical and anamnestic data of patients with and without PID is presented in Table 2.

According to Table 2, patients with PD in both groups (with and without LID) did not differ in age, gender, ethnicity, stage of disease, non-motor symptoms, and cognitive impairment. At the same time, patients with LID are more likely to have motor fluctuations, a longer duration of levodopa therapy, and a higher levodopa equivalent daily dose.

We studied the relationship of DRD3 and LINGO1 gene polymorphisms with LID in PD (Table 3) and did not find such an association.

Discussion. The dopamine D3 receptor gene (DRD3) is located on chromosome 3 (3q13.3) [10]. The rs620 (Ser-8Gly) polymorphism of the DRD3 gene is a replacement of C allele encoding serine with T allele encoding glycine. As a result, the affinity of the D3 receptor for dopamine decreases, and patients with Gly / Gly genotype require a higher dose of dopamine receptor agonists [9]. J.Y. Lee et al. found the effect of TT genotype of this polymorphism on the development of biphasic LID (OR 3.1; 95% CI 1.4–6.5) [13]. Similar results were obtained in patients with PD from Italy (OR 4.9; 95% CI 2, 0-12.2) [25].

The LINGO1 gene is located on chromosome 15q24 and encodes a transmembrane glycoprotein of the central nervous system that plays a role in the structural plasticity and survival of dopaminergic neurons [18, 29]. It has now been established that mutations in the LINGO1 gene are associated with the development of essential tremor [2, 20, 31]. About Parkinson's disease, the results are different. In a study by Carles Vilarino-Guell and colleagues involving 426 patients with PD, an increased risk of disease was reported with the carriage of AA genotype of the rs9652490 polymorphism [20]. On the contrary, a group of scientists from China, as a result of genotyping 425 patients with PD, did not reveal the effect of mutations in this gene on the development of the disease [26]. A similar result was obtained by Yih-Ru Wu and colleagues, who studied the effect of this polymorphism on 649 patients with PD from Taiwan and Singapore [17]. In 2020, Ting Gao and colleagues also reported that there was no effect of the rs9652490 polymorphism on the development of PD [8]. At the same time, we found no studies on the effect of mutations in the LINGO1 gene on the development of LD.

We did not find the effect of both the Ser9Gly (rs6280) polymorphism of the DRD3 gene and the rs9652490 polymorphism of the LINGO1 gene on the development of LID in PD patients.

One of the significant limitations of our study is a small sample, which allows us to classify this study as a pilot study. Despite this, it was rightly revealed that long-term levodopa therapy and a high daily dose of levodopa are statistically significantly associated with the development of LID. Therefore, the absence of a relationship between the development of LD in PD with the Ser9Gly (rs6280) polymorphisms of the DRD3 gene and rs9652490 of the LINGO1 gene should be taken as a true negative result.

Conclusion. The results of a pilot study indicate the absence of a predictive role of the carriage of SNV rs6280 (DRD3 gene) and rs9652490 (LINGO1 gene) on the development of LID in PD patients living in the Republic of Sakha (Yakutia). However, the authors do not exclude the influence of a small sample size on the results of the associative genetic study.

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A SEROLOGICAL SURVEY OF ZOONOTIC INFECTIONS IN YAKUTIA

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The article analyzes the results of three field surveys concerning the seroepidemiology of various zoonoses in the Sakha Republic (Yakutia). These studies were carried out from 2007 to 2018 in Artic (Verkhoyansk area) and subarctic (Vilyuysk area then Central Yakutia) regions. The first major finding was the presence of Lyme borreliosis in both Vilyuysk and Verkhoyansk areas. Then an elevated incidence rate of Q fever was observed in the Verkhoyansk area. Transmission of saprozoonotic toxocariasis and food-borne trichinellosis was remarkably low in the three surveyed places. Finally, the epidemiological status of echinococcoses, alveolar and cystic, must be clarified by further field studies which will have to assess the etiology and the respective part of these major zoonoses.

Keywords: zoonoses, seroepidemiology, Yakutia.

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Introduction. According to One Health initiative [1], the current concept of the epidemiology of zoonoses focuses onto interactions between and among humans, animals, plants, parasites, microbes, and chemical contaminants in terrestrial, aquatic, and marine ecosystems [2]. Also the diversity and the complexity of cultural factors should be addressed in such an approach. Contact with either domestic or pet animals or with wildlife [3-4] is considered an important source of disease [5] and zoonoses represent an increasing threat for human health [6]. Therefore, the results from any survey about zoonoses should be analyzed according to One Health principles [7].

Globally, the growing need for resources leads to increased human pressure on the environment, thus inducing changes that are particularly apparent in Arctic and subarctic regions. This pressure may be local, due to logging, mining, gas or oil extraction, or global, from climate change in particular. "*With the Arctic warming about twice as fast as anywhere else on the planet, and the prospect of an ice-free Arctic summer now all but inevitable, scientists fear that zoonoses will spread, threatening the indigenous people and wildlife that inhabit the region*" [8].

The Sakha Republic (Yakutia), in Eastern Siberia, is a member of the Russian Federation. Its surface area is 3,083,523 square kilometers, of which approximately 1/3 lays beyond 60° North and belongs therefore to subarctic or Arctic regions. In 2004, the French Archaeological Missions in Eastern Siberia research group, a partnership between Paul-Sabatier University, Toulouse, the French National Center for Scientific Research, Paris, and the Northeastern Federal University, Yakutsk, was formed for the purpose of studying the peopling of Eastern Siberia. The focus of this collaboration was subsequently extended to include the diversity of modern Yakutian populations and human ecology. Particularly, studies about zoonoses among Yakut people appeared to be of great interest, because the Yakut way of life still includes in most cases strong interactions between humans and animals [9-10]. Moreover, given the geographical situation of Yakutia, climate change could potentially affect the time and space distribution of zoonotic agents, along with that of hosts and vectors (if involved) [11].

Materials, subjects and methods. Three field surveys concerning the epidemiology of zoonoses in subarctic or Arctic Yakutia were carried out, first in 2007 [12], then in 2012 [13] and finally in 2018 [14]. The first survey was conducted in 2007 in Vilyuysk city, which is located in the Northwestern part of the Sakha Republic (latitude: 63° 45' North - longitude: 121° 27' East - 10,529 inhabitants). The second survey took place in the Arctic area of Verkhoyansk, more precisely in the villages of Suordakh (66° 40' North - 131° 46' 16" East - 325 inhabitants) and Tomtor (67° 12' 17" North - 132° 8' 10" East - 282 inhabitants). The third survey was carried out in 2018 in Central Yakutia and concerned three localities: Maralayy village (61°59' North - 131°55' East - 837 inhabitants) in the Churapchinsky *ulus*, Pavlovsk village (61°52' North -129°53' East - 2091 inhabitants) in the Megino-Kangalassky ulus, and Borogontsy town (62°40' North - 131°08' East - 5,222 inhabitants) in the Ust-Aldansky *ulus*.

Given technical limitations due to the remote situation of the surveyed areas, all studies relied only upon serology of various bacterial, parasitic or viral agents of zoonoses (Table). Blood samples were obtained from 90 subjects in Vilyuysk, from 77 subjects at Suordakh and Tomtor, and from 90 subjects in Central Yakutia. All subjects were volunteers, adult and apparently healthy. Every volunteer

had to give a written informed consent then to reply an oral questionnaire which inquired about demographic, occupational and environmental characteristics, and also about food habits. For Vilyuysk and Verkhoyansk surveys, immunodiagnoses of bacterial and parasitic diseases were performed in the Department of Parasitology and Mycology, Toulouse University Hospitals, France, whereas those for viral diseases were carried out at the National Reference Center for Arboviruses, IRBA, Marseilles, France. Concerning the study in Central Yakutia, serodiagnoses of three surveyed helminthiases were carried out in the Laboratory of Molecular Genetics at the Northeastern Federal University in Yakutsk. Laboratory procedures have been detailed in the relevant articles [12-14].

Results. The seroprevalence rates of the surveyed zoonoses appear in Table. Statistical analysis did not find any significant relation between the recorded personal or environmental characteristics and the incidence (Q fever) or prevalence (other diseases) of the studied zoonoses. Moreover, no significant difference ($\chi 2$ or Fisher's exact tests) in the distribution of the positive results was found between the surveyed areas.

Discussion. The seroprevalence of partially (toxoplasmosis) or totally (alveolar or cystic echinococcosis, cysticercosis, toxocariasis) soil-transmitted zoonoses (saprozoonoses) was remarkably low in Yakutia (Table). This finding was particularly surprising for cystic echinococcosis and toxocariasis, given the high degree of *Echinococcus sp*. or *Toxocara canis* infection in Yakut dogs [15]. This very low level of transmission for the investigated saprozoonoses was likely the positive consequence of the high pressure exerted by the peculiar Siberian environment. Climatic conditions in surveyed areas are characterized by very low temperatures (below -55°C) during the winter period, approximately 320 frost days per year, and an annual mean temperature ranging from -10°C in Vilyuysk to -15°c in the Verkhoyansk area. In such a harsh climate, propagules that are spilled on the soil, namely, helminth ova or oocysts of *Toxoplasma gondii*, are destroyed. However, this explanation applies only in part to echinococcoses, since *Echinococcus* sp. eggs that would be spread in humid soil are very resistant to temperatures up to -30°C [16]. Another reason may lie in the genetic diversity of *Echinococcus* sp. in Russia. A recent study has demonstrated that the species predominant in Yakutia, where husbandry concerns mainly cattle or horses, is *E. canadensis* including the genotypes G6, G8 and G10 [17]. In the human intermediate host, this species elicits predominantly pulmonary cysts [18] that are less detectable by serology than hepatic involvement.

Interestingly, similar low incidence or seroprevalence rates for saprozoonoses have been observed in Arctic or subarctic Canada. For example, only 108 cases of cystic echinococcosis have been reported between 2001 and 2005 for the whole country [19]. Concerning toxocariasis, the seroprevalence ranged from 0% to 10% in the Cree communities of the subarctic area of James Bay [20].

In Yakutia, the seroprevalence of trichinellosis appeared to be very low whatever the place of survey. The food-borne route, through ingestion of meat from various domestic or wild carnivorous or omnivorous mammals is the major route of contamination for this zoonotic helminthiasis. Human infections originates frequently in the use of meat from brown bear (*Ursus arctos collaris*) or polar bear (*Ursus maritimus*) that accounted for 60.2% of the outbreak cases recorded between 1998 and 2002 [21]. Consumption of pork was the second major risk factor, followed by badger meat (*Meles leucurus sibiricus*). In the three above-cited surveys, the questionnaire about volunteers' culinary habits found that pork or bear meat always was used well cooked, which certainly reduced drastically the risk of contamination. Moreover, in the rural areas of Yakutia, people used to store meat from game or domestic animals outside the home for weeks, in pantries that are therefore exposed for at least 8 months per year to Siberian cold. In Eastern Siberia, the prevalent species of *Trichinella* is *T. nativa* [22]. Inside muscles, *T. nativa* larvae survive easily to temperatures between 0°C and −20°C but, at lower temperatures, survival time reduces rapidly [23]. Therefore the traditional storage habit for meat could achieve natural sanitization.

Whether the ongoing global warming could reduce this sanitizing effect of the harsh Siberian climate is a great question. In the Canadian Arctic, Inuit people have suffered epidemics due to *Clostridium botulinum* because the summer temperatures were over 4°C, thus making their traditional practices of fermentation or smoking for the conservation of meat or fish less efficient [24]. Although the annual average temperature has increased in Yakutia by 1.1°C between 1955 and 2000 [25], the risk of diminished efficacy of the traditional deep-freeze outside storage appears to be low, at least in the near future.

The results of the serological investigations for tick-borne zoonoses were more unexpected. Lyme borreliosis was found to be present in Vilyuysk and, more surprisingly, also in the Verkhoyansk area. Antibodies to the viral agent of tick-borne encephalitis (TBE) were found by the Vilyuysk surveys but were not detected by

Seroprevalence of various zoonoses in Yakutia

Agents	Disease	Vilyuysk. 2007 90 subjects		Verkhoyansk. 2012 77 subjects		Central Yakutia. 2018 90 subjects	
		Rate (%) [a]	95% CI [b]	Rate (%) [a]	95% CI [b]	Rate (%) [a]	95% CI [b]
Bacteria *Borrelia burgdorferi s.l.* *Coxiella burnetii*	Lyme borreliosis Q fever	3.3 NA [c]	0.7 – 9.4	10.4 2.6	1.4 – 12.8 0.3 – 9.1	NA [c] NA [c]	
Parasites *E. granulosus* *E.multilocularis* *Taenia solium* *Toxocara spp.* *Toxoplasma gondii* *Trichinella spp.*	CE [d] AE [e] Cysticercosis Toxocariasis Toxoplasmosis Trichinellosis	0 0 NA [c] 4.4 8.8 4.4	0.0 – 4.0 0.0 – 4.0 1.2 – 11.0 3.9 – 16.8 1.2 – 11.0	0 1.3 0 0 5.2 0	0.0 – 4.8 0.0 – 7.0 0.0 – 4.8 0.0 – 4.8 1.4 – 12.8 0.0 – 4.8	4.4 NA [c] NA [c] 1.1 NA [c] 2.2	1.2 –11.0 0.0 – 6.0 NA [c] 0.3 – 7.8
Viruses	TBE [f]	3.3	0.7 – 9.4	0	0.0 – 4.8	нт	

a seroprevalence - b 95% confidence interval - c not available data (not tested)
d cystic echinococcosis - e alveolar echinococcosis - f tick-borne encephalitis

the Verkhoyansk study. The indisputable presence of Lyme borreliosis in these areas was notable since Northeastern and Arctic Siberia were considered free of this zoonosis [26]. However, a previous study which had been carried out in the countryside around Vilyuysk in 2006 had found a 19.5% positivity rate [27]. These findings confirm the presence in Arctic and subarctic Yakutia of infected ticks that likely belonged to the cold-resistant species *Ixodes persulcatus*. Since no information was available in the international literature about the epidemiology in Yakutia of the above-cited tick-transmitted zoonoses, it was not possible to draw firm conclusions from our surveys.

The investigations on the epidemiology of Q fever were carried out only in the Verkhoyansk area. They were prompted by the long tradition of cattle breeding among the Yakut people, extending to the Arctic regions. Q fever is a bacterial zoonosis of which the transmission is either direct, between humans, from the inhalation of bacteria, or indirect, from contact with the milk, urine, feces, vaginal mucus, or semen of infected animals [28]. Unsurprisingly, we found a substantial incidence rate. Although no retrospective analysis was possible given the lack of any information about the historical incidence of *Coxiella burnettii* infection in Yakutia [29], it seems likely that Q fever in this region is not an emerging zoonosis, but rather that its transmission occurred on a regular basis.

Conclusion. Concerning Lyme borreliosis, it was not possible to firmly conclude that the presence of tick-transmitted zoonoses in subarctic and particularly in Arctic Yakutia was a "newly emergent" phenomenon or, rather, a "new recognition" due to the lack of previous studies in these areas. Should the first hypothesis be verified in the future, global climate change favoring the spread of the tick vector beyond its current more Southern distribution might be retained as plausible explanation. Anyway, along with Q fever, Lyme borreliosis would represent a major threat to the health of people living, sporting or working in Upper subartic or Arctic Yakutia.

Among the investigated helminth zoonoses, clearly toxocariasis does not represent a health problem in the Sakha Republic. For alveolar (AE) or cystic (CE) echinococcosis, the results of our studies should be confirmed by field surveys combining serology and ultrasonography in the human intermediate host [30]. Respective parts of AE or CE have to be clarified by checking by computerized tomography any subject found positive by ultrasonography and by using western-blot to confirm the positive results from ELISA [31]. These future studies also will have to investigate concomitantly the canine definitive hosts. The search for *E. granulosus* DNA in the feces will yield crucial information for assessing the transmission pressure. Moreover, genotype studies will have to be carried out on this fecal material in order to clarify the role of *E. canadensis*.

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IMPACT OF MEDICAL AND SOCIAL FACTORS ON OBESITY IN PRESCHOOL CHILDREN IN THE NORTH OF VIETNAM: A CASE-CONTROL STUDY

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Obesity in children increases the risk of overweight and obesity in adults and leads to several complications, both physical and mental, which put a strain on public health. A case-control study of 360 obese children and 786 normal children (according to WHO 2006 criteria) as a control group helped to elucidate the influence of medical and social factors on the development of childhood obesity. Univariate regression analysis showed the following risk factors: overweight/obesity of the father with OR = 5.1 (95% CI: 3.8 – 6.9): mother's overweight/obesity with OR = 6.1 (95% CI: 3.8 – 9.7); stress during pregnancy of mother with OR = 1.5 (95% CI: 1.1 – 2.1); excessive weight gain of mother during pregnancy (≥ 12 kg) with OR = 1.75 (95% CI: 1.3 – 2.3). Risk factors related to nutrition and physical activities were found: fast eating speed with OR = 2.1 (95% CI: 1.2 – 3.9); soft drinks with OR = 1.6 (95% CI: 1.1 – 2.3); time for TV watching if ≥ 2 hours per day with OR = 4.7 (95% CI: 3.4 – 6.5) or during 1 – 2 hours per day OR = 2.6 (95% CI: 1.9 – 3.5), respectively. Multivariate regression analysis identified the most significant risk factors for obesity in children: BMI of the father and mother, time for TV watching per day and eating speed, with the further development of a formula for calculating the probability of obesity, which can be used in organizing a preventive program for obesity in children.

Keywords: children, obesity, social, feeding rate, BMI, risk factor, Vietnam.

Introduction. According to WHO, in 2019, about 38 million children under the age of 5 were overweight or obese and it is estimated that by 2030 almost a third of the world's population may be overweight or obese [1]. In the USA for 2017 - 2018 obesity was specified in 13.4% of children 2 - 5 years old; in 20.3% of children 6-11 years old and in 21.2% of children 12-19 years old [2]. In Russia, the number of children and overweight and obese children is also increasing over a ten-year period [3, 4]. In Vietnam, from 2000 to 2015, the proportion of overweight children more than quadrupled from 3.3% to 17.5% related to urbanization and food transitions [5]. Childhood obesity is becoming a global public health problem around the world.

Obesity in children increases the risk of adult overweight and obesity by 70 - 80%, accompanying a number of complications such as metabolic disorders, vascular – cardiac diseases, environmental diseases, diseases of the musculoskeletal system and psychological disorders [6]. Ethnic variations in prevalence of overweight and obesity between Kinh, Muong, Tay (as main ethnic groups in Vietnam) children under 5 years old [7] were identified in a study in 2019 suggested that childhood obesity depends on the genotype and the environmental factors. In Vietnamese children primary school was found the significant relationship between delivery method, birthweight, night sleep duration and *BDNF* Val66Met polymorphism to adiposes [8]. In a review, degree of dependence of obesity on susceptible genes may be different in various populations [9] but in general, childhood obesity more depends on a number of reversible cultural and environmental factors such as diet, physical activity, lifestyle... [10].

Identifying reversible medical and social risk factors for obesity in preschool children could stands the base for reducing the risk interventions on individual level and provide a rationale for developing an obesity prevention program from an early age on population level.

Methodology and materials. This study was conducted from 1/1/2019 to 31/12/2019 on 16175 children aged from 24 to 60 months old living in the north of Vietnam. Participants were chosen from various randomly selected kindergartens located in 6 north cities and provinces of Vietnam including Hanoi, Thanhhoa, Namdinh, Phutho, Caobang, Hoabinh, to ensure the representative characteristics for North Vietnam of sample.

Nutritional status as malnutrition, norm, overweight and obesity was classified according to the WHO 2006 criteria. Obesity was defined if the child had the

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Z-score weight/ height/ age ≥3SD. Sample were selected using matched pair case-control method, and sample size was calculated using program sourceforge.net with the power = 80%, anpha risk = 5%, OR = 2, ratio control/case = ½, and estimated prevalence of childhood obesity 6.1% [11], resulting in 311 case and 622 control. In fact, two groups of children were compiled: obesity (n = 360) and a control group (normal nutritional status: n = 786) with Z-score weight/ height/age in interval (-1; 1) and the "control" child would in the same class and the same gender with the "case" child for the purpose of clarifying the effect of independent factors on obesity.

The parents of the subjects were given a questionnaire to identify the main social factors, related to family lifestyle and its impacts to childhood obesity. The research method was an open comparative retrospective case - control. The inclusive criteria were children aged from 24 - 60 months old with the written consent of their parents. The exclusive criteria were children with obesity due to confirmed genetic and endocrine disorders; obesity related to medications (corticosteroid, antidepressant…); children with acute and chronic diseases and all cases without the written consent of the parents. This study used part of the data from the Vietnamese Ministry of Education and Training project "Building a Model to Predict the Risk of Obesity in Preschool Children Based on Selected Genes, Diet and Physical Activity Habits," Grant No. B2018-SPH-50. The study was approved by the Ethics Committee of the Medical Institute of the Northeastern Federal University. M.K. Ammosov on October 6, 2019.

The data were processed using Microsoft Excel and SPSS version 16.0. Qualitative variables are represented by the number (n) and shares (%), the comparison was made according to the X^2 criteria. Quantitative variables were tested for normal distribution and compared by Student's t-test/ Mann-Whitney test. Univariate and multivariate regression analysis was used to determine the effects of risk factors on obesity. A predictive model was created and then an R-curve with area under the curve was constructed with p-values <0.05 were considered statically significant from both sides.

Results and discussion

1. Participants' characteristics. The general characteristics of the students were indicated in Table 1. Two groups of obese children and normal nutritional status are identical in gender and age structure (p> 0.05). All anthropometric indicators (weight, height, Z-score of weight / age, height / age, weight / height / age and BMI / age) in children with obesity were much higher than the control group, including Z-score of weight / height / age and Z-score for BMI / age, respectively.

The similarity in sex and age of the two groups can be explained by the method of sample selection for case – control study.

2. The influence of medical and social factors. Various medical and social risk factors of obesity have been identified in children. The results were

Table 1

Main characteristics of students

Characteristics		Obese group (360)	Control group (786)	p
Gender	Boys	277 (76.9%)	569 (72.4%)	0.111*
	Girls	83 (23.1%)	217 (27.6%)	
Age groups	2 – 3 years old	22 (6.1%)	55 (7%)	0.792*
	3 – 4 years old	41 (11.4%)	95 (12.1%)	
	4 – 5 years old	297 (82.5%)	636 (80.9%)	
Anthropometric indices	Weight	24.3±3.9	16.3±2.6	<0.001**
	Height	105.2±7.8	102.4±6.8	<0.001**
	Z-score weight for age	2.77±0.92	-0.20±0.98	<0.001**
	Z-score height for age	0.29±1.21	-0.35±1.15	<0.001**
	Z-score weight for height for age	3.69±0.60	-0.01±0.92	<0.001**
	Z-score BMI for age	3.81±0.74	0.02±0.92	<0.001**

* p obtained from test χ2; ** p obtained from t-test.

Table 2

Several medical and social risk factors and their impacts on obesity in preschool children in North Vietnam

Risk factors		n (%)		Univariate analysis
		Obese group	Control group	OR (95% CI)
BMI of father	BMI <25	213 (59.2)	292 (88)	1
	BMI ≥25	147 (40.8)	94 (12)	**5.081 (3.760 – 6.865)**
BMI of mother	BMI <25	296 (82.2)	759 (96.6)	1
	BMI ≥25	92 (17.8)	27 (3.4)	**6.078 (3.801 – 9.719)**
Living place	Countryside	90 (25)	197 (25.1)	1
	Suburban	152 (42.2)	324 (41.2)	1.027 (0.749 – 1.407)
	Urban	118 (32.8)	265 (33.7)	0.975 (0.7 – 1.356)
Mother was stressed during pregnancy	No	276 (76.7)	654 (83.2)	1
	Yes	84 (23.3)	132 (16.8)	**1.508 (1.109 – 2.051)**
Weight gain of mother during pregnancy	10 – 12 kg	156 (43.3)	403 (51.3)	1
	Less than 10kg	41 (11.4)	101 (12.8)	1.005 (p=0.98)
	More than 12 kg	163 (45.3)	282 (35.9)	**1.75 (1.34 – 2.287)**
Birth method	Normal	131 (36.4)	248 (31.6)	1
	Caesarean section	229 (63.6)	538 (68.4)	0.806 (0.620 – 1.047)

ОШ – отношение шансов.

reported in Tables 2 and 3. A father and mother BMI ≥ 25, considered as overweight or obese, increases the risk of childhood obesity by 5 and 6 times. This is similar to the results of many studies around the world that have shown a link between parental BMI and the risk of obesity in children [12, 13]. Obesity is transmitted through generations due to the role of genetic predisposition, and the lifestyle of the family and the nutrition of children under 5 years old strongly depends on those of parents.

During pregnancy, the mother experienced stress or added weight dramatically (more than 12kg) also increases the risk of obesity in children by 50% and 75%, respectively. Research by Lampard [14] and Voerman [13] also showed this strong correlation, which can be explained by the fact that stress increases the mother's appetite, as well as the role of the first 1000 days (including the time during pregnancy) in programming the child's development. The method of birth in this study did not show an effect on obesity in children, similar other studies [15, 16]. In our study, the living place did not increase the risk of obesity, although the prevalence of obesity was increasing in rural areas nowadays, but there is still a gap in obesity among rural and urban residents in a separate health region [17].

The parental characteristics of preschool children have had a major impact on the risk of obesity. The data were shown in Table 3. A child drinking a lot of soft drinks or eating too fast (time per meal less than 20 minutes) can have the increased risk of obesity by 1.6 and 2.1 times, respectively. Our result is resemble a study on over 4000 children aged 9–10 years in Japan, which showed fast speed increased the risk of obesity by 1.5 times in comparison with children who eat normally [18]. It is possible that when children eat quickly, blood glucose levels rise rapidly, causing excessive insulin secretion and consistently insulin resistance in many cell types. Decreased insulin sensitivity leads to overeating and obesity.

The time children spend watching TV and the nature of children (hyperactivity, shyness) indicate the degree of physical activity of children. Consequently, if children are not active increases his risk of obesity by 1.5 or 1.8 times, depending on the frequency. On the contrary, if children are not shy, the risk of obesity decreases by 30 - 70%, depending on the regularity. According to a 2018 Gobadi study of 607 children aged 6 to 10, children who watched TV for more than 2 hours a day

Table 3

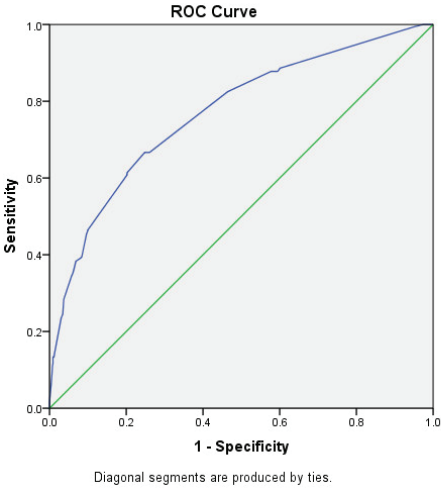
Influence of the characteristics of upbringing and the trait of children on the obesity in preschool children in the north of Vietnam

Risk factors		Univariate analysis	
		OR (95% CI)	p
Feeding children by desiration	No	1	0.073
	Yes	0.722 (0.506 – 1.031)	
Time for 1 meal	20 – 40min	1	
	<20 min	**2.128 (1.15 – 3.935)**	**0.016**
	>40 min	**0.307 (0.158 – 0.592)**	**0.001**
Soft drink	Little	**1**	**0.007**
	Much	**1.611 (1.140 – 2.278)**	
Time for TV watching /day	Less than <60 min	1	
	Medium (60 – 120 min)	**2.56 (1.881 – 3.484)**	**<0.001**
	Much (>120 min)	**4.663 (3.368 – 6.456)**	**<0.001**
Hyperactiveness	Regularly	**1**	
	Sometimes	**1.543 (1.061 – 2.243)**	**0.023**
	Never	**1.845 (1.331 -2.559)**	**<0.001**
Shyness	Regularly	**1**	
	Sometimes	**0.723 (0.553 – 0.944)**	**0.017**
	Never	**0.318 (0.109 – 0.928)**	**0.036**

Table 4

Risk factors of obesity in preschool children living in North Vietnam with coefficients in multivariate regression analysis

Risk factors		Coefficient	OR	p
Sex	Boys	-0.094	1	0.576
	Girls		0.91	
Age (months)		0.006	1.006	0.394
Stress in pregnancy	No		1	0.104
	Yes	0.307	1.36	
Weight gain during pregnancy	10-12 kg		1	
	< 10 kg	0.114	1.12	0.629
	> 12 kg	0.260	1.3	0.99
Time for 1 meal	Normal		1	
	<20 min	**0.946**	**2.6**	**<0.001**
	>40 min	**-1.194**	**0.3**	**0.001**
Soft drink	Little		1	**0.021**
	Much	**0.48**	**1.6**	
Time for TVwatching /day	Less		1	
	Medium	**1.079**	**2.9**	**<0.001**
	Much	**1.551**	**4.7**	**<0.001**
Overweight/obese mother		**1.191**	**3.3**	**<0.001**
Overweight/obese father		**1.394**	**4.0**	**<0.001**
Hyperactiveness	Regularly		1	
	Sometimes	0.058	1.059	0.747
	Never	-0.301	0.740	0.187
Shyness	Regularly		1	
	Sometimes	-0.320	0.726	0.056
	Never	-0.878	0.416	0.144



ROC Curve of predicting model for obesity in preschool children based on medical and social risk factors.

were 1.99 times more likely to become obese than those who watched TV for less than 2 hours a day. In addition to the fact that a lot of time spent by children in front of the TV reduces physical activity, but the influence of electronic devices on the risk of developing obesity has also been revealed. [19].

The time children spend watching TV and the nature of children (hyperactivity, shyness) indicate the degree of physical activity of children. Consequently, if children are not active increases his risk of obesity by 1.5 or 1.8 times, depending on the frequency. On the contrary, if children are not shy, the risk of obesity decreases by 30 - 70%, depending on the regularity. According to a 2018 Gobadi study of 607 children aged 6 to 10, children who watched TV for more than 2 hours a day were 1.99 times more likely to become obese than those who watched TV for less than 2 hours a day. In addition to the fact that a lot of time spent by children in front of the TV reduces physical activity, but the influence of electronic devices on the risk of developing obesity has also been revealed. [19].

3. Multivariate regression analysis risk factors of childhood obesity. Risk factors revealed by univariate regression analysis were put in a multivariate regression, including sex and age of children, the result was shown in Table 4.

Multivariate analysis revealed the following risk factors for obesity in preschool children in Vietnam: time per meal (less than 20 minutes increases the risk of obesity by 1.36 times, more than 40 minutes decreases the risk by 0.3 times); TV time (more than 60 minutes will increase the risk by 2.9 times, more than 120 minutes - 4.7 times); A father's BMI over 25 increases the risk by 4 times and an overweight/obese mother increases the risk by 3.3 times.

From the 4 above mentioned risk factors, it is possible to construct a prediction model with an area under the curve (0.769). This model with AUC = 769 showed high reliability and can be used in a preclinical setting to predict the risk of obesity in children, thereby warning young parents and educational institutions for preschool children. also take timely interventions to prevent early obesity.

Formula for predicting obesity in preschool children based on medical and social factors:

$P = e^y/(1+e^y)$

P – probability of obesity in children

e - Euler's number (≈2,718)

y= k1*βtime_for_1_meal+ k2*βtime_for_TV watching /day + 1,394*βBMI_father + 1,191*βBMI_mother - 2,089

k1= 0,946 if time for 1 meal less 20 min

k1=-1,194 if time for 1 meal more 40 min

k2= 1,079 if time for watching TV/day in 60 – 120 min

k2=1,551 if time for watching TV/day more than 120 min

For example: A child (code number 318402: BMI of father = 29,07; BMI of mother = 20,3; time for watching TV/day more than 120 min, time for 1 meal in 20 – 40 min, we can calculate y = (0+1.551+1.394+0)-2,089 = 0.856 => obese probability = 70.2%.

and in facts this boy, age 57.6 months, with a BMI / age z-score = 4.85, is obese.

This formula can be used easily in population studies related to childhood obesity.

The strengthens of this study were good design with large sample size calculated for case-control study with power > 80%. Addition, the statistical analysis approach with a probabilistic model constructed by multilevel analysis provided the effect sizes without bias. The limitation was almost all qualitative variates collected through questionnaire and retrospective method that could lead to some individual errors due to memories, that open up a new research direction with more quantitative studies in future.

4. Conclusions. The obesity of Vietnamese preschool children depends on various medical and social risk factors that are subject to possible changes. To reduce the risk of early obesity, the following measures should be taken: normalize the BMI of the father and mother, organize the correct diet for children, and limit a sedentary lifestyle. Enhancing physical activity and controlling nutrition is critical in preventing obesity in young children.

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Conflict of interest. The authors declare no conflicts of interest.

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CONTRIBUTION OF *IL12A*, *IL12B*, *IL13* AND *IL12RB2* GENE POLYMORPHISMS TO THE DEVELOPMENT OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Chronic obstructive pulmonary disease (COPD) is a chronic inflammatory lung disease affecting primarily distal respiratory pathways and lung parenchyma. An abnormal inflammatory response to inhaled harmful particles and tobacco smoke leads to airway remodeling and is thought to be a main mechanism of COPD development.

This study aimed to determine possible genetic association of *IL12A* (rs568408, rs2243115), *IL12B* (rs3212227), *IL13* (rs20541), *IL12RB2* (rs3762317) genes polymorphisms with COPD and studied the relationship between selected candidate genes variants with quantitative lung function parameters and smoking index in a Tatar population from Russia.

SNPs of *IL12A, IL12B, IL13, IL12RB2* genes were analyzed for association with COPD in cohort of 601 patients and 617 controls. SNPs were examined by the real-time polymerase chain reaction (PCR), with the use of TaqMan SNP discrimination assays.

As a result statistically significant associations with COPD in the study group under the biologically plausible assumption of additive genetic model were identified in *IL12A* (rs568408G>A) (P = 0.00001, OR = OR=2.07), *IL12A* (rs2243115T>G) (P = 0.00001, OR = 2.85), *IL13* (rs20541A>G) (P = 0.00001, OR = 1.58). A relationship between smoking index and *IL12A* (rs568408G>A) (P=0.027), *IL12A* (rs2243115T>G) (P=0.0038) was revealed. A significant genotype-dependent variation of Forced Vital Capacity was observed for *IL12A* (rs568408G>A) (P=0.045), *IL12A* (rs2243115T>G) (P=0.013) and *IL13* (rs20541A>G) (P =0.0051). Vital Capacity was affected by *IL12A* (rs2243115T>G) (P =0.0019).

Our data confirm the assumption about the essential role of genes responsible for the synthesis of α- and β-subunits of IL12, structural α-helices of IL13 to increased risk of COPD.

Keywords: chronic obstructive pulmonary disease, inflammation, interleukins 12 and 13.

Introduction. Chronic obstructive pulmonary disease (COPD) is a common chronic inflammatory disease that characterized by partly reversible airflow limitation, chronic inflammation, fibrosis of small airways, and destruction of lung parenchyma [15]. Today, the total number of people suffering from this disease is estimated at 251 million people, while COPD is the third most common cause of death in the world, which leads to a high demand for studies of the mechanisms of pathogenesis, new methods of therapy and early diagnosis of the disease [15]. An abnormal inflammatory response to inhaled harmful particles and tobacco smoke leads to airway remodeling and is thought to be a main mechanism of COPD development. In this case, the most important part of the mechanism of its development are inflammatory mediators, chemokines and interleukins, in particular IL12 and IL13 and their receptors [7,15].

IL-12 is a heterodimer consisting of protein α (IL-12p35) and β (IL-12p40)

subunits connected by a covalent disulfide bond [1]. The α-subunit is encoded by the IL-12A gene located in the 3q25.33 locus and consisting of 7185 base pairs [12]. While the β-subunit is encoded by the IL-12B gene located in the 5q33.3 locus and comprising 15708 base pairs [12]. The main function of IL-12 is induction of immune and immune-mediated inflammatory responses to pathogenic microorganisms and intracellular pathogens by activating the differentiation of immature T-lymphocytes into memory T-cells and Th1, Th17 effector cells with their further proliferation. Also IL-12 increases the cytotoxicity of cytotoxic T-cells and natural killer cells, stimulates the synthesis of γ-IFN by the latter [1]. The association of IL-12 secretion and the development of chronic progressive inflammation in COPD has been proven, including due to the activation of T-cell immunity [9].

The IL-12 receptor is a heterodimeric protein complex that includes β1 and β2 subunits (IL-12Rβ1; IL-12Rβ2) [1]. The β1-subunit gene is located in the 19p13.11 region of the 19th chromosome, the β1-subunit gene - in the 1p31.3 region; their size is about 91,000 and 40,000 base pairs, respectively [14].

IL13 is a 13 kDa protein molecule with 4 α-helices in its structure [1]. This protein is encoded by the IL13 gene located at the 5q31.1 locus, which has 4848 base pairs [12]. IL13 is produced by Th2, eosinophils, natural killer cells, T-lymphocytes and is responsible for the development of airway hyperresponsiveness, alternative activation of tissue macrophages with the subsequent development of subepithelial fibrosis of the airways and emphysema, mucus hypersecretion, IgE synthesis, IL13 plays an important role in the development of bronchial asthma, lungs cancer, COPD and pulmonary fibrosis [1, 6, 7].

This work was designed as case-control study aimed at investigating the association of *IL12A* (rs568408, rs2243115), *IL12B* (rs3212227), *IL13* (rs20541), and *IL12RB2* (rs3762317) polymorphisms with COPD in the Tatar population from Russia.

Materials and methods. We used DNA samples from unrelated individuals, Tatars by ethnicity, living in the Republic of Bashkortostan. The COPD patients were selected and collected from 2010 to 2019 years in the pulmonary department of Ufa City Hospitals №21 (Ufa, Russia). The COPD patients were recruited randomly according to the International Classification of Diseases tenth revision (ICD 10) (http://www.who.int/classifications/icd/en/) and following the recommendations of the Global Initiative for Chronic Obstructive Lung Disease (Global Strategy for the Diagnosis, Management and Prevention of Chronic Obstructive Pulmonary Disease, 2011-2019). The group of patients included 601 individuals (522 men (86.85%) and 79 women (13.15%)), the average age was 63.38 ± 11.81 years. Among patients with COPD, the number of smokers and former smokers was 484 people (80.53%), non-smokers - 117 (19.47%). The smoking index for current and former smokers was 44.58 ± 25.92 pack-years. Subjects performed standardized pre-bronchodilator and post-bronchodilator spirometry. Vital capacity (VC), forced vital capacity (FVC), forced expiratory volume in the first second (FEV1), and the ratio of this volume to lung vital capacity (FEV1/VC) were evaluated as well. In the group of patients, the indices (in % of the norm) were FEV1 = 41.68 ± 19.32, FVC = 44.22 ± 17.88, VC = 49.02 ± 15.54, and FEV1/FVC = 58.66 ± 13.66. The control group included 617 individuals (548 men (88.88%) and 69 women (11.12%)), the average age was 58.44 ± 14.79. The number of smokers and former smokers was 517 (83.79%) and non-smokers - 100 (16.21%)); the smoking index in smokers was 38.54 ± 23.12 pack-years. Inclusion and exclusion criteria in the COPD group and control are described earlier in our previous works [13]. Informed voluntary consent was obtained from each of the participants in the study. Study was approved by the Local Ethical Committee of Institute of Biochemistry and Genetics of Ufa Scientific Center of Russian Academy of Sciences (IBG USC RAS), Ufa, Russia (Ufa, Protocol No 17, December 7, 2010).

DNA was isolated from peripheral blood leukocytes using phenol-chloroform extraction. For our study were selected the following polymorphic loci: *IL12A* (rs568408, c.* 121G> A; rs2243115, c.-564T> G), *IL12B* (rs3212227, c. *159A> C), *IL13* (rs20541, c.236A> G, p.Gln79Arg), *IL12RB2* (rs3762317, c.-1105A> G). SNPs were examined by the real-time polymerase chain reaction (PCR), with the use of TaqMan SNP discrimination assays (https://www.oligos.ru, OOO DNA-Sintez, Russia). Accumulation of specific PCR-product by hybridization and cleavage of double-labelled fluorogenic probe during amplification was detected with BioRad CFX96 instrument (Bio-Rad Laboratories Inc., USA), using CFX Manager software. The methods of analysis were described in detail by us earlier [13]. Statistical data processing was carried out using the Statistica v. 6.0 (StatSoft Inc., USA) and PLINK v. 1.07. A detailed description of the methods of statistical analysis are described earlier in our previous works [13].

Results. Systematic quality control procedures were performed to guarantee a high quality of the data. Subsequently, SNPs were filtered according to their proportion of missing data, MAF or deviation from Hardy–Weinberg equilibrium within the controls. For the control group, the following results were obtained: *IL12A* (rs568408) (P^{X-B}=0.24), *IL12A* (rs2243115) (P^{X-B}=0.81), *IL12B* (rs3212227) (P^{X-B}=0.07), *IL13* (rs20541) (P^{X-B}=0.43), *IL12RB2* (rs3762317) (P^{X-B}=0.14).

Statistically significant differences in the genotypes and alleles frequencies distribution of the *IL12A* (rs568408G> A) gene were revealed between the COPD and controls group (P = 0.001 and P = 0.00001) (Table 1). The frequency of the rare allele A of *IL12A* (rs568408G> A) was significantly higher in the COPD group (OR = 1.38 95% CI 1.16-1.64).

Association between COPD and *IL12A* (rs568408 G>A) in the dominant (P=0.00001, $P_{cor-FDR}$=0.00002, OR=2.31 95%CI 1.62-3.28), recessive (P=0.0014, $P_{cor-FDR}$=0.0022, OR=2.87 95%CI 1.49-5.5) and additive (P=0.00001, $P_{cor-FDR}$=0.00002, OR=2.07 95%CI 1.56-2.75) models was revealed.

The COPD group significantly differed from the control group by the genotypes and alleles frequencies distribution of the *IL12A* (rs2243115 T>G) (P = 0.00001 and P = 0.00001). The frequency of the rare G allele of *IL12A* (rs2243115 T> G) was significantly higher in the group of COPD (21.31% vs 9.97% in the control, OR = 2.45 95% CI 1.94-3.08). The *IL12A* rs2243115 T> G) was associated with COPD in the dominant (P=0.00001, $P_{cor-FDR}$=0.00002, OR=2.93 95%CI 2.00-4.30), recessive (P=0.00001, $P_{cor-FDR}$=0.00002, OR=16.3 95%CI 5.09-52.17) and the additive (P = 0.00001, $P_{cor-FDR}$ = 0.00002, OR = 2.85 95% CI 2.05-3.95) models. This was due to an increase in the proportion of heterozygotes and homozygotes for the rare G allele in the group of patients.

Significant differences in the frequency distribution of genotypes and alleles of the *IL13* (rs20541 A> G) were revealed between the studied groups (P = 0.002 and P = 0.002). Considerable associations between the COPD development and *IL13* (rs20541 A> G) were obtained in the dominant (P = 0.012, $P_{cor-FDR}$ = 0.0152, OR = 1.56 95% CI 1.10-2.20), recessive (P = 0.00001, $P_{cor-FDR}$ = 0.00002, OR = 2.54 95% CI 1.60-4.03) and the

additive (P = 0.00001, $P_{cor-FDR}$ = 0.00002, OR = 1.58 95% CI 1.24-2.01) models, which was due to an increase in the frequency of the AA genotype in the group of patients.

No significant associations were observed between *IL12B* (rs3212227), *IL-12RB2* (rs3762317) gene polymorphisms and COPD.

We investigated the relationship between the candidate gene polymorphisms and smoking index (in pack-years) in smoking subjects (Table 2). The smoking index was affected by the genotypes of *IL12A* (rs568408 G>A and rs2243115 T>G). In specific, the smoking index was significantly higher in carriers of the GG genotypes of *IL12A* (rs568408 G>A) and *IL12A* (rs2243115 T> G) (P = 0.27 and P = 0.0038).

We investigated the relationship between the studied genes polymorphisms and lung function parameters: Forced Vital Capacity (FVC), Forced Expiration Volume in 1 s (FEV1), and FEV1/FVC ratio in COPD patients (Table 2).

As shown in Table 2, the allele A carriers of the *IL12A* (rs568408 G>A) (P = 0.045), the GG genotype of the *IL12A* (rs2243115 T>G) (P = 0.013), and the AA genotype of the *IL13* (rs20541 A>G) (P = 0.0051) were associated with a decrease in FVC values. Carriers of the

Table 1

Frequency distribution of genotypes and polymorphic loci alleles of candidate genes in COPD and control groups and association of candidate genes polymorphic loci with the development of COPD (log regression analysis)

Gene, polymorphic locus	rare allele	Genotypes, alleles	COPD n (%) (N=601)	Control n (%) (N=617)	P / P_{adj}	OR (95% CI)
IL12A rs568408 G>A	A	GG/GA/AA	280/273/57 (45.90/44.75/9.34)	339/245/33 (54.94/39.71/5.35)	0.001	-
		G/A	833/387 (68.28/31.72)	923/311 (74.80/25.20)	0.00001	1.38 (1.16-1.64)
		GG GA+AA dominant	280 (45.90) 330 (54.1)	339 (54.94) 278 (45.06)	0.00001	1.00 2.31 (1.62-3.28)
		GG+GA AA recessive	544 (90.66) 57 (9.34)	584 (94.5) 33 (5.35)	0.0014	1.00 2.87 (1.49-5.5)
		additive	-	-	0.00001	2.07 (1.56-2.75)
IL12A rs2243115 T>G	G	TT/TG/GG	406/148/56 (66.56/24.26/9.18)	499/113/5 (80.88/18.31/0.81)	0.00001	-
		T/G	960/260 (78.69/21.31)	1 111/123 (90.03/9.97)	0.00001	2.45 (1.94-3.08)
		TT TG+GG dominant	406 (66.56) 154 (33.44)	499 (80.88) 118 (21.12)	0.00001	1.00 2.93 (2.00-4.30)
		TT+TG GG recessive	554 (90.82) 56 (9.18)	612 (99.19) 5 (0.81)	0.00001	1.00 16.3 (5.09-52.17)
		additive	-	-	0.00001	2.85 (2.05-3.95)
IL12B rs3212227 A>C	C	AA/AC/CC	459/129/22 (75.25/21.15/3.61)	436/156/25 (70.66/25.28/4.05)	0.192	-
		A/C	1 047/173 (85.82/14.18)	1 028/ 206 (83.31/16.69)	0.096	0.82 (0.66-1.03)
IL13 rs20541 A>G	A	GG/GA/AA	235/254/121 (38.52/41.64/19.84)	270/269/78 (43.76/43.60/12.64)	0.002	-
		G/A	724/496 (59.34/40.66)	809/425 (65.56/34.44)	0.002	1.30 (1.11-1.54)
		GG GA+AA dominant	235 (38.52) 375 (61.48)	270 (43.76) 347 (56.24)	0.012	1.00 1.56 (1.10-2.20)
		GG+GA AA recessive	489 (80.16) 121 (19.84)	539 (87.36) 78 (12.64)	0.00001	1.00 2.54 (1.60-4.03)
		additive	-	-	0.00001	1.58 (1.24-2.01)
IL12RB2 rs3762317 A>G	G	AA/AG/GG	419/155/36 (68.69/25.41/5.90)	400/186/31 (64.83/30.15/5.02)	0.166	-
		A/G	993/227 (81.39/18.61)	986/248 (79.90/20.10)	0.377	0.91 (0.74-1.11)

Note: P- is the significance for X2 test for allele or genotypes frequency difference between COPD and control). OR - odds ratio for a rare allele (basic allelic test) or regression model; CI95% - 95% confidence interval for OR; Padj, significance in the likelihood ratio test for the regression model adjusted for age, sex, BMI, smoking status and pack-years.

Table 2

The relationship between chemokine and chemokine receptor genes polymorphisms and quantitative phenotypes (lung function parameters and pack-years)

Gene, polymorphic locus	Genotype	M± S.E	P	beta (CI 95%)
Smoking index (pack-years) in the total smokers group (N=1001)				
IL12A rs568408 G>A	GG GA+AA	32.87 (1.34) 28.87 (1.12)	0.027	0.00 -3.90 (-7.35 – (-0.44)
	GG+AA AG	32.39 (1.23) 28.73 (1.2)	0.042	0.00 -3.66 (-7.18 – (-0.14)
IL12A rs2243115 T>G	TT+TG GG	31.08 (0.87) 43.97 (5.39)	0.0038	0.00 12.93 (4.19-21.68)
FVC (Forced Vital Capacity) (N=601)				
IL12A rs568408 G>A	GG GA+AA	57.02 (1.74) 52.41 (1.51)	0.045	0.00 -4.61 (-9.11 – (-0.11)
IL12A rs2243115 T>G	TT TG+GG	57.04 (1.44) 47.8 (2.65)	0.0001	0.00 -9.24 (-13.78- (-4.7)
	TT+TG GG	54.81 (1.21) 45.84 (2.65)	0.013	0.00 -8.97 (-16.04- (-1.9)
IL13 rs20541 A>G	GG+GA AA	55.29(1.33) 47.16 (2.14)	0.0051	0.00 -8.13 (-13.78- (-2.48)
VC (Vital Capacity) (N=601)				
IL12A rs2243115 T>G	TT+GG TG	56.62 (1.33) 48.41 (1.84)	0.0012	0.00 -8.20 (-13.11- (-3.29)

Note: M ± S.E - mean values and standard error of the mean, P - significance level for the regression equation, beta (CI 95%) - regression coefficient and 95% confidence interval for coefficient.

IL12A (rs2243115T>G) TG genotype are characterized by lower VC values (P = 0.0019).

Discussion. In this work, *IL12A*, *IL12B, IL13, IL12RB2* genes polymorphisms were tested for association with COPD in the Tatar population from Russia. We studied the relationship between selected candidate genes variants with quantitative lung function parameters and smoking index.

The risk of COPD developing in our study was associated with the A allele of the *IL12A (rs568408)*. Further analysis showed that rare A allele carriers had a reduced Forced Vital Capacity. On the other hand, the rare A allele carriers have a lower smoking index, which may indicate that the development of the disease in these individuals is not associated with prolonged exposure to cigarette smoke. A number of studies have shown that the A allele of the *IL12A (rs568408)* is associated with the development of bronchial asthma, its severity [3, 4] and lung cancer [8, 10, 11] in the populations of China, Taiwan, Italy, Tunisia, Great Britain, USA.

In our sample, an association with COPD was established for the *IL12A* (rs2243115). The risk of disease development was associated with a rare G allele. Our results confirm the data on the association of the *IL12A* (rs2243115) with COPD in the Chinese population, where the risk was also associated with rare G allele [16]. We have shown that in rare G allele carriers Forced Vital Capacity and Vital Capacity are significantly decreased, and in GG homozygotes the smoking index is increased, which indicates a possible interaction of the environmental factor (smoking) and the *IL12A* (rs2243115) during the development of COPD.

The rare A allele of the *IL13* (rs20541) was identified as a risk marker of COPD development; the highest risk was found for AA homozygotes. Moreover, homozygotes for the rare A allele have significantly decreased of Forced Vital Capacity, which indicates the role of this SNP in the progression of airway obstruction in patients with COPD. Our results are consistent with the data obtained in other studies, as the A allele of the *IL13* (rs20541) was associated with the development of bronchial asthma and COPD in the Asian and Caucasians populations [2, 5].

In conclusion, our data confirm the assumption about the essential role of genes responsible for the synthesis of α- and β-subunits of IL12, structural α-helices of IL13 to COPD development and progression. The data obtained indicate the contribution of genes *IL12A* (rs568408, rs2243115) and *IL13* (rs20541) polymorphisms to this disease.

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SEARCH FOR ASSOCIATION OF DELETION POLYMORPHISMS OF GLUTATHIONE-S-TRANSFERASE *GSTM1* AND *GSTT1* GENES WITH RISK OF LUNG CANCER IN THE YAKUT POPULATION

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In the structure of oncological morbidity, lung cancer occupies one of the leading positions. According to scientific sources, lung cancer is a multifactorial disease in which both external and internal factors are involved. The aim of this work is to search for an association of deletion polymorphisms of the enzyme glutathione-S-transferase *GSTM1* and *GSTT1* with the risk of lung cancer in the Yakut population. Analysis of polymorphic variants of specific loci of genes *GSTM1*, *GSTT1* was carried out in a sample of patients with lung cancer (n = 112) and control (n = 65). In our study, in the Yakut population, we did not find a significant association between the null genotypes *GSTT1* and *GSTM1* and their combinations. We found that the genotype *GSTM1* * + / *GSTT1* * 0 in the group of patients with non-small cell lung cancer occurred 3.7 times less frequently than in the control group (OR 0.226 (CI 95%: 0.0609-0.841); $\chi 2 = 5.621$, $p = 0.0177$).

Keywords: glutathione-S-transferase, genes for biotransformation of xenobiotics, xenobiotic detoxification enzymes, lung cancer, isozymes, deletion.

Introduction. Lung cancer occupies a leading position in the structure of cancer morbidity in Yakutia. In the Sakha Republic (population: 982.1 thousand) lung cancer affects approximately 400 people yearly [1]. The severity of the problem is due not only to the high prevalence of the disease, but also to late diagnosis, unsatisfactory treatment results and, as a result, high mortality.

According to our sources, the development of lung cancer can be promoted by external background factors, such as: asbestos [15], radon [27], arsenic [28] polycyclic aromatic hydrocarbons [19], etc. According to many researchers, one of the most important causes of lung cancer worldwide is smoking [7,9,24], but lung cancer does not develop in all smokers, only in 5-10% [22]. Tobacco smoke contains about 4,000 known chemical substances. It has been established that 60 of them cause oncological diseases [29]. These carcinogenic substances are neutralized by enzymes of the xenobiotic detoxification system. An important role in this process is played by enzymes of the glutathione-S-transferase family [16; 26].

Glutathione-S-transferases (GST; EC 2.5.1.18) are enzymes of the second phase of xenobiotic biotransformation that catalyze the conjugation reaction of glutathione with a wide range of nonpolar compounds of endogenous and exogenous origin containing electrophilic carbon, sulfur, nitrogen and phosphorus atoms [20]. In humans, GST enzymes are represented mainly by the cytosolic GST family, while there are eight classes of dimeric enzymes, which are classified based on the basis of their amino acid sequence and substrate specificity of α (A), (K), μ (M), π (P), θ (T), σ (S), ω (O), ζ (Z) [25]. The spectrum of substrates of these isoenzymes partially overlaps. For example, for all GST isoenzymes, the substrate is the substance 1-chloro-2,4-dinitrobenzene, the only exception is the isoenzyme GSTT1 [23]. But despite this, GST isoforms show their specificity, as

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class-A enzymes predominantly bind to cumene hydroperoxide, class-P - ethacrynic acid, class-M - epoxides, benzo(a) pyrene, styrene-7, 8-oxide, trans-stilbene oxide, class-T - epoxybutane, ethylene oxide, halomethane and methyl bromide, etc. [12].

It is assumed that disorders in the detoxification system enzymes' functioning can provoke the development of oncological diseases. Particularly interesting for studying the association with oncological diseases are two polymorphic genes *GSTT1* and *GSTM1,* which have a mutation in the form of an extensive deletion, which is characterized by a complete absence of expression of the corresponding forms of enzymes. According to some references, it is known that carriers of these mutations have a higher risk of developing oncopathologies [22; 10]. The prevalence of null alleles *GSTM1* and *GSTT1* varies greatly among different ethnic groups [5].

The aim of this study is to search for associations of deletion polymorphisms of the enzyme glutathione-S-transferase *GSTM1* and *GSTT1* with the risk of lung cancer in the Yakut population.

Material and methods of researc. This work was carried out within the framework of research titled: "Epidemiological aspects of malignant tumors in the Far North, the development of modern methods of early diagnosis and prevention using highly informative fundamental research methods" in the Department of Adaptation Mechanisms Research of the Yakut Science Centre of Complex Medical Problems.

112 patients with lung cancer were examined. The patients were diagnosed with lung cancer based on histopathological examination. Histological types of lung cancer included non-small cell lung cancer, squamous cell lung cancer and lung adenocarcinoma. Other types of lung cancer included: large cell carcinoma, mesothelioma and bronchial carcinoid, etc. As a control group, 65 people were studied without signs of oncological and pre-oncological or any chronic or acute inflammatory diseases. Obtaining the informed consent of the respondents to the study (according to the protocol of the Ethics Committee of the YSC CMP No. 49 of 25.03.2018) was mandatory.

Table 1 presents the general characteristics of the studied groups. According to the questionnaire data, almost all lung cancer patients are long-term smokers, of which only 15 people (13.4%) are non-smokers. Compared with cancer patients, the control group had a greater proportion of non-smokers – 23 people (35.4%),. The patients were divided into three groups according to their histological type of tumor.

Venous blood was taken on an empty stomach from the median cubital vein. DNA was isolated using the standard method of phenol-chloroform extraction [14]. The analysis of polymorphic variants of specific regions *of the GSTM1 and GSTT1* genes was carried out using polymerase chain reaction methods using the structure of primers described earlier [21]. The amplification products were detected in a 7% polyacrylamide gel. The presence of null deletion polymorphisms *of the GSTM1* and *GSTT1 genes* was indicated by the absence of the corresponding bands 271bp and 480bp. The 183bp-sized CYP1A1 gene was amplified as an internal control (Fig. 1, 2).

The study used computational methods of mathematical statistics implemented in the licensed integrated statistical package for complex data processing SPSS for Windows 10.0. To check the reliability when comparing the frequency of occurrence of genotypes in groups, the standard Pearson's $\chi 2$ criterion or the Fisher's exact test for small samples were used. The relationship between genotypes and the risk of lung cancer was evaluated by the odds ratio (OR) with a 95% confidence interval (95% CI)

Results. The frequency of occurrence of null deletion polymorphisms *of the GSTM1*0 and GSTT1*0* genes in patients with lung cancer (n=112) was 50.9% and 32.1%, in the control sample (n=65) - 41.5% and 36.9%, respectively.

According to the data obtained by us, in the total sample of patients with lung cancer, the frequency of occurrence of the "null" genotype *GSTM1*0* was 1.2 times higher compared to healthy individuals, but the differences between the groups did not reach the level of statistical significance. At the same time, the odds ratio of developing lung cancer when carrying the null genotype *GSTM1*0* was 1.5 times higher (OR 1.458 (CI 95%: 0.787-2.703)) (Table 2). According to the frequency of occurrence of the "null" genotype *GSTT1*0*, there were also no statistically significant differences between the total sample of lung cancer patients and the control at OR 0.809; CI 95% (0.426-1.536) (Table 2).

When studying the relationship between the genotypes *GSTT1, GSTM1* and various histological types of lung cancer, it was found that in the group of patients with squamous cell lung cancer, the chance of meeting the null genotype *GSTM1*0* is 2.8 times higher than in persons without cancer (OR 2.814; 95% CI (0.769-10.304)), but these differences did not reach the level of statistical significance. The frequency of occurrence of the null genotype *GSTT1*0* in the groups of patients with adenocarcinoma was 1.7 times higher compared to the control (OR 1.708; CI 95% (0.319-9.145)), but these differences were also not significant (Table 2).

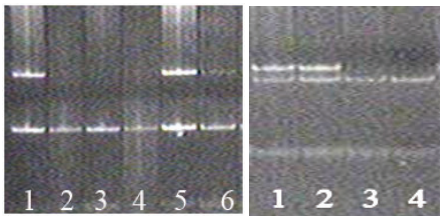
We analyzed the combined occurrences of the *GSTM1* and *GSTT1* genotypes in the control group and in patients suffering from lung cancer. The combined null genotype *GSTM1*0/GSTT1*0* was 1.2 times more common in the total group of patients compared to healthy ones (OR 1,200 (CI 95%: 0.540-2.666);, but the differences did not reach the level of statistical significance (p=0.654). If we look at the histological types of cancer, then in patients suffering from non-small cell lung cancer, adenocarcinoma and squamous cell cancer, the frequency of occurrence of the combined null genotype was similar to the control group and did not differ significantly. However, in other types of cancer, the incidence of the zero combined genotype *GSTM1*0/GSTT1*0* was 1.5 times higher than the control (OR 1.753 (95% CI: 0.664-4.627); χ^2=1.303, p=0.253).

Among all combinations of the studied genotypes, a significant change in the frequency of occurrence of the *GSTM1*+/GSTT1*0* genotype was noted by us in people suffering from non-small cell lung cancer. The combination of genotypes *GSTM1*+/GSTT1*0* was 3.7

Table 1

Characteristics of the control group and patients with lung cancer by histological type

Groups	Men n (%)	Women n (%)	n	Age X * SD
All patients	84 (75.0)	28 (25.0)	112	59.99±0.80
Non-small cell lung cancer	43 (76.8)	13 (23.2)	56	59.68±0.96
Adenocarcinoma	2 (33.3)	4 (66.7)	6	69.83±3.90
Squamous cell lung cancer	10 (83.3)	2 (16.3)	12	56.82±2.45
Other types of cancer	29 (76.3)	9 (23.7)	38	59.74±1.26
Control	49 (75.4)	16 (24.6)	65	55.69±1.02



a b

Examples of identification: a - GSTT1 * 0 and GSTT1 * + genotypes for the *GSTT1* gene; CYP1A1 - internal PCR control (183bp); 2, 3, 4, 6 - "GSTT1 * 0" genotype; 1, 5 - "GSTT1 * +" genotype; b - GSTM1 * 0 and GSTM1 * + genotypes for the *GSTM1* gene; CYP1A1 - internal PCR control (183bp); 3, 4 - "GSTM1 * 0" genotype; 1,2 - "GSTM1 * +" genotype.

times less common in the group of patients with non-small cell lung cancer (OR 0.226 (CI 95%: 0.0609-0.841); χ^2=5.621, p=0.0177) compared with the control group. In other combinations, we did not find an association of deletion genotypes of the *GSTM1* and *GSTT1 genes* with the risk of developing different histological types of lung cancer (Table 3).

Discussion. The lungs are most vulnerable to the action of carcinogens contained in polluted air, since they are directly in contact with them. The tissues and cells of each organ have a unique set of isozymes of the detoxification system. Both *GSTM1* and *GSTT1* genes are actively expressed in lung tissues, which is confirmed by the data of the UniProt consortium [https://www.uniprot.org].

The *GSTM1* gene is located on the short arm of the 1st chromosome (1p13. 3), has a length of 5,929 kb and consists of 8 exons. The *GSTT1* gene (22q11.23) is located on chromosome 22, it occupies about 8,179 kb and consists of 6 exons (https://www.ncbi.nlm.nih.gov). A feature of these genes is the presence of extensive deletions having sizes of 15kb *(GSTM1)* and 50kb *(GSTT1)*. *GSTM1* and *GSTT1* mutations are polymorphic, occurring in different populations of the world with a frequency of 37-53% and 18-48%, respectively (Table 4). The phenotypic manifestation of deletions is the complete absence *of GSTM1* and *GSTT1 enzymes* [11].

Some researchers claim that deletion polymorphisms in *the GSTM1* and *GSTT1 genes* reduce the overall enzymatic activity of GST [31]. It should be noted that a decrease in the activity of important enzymes necessary for the neutralization of carcinogens can lead to an increased risk of developing oncological diseases. In this regard, many authors are looking for a relationship between the null genotypes *of GSTM1, GSTT1* and the development of oncopathologies [10,22].

Our results showed that the null genotypes *GSTM1* and *GSTT1* have no significant association with the risk of lung cancer in the Yakut population, as well as their null combinations *GSTM1*0/ GSTT1*0*. We found that in the Yakut population, the genotype *GSTM1*+/ GSTT1*0* in the group of patients with non-small cell lung cancer was 3,7 times less common than in the control group, and this value reached statistical significance. When analyzing studies conducted earlier in different populations, it became clear that many of the results contradict each other. For example, in the work of Carlsten et al., (2008) was found a significant association between the null genotype of *GSTM1* and the development of lung cancer pathology, while Liu et al., (2015) did not find a reliable association, and both studies took into account the Chinese population. In the study of Liu et al., (2020), the authors note that a reliable association was found between the zero genotype *of GSTT1* and lung cancer in the Asian population, but no reliable association was found in the populations of Europeans and Africans.

According to the results of a meta-analysis of the association of lung cancer risk with null genotypes *GSTM1* and *GSTT1* and their combinations conducted by a group of researchers Zhang et.

Table 2

Incidence of null genotypes of *GSTTM1* and *GSTT1* in patients with lung cancer and the control group

Groups	n	n (%)	OR	95% CI	χ^2	p
GSTM1*0						
All patients	112	57 (50,9)	1,458	0,787-2,703	1,443	0,229
Non-small cell lung cancer	56	27 (48,2)	1,31	0,637-2,691	0,542	0,461
Adenocarcinoma	6	3 (50,0)	1,407	0,264-7,511	0,161	0,688
Squamous cell lung cancer	12	8 (66,7)	2,814	0,769-10,304	2,58	0,108
Other types of cancer	38	19 (50,0)	1,407	0,629-3,147	0,694	0,404
Control	65	27 (41,5)				
GSTT1*0						
All patients	112	36 (32,1)	0,809	0,426-1,536	0,419	0,517
Non-small cell lung cancer	56	12 (21,4)	0,466	0,206-1,050	3,455	0,063
Adenocarcinoma	6	3 (50,0)	1,708	0,319-9,145	0,398	0,527
Squamous cell lung cancer	12	4 (33,3)	0,854	0,232-3,139	0,056	0,812
Other types of cancer	38	17 (44,7)	1,383	0,613-3,121	0,611	0,434
Control	65	24 (36,9)				
GSTM1*0/GSTT1*0						
All patients	112	22 (19,6)	1,2	0,540-2,666	0,2	0,654
Non-small cell lung cancer	56	9 (16,1)	0,94	0,358-2,502	0,015	0,899
Adenocarcinoma	6	1 (16,7)	0,981	0,104-9,248	0,0003	0,987
Squamous cell lung cancer	12	2 (16,7)	0,981	0,188-5,116	0,0005	0,982
Other types of cancer	38	10 (26,3)	1,753	0,664-4,627	1,303	0,253
Control	65	11 (16,9)				

Table 3

Combined incidences of *GSTTM1* and *GSTT1* genotypes in patients with lung cancer and the control group

Groups	n	Genotype (*GSTM1*+ / GSTT1*+*) n (%)	Genotype (*GSTM1*0 / GSTT1*+*) n (%)	Genotype (*GSTM1*+ / GSTT1*0*) n (%)	Genotype (*GSTM1*0 / GSTT1*0*) n (%)
All patients	**112**	**41 (36.6)**	**35 (31.3)**	**14 (12.5)**	**22 (19.6)**
Non-small cell lung cancer	56	26 (46)	18 (32.1)	3 (5.4)*	9 (16.1)
Adenocarcinoma	6	1 (16.7)	2 (33.3)	2 (33.3)	1 (16.7)
Squamous cell lung cancer	12	2 (16.7)	6 (50.0)	2 (16.7)	2 (16.7)
Other types of cancer	38	12 (31.6)	9 (23.7)	7 (18.4)	10 (26.3)
Control	**65**	**25 (38.5)**	**16 (24.6)**	**13 (20.0)**	**11 (16.9)**

* $p \leq 0.05$.

Table 4

Frequency of occurrence of deletion polymorphisms of the *GSTM1* and *GSTT1* genes in different populations

Populations	n	Genotype (*GSTM1*0*) n (%)	n	Genotype (*GSTT1*0*) n (%)	n	Genotype (*GSTM1*0/ GSTT1*0*) n (%)	Sources
Yakuts	65	27 (41.5)	65	24 (36.9)	65	11 (16.9)	
Buryats	130	49 (37.7)	130	53 (40.8)	129	21 (16.3)	Tabixanova et al., (2019)
Kazakhs	220	118 (46.4)	220	56 (25.5)	220	27 (12.3)	Balmukhanov et. al, 2013
Chinese	412	194 (47.1)	412	198 (48.1)	412	90 (21.8)	Zhang et. al, 2011
Japanese	457	236 (51.6)	457	205 (44.9)	457	333 (72.9)	Hidaka et. al, 2016
Turks	231	124 (53.6)	231	43 (18.6)	108	22 (20.40)	Ada et. al, 2012
Russians	341	164 (48.1)	341	132(38.7)	341	78 (22.9)	Korchagina et al., 2011

al, (2021), quite interesting results were obtained on a very large sample. Statistically significant associations with the development of lung cancer were found in the Japanese population for the null genotype *GSTM1*, and in the Chinese population for the null genotype *GSTT1*. A significant association between lung adenocarcinoma and null genotypes *of GSTM1* and *GSTT1* was found by Zhang et. al, (2021) in Asian populations

Thus, based on the data we have obtained, we can conclude that *the GSTT1* and *GSTM1 genes* may play different roles in cancer predisposition in different populations. This is explained by the fact that oncological diseases are multifactorial. The layering of various factors creates conditions in which the same combinations of zero genotypes *of the GSTT1* and *GSTM1* genes can be risk factors or have no association with the development of lung cancer. In the Yakut population, we did not find a significant association between the null genotypes *GSTT1* and *GSTM1* and their combinations with the risk of developing lung cancer, but we found that the genotype *GSTM1*+/GSTT1*0* was significantly less common in the group of patients with non-small cell lung cancer compared to the control group.

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FEATURES OF IMMUNE REACTIONS IN AUTOIMMUNE THYROIDITIS IN RESIDENTS OF THE NORTHERN REGION

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Aim: to reveal the features of immune responses in autoimmune thyroiditis (AIT) in residents of the northern region.

Materials and methods: We examined 223 people living in the city of Arkhangelsk, aged 21 to 55, including 108 patients with AIT and 115 practically healthy people. A database was formed, including the data of the subject: date of birth, date of examination, age, sex and indicators of the immune background. Statistical analysis of the data was carried out using the Statistics 21.0 software package.

Results. In patients with AIT, compared with the level of the studied parameters in practically healthy individuals, an increase in the content of mature T-lymphocytes (CD3+), T-helpers (CD4+), cytotoxic T-lymphocytes (CD8+), activated cells with a transferrin receptor (CD71+) and cells labeled for programmed cell death (CD95+). The inflammatory process in AIT is manifested by systemic reactions - thrombocytopenia (27%), an increase in the content of IL-6 and TNF-α (26 and 18%, respectively). In patients with AIT, in addition to increased concentrations of antibodies to thyroid peroxidase, a high frequency of detection of elevated levels is recorded. antibodies to DNA (ds-DNA) (52.3%), RNA (RNP) (60.3%), antiphospholipids (aPhL) of the IgM and IgG classes (16.17%).

Conclusion. It was found that the inflammatory process in AIT in 15-27% of cases is manifested by systemic reactions-thrombocytopenia, increased levels of IL-6 and TNF-α, lymphocytosis and monocytosis. With AIT, the concentration of cytotoxic T-lymphocytes increases 3 times with a low activity of natural killer cells and the phagocytic ability of neutrophilic granulocytes. The development of cell-mediated cytotoxicity is formed at a low background level of activity of mature T-lymphocytes (CD3+), natural killer cells (CD3-CD16+CD56+), activated T-lymphocytes with a transferrin receptor (CD71+) and lymphocytes capable of proliferation (CD10+).

Keywords: autoimmune thyroiditis, antibodies, T-lymphocytes, pro-inflammatory cytokines, inhabitants of the north.

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Introduction. The autoimmune process is a physiological process that regulates secretion, tissue metabolism, use, and clearance of hormones and other biologically active substances in the body [9, 18]. Currently, the understanding of the spectrum of autoantibodies, their concentrations and physiological regulatory role is expanding [3]. It was revealed that autoimmune processes have a clear tendency to increase [12]. Increased concentrations of autoantibodies to thyroid peroxidase are detected in residents of areas affected by the Chernobyl accident [2], as well as in unfavorable climatic conditions [9, 10], during physical exertion [11], stressful situations [4], and also depends on the degree tissue metabolism of the amount of metabolic products entering the blood [10].

In addition, with pathology, new structures with antigenic properties may appear as a result of conformational changes in the formed complex or destruction of any biologically active substance with antigenic properties [7].

In connection with the above, the aim of the work is identification of the features of immune responses in autoimmune thyroiditis (AIT) in residents of the northern region.

Materials and methods. The objects of the study were residents of the city of Arkhangelsk. The work includes the results of an immunological examination of 223 people aged 21-55 years, including 108 people with AIT, who applied to the

center of professional diagnostics "Biolam" and 115 practically healthy people. Inclusion criteria for two groups: living in Arkhangelsk, the first group - practically healthy persons at the time of the study, the second group - patients with a diagnosis of autoimmune thyroiditis. The average age for the first group is 37.62 ± 1.53, for the second 44.93 ± 1.79. All studies were carried out with the consent of the surveyed and in accordance with the requirements of the World Medical Association Declaration of Helsinki on the Ethical Principles of Medical Research (2000).

All stages of clinical laboratory examination were carried out by medical workers of the laboratory of the "Biolam" center and research workers of the Institute of Physiology of Natural Adaptations of the Federal State Budgetary Institution FITSKIA UB RAS: instruction on the rules of preparation for laboratory research, taking of biomaterial and its preliminary processing, application of analytical technology using appropriate reagents and equipment, obtaining survey results. Clinical and diagnostic interpretation of the examination results, identification of risk factors and causes of the disease, the formation of recommendation protocols were carried out by the doctor of medical sciences, professor, immunologist Dobrodeyeva Lilia Konstantinovna. Before the clinical and laboratory examination, everyone was informed about the possibility of using the examination results for research purposes by the staff of the Institute of Physiology of Natural Adaptations of the FGBUN FITSKIA of the UB RAS while maintaining the confidentiality of the results.

A database was formed, including the data of the subject: date of birth, date of examination, age, sex and indicators of the immune background.

The complex of immunological research included the study of hemogram (number of platelets, erythrocytes, leukocytes, total hemoglobin in the blood, leukogram with 5-component differentiation of leukocytes) on an automatic hematological analyzer XS-500i (Japan), phagocytic activity of neutrophilic leukocytes in peripheral blood in blood smears stained by the Romanovsky-Giemsa method. Phagocytic activity of blood neutrophils was determined by the absorption of latex particles with a diameter of 0.9 μm. Under a microscope (magnification x 1000), the percentage of neutrophils that included latex particles (phagocytic index - PI, %) and the average number of granules per phagocytic neutrophil (phagocytic number - FP) were determined.

The content of lymphocyte phenotypes (CD3+,CD4+, CD8+, CD3+CD16+CD56+, CD3-CD16 +CD56+, CD10+, CD95+, CD71+) was studied by flow cytometry using an Epics XL apparatus from Beckman Coulter (USA) with Immunotech and Beckman Coulter Company reagents "(France) and by the method of indirect immunoperoxidase reaction using monoclonal antibodies (" MedBioSpektr "," Sorbent ", Moscow).

The concentration of cytokines (TNF-α, IL-6) in the blood serum was determined by enzyme-linked immunosorbent assay. Reactions were assessed using a Multiskan MS photometer (Labsystems, Finland) and an Evolis automatic immunoassay analyzer (Bio-RAD, Germany).

Quantification of circulating IgG antibodies to thyroid peroxidase (antibodies to TPO) was carried out using the test systems of the company "Orgentec" (Germany), autoantibodies to double-stranded DNA (ds-DNA) and to nucleoproteins (RNP) - a kit "Bio Rad", USA; autoantibodies of IgM / IgG classes to phospholipids (aPL) - "Orgentec", Germany.

The results of the study were statistically processed with the determination of mean values and are presented as the arithmetic mean ± error of the mean (M ± m), reliability differences were assessed using Student's t-test. The package of the computer program "Microsoft Excel MX" was used. Statistical analysis of the data was carried out using the Statistics 21.0 software package.

Results and discussion. Pathological processes in AIT are distinguished by insignificant reactions of an increase in the content of leukocytes (from 4.34±0.16 to 6.48±0.19x10^9 cells/l; p<0.001), lymphocytes (from 1.73±0.08 to 2.78±0.11x10^9 cells/l; p<0.001), neutrophilic granulocytes (from 2.6±0.09 to 3.70±0.17x10^9 cells/l; p <0.001) and platelets (from 156.52±10, 37 to 204.63±5.45x10^9 cells/l; p<0.001). In some cases, thrombocytopenia (27.78 ± 0.76%), monocytosis (20.37±0.66%) and lymphocytosis (15.74±0.58%) were recorded. The frequency of registration of hemoglobin deficiency in patients with AIT, compared to that in practically healthy individuals, is 4.7 times higher (21.30±0.67 and 4.35±0.48%, respectively); erythrocytosis was recorded 2.5 times more often (16.67±0.60 and 6.96±0.59%), respectively, which indicates a compensatory response to tissue oxygen supply. The inhabitants of the North have a lower life span of an erythrocyte, the average content of hemoglobin in them, a change in the shape of an erythrocyte and a thickening of its cell wall have been established, which reduces the activity of providing oxygen to tissues [7]. An increase in the microviscosity of lipids with an increase in the content of cholesterol and monounsaturated fatty acids in membranes slows down the release of O2 from the erythrocyte, impairs the rheological properties of blood, and reduces the rate of deoxygenation of intracellular Hb [13]. In venous blood, regardless of age and length of residence in the North, oxygen tension and blood saturation are markedly reduced compared to those in the control group [5]. As a result, the capillary-venous difference in residents of the northern regions is reduced. Thus, in northerners, the difference in the venous-capillary partial pressure of CO2 and O2 is higher, but the partial pressure in the venous blood is lower. One of the reasons for this phenomenon may be the increased activity of erythrocyte aggregation in northerners; there is evidence that the aggregation of erythrocytes is accompanied by a decrease in tissue oxygenation [12].

In patients with AIT (table), compared with the level of the studied parameters in practically healthy individuals, an increase in the content of mature T-lymphocytes (CD3+), T-helpers (CD4+), cytotoxic T-lymphocytes (CD8+), activated cells with a transferrin receptor (CD71+) and cells labeled for programmed cell death (CD95+). Attention is drawn to the lack of response from lymphocytes ready for proliferation (CD10+), T NK cells (CD3+CD16+CD56 +) and a decrease in the content of NK cells (CD3-CD16+CD56+) in persons with AIT.

The activation of cell-mediated and antibody-dependent immune responses in AIT is rather low. A statistically significant increase in the content of T-helpers was confirmed on average only in 5 patients (4.63±0.31%), an increase in the concentration of cytotoxic T-lymphocytes was found in 8 cases (7.41±0.40%). This situation is possible with the formation of a low initial background of the content of immunocompetent cells in the circulation. Thus, the deficit in the content of mature T-cells in patients was established in 57.14±1.09% (60 patients), the deficiency of T-helpers was detected in 20 cases (18.52±0.64%); a low content of activated T-lymphocytes with a transferrin receptor was in 76 patients (70.37±1.23%). It is unlikely that apoptosis of lymphocytes is the main mechanism for the formation of a low immune background; an increased level of cells labeled for apoptosis was not recorded, their low content was found in 72 patients (66.67±1.20%).

The content of cells capable of proliferation (CD10+) [6] in all patients did not exceed the levels of their concentration in practically healthy individuals. Thus, a low initial background of the level of immune reactivity could be provided by a decrease in the activity of proliferative processes.

The activation of natural killer cells with an increase in their concentration >0.4x109 cells/l is recorded very rarely (in 5 patients, 4.63±0.16%). There is evidence that a decrease in the number of NK cells correlates with the loss of immunological control over autoreactive clones of lymphocytes and affects the activity of autoimmune inflammation in AIT [15].

Damage to thyroid cells in autoimmune processes can be provided by antibody-dependent cytotoxicity, which can be manifested by free membrane antibodies, activating the complement system, as well as immunoglobulin complexes on the phagocyte membrane [1]. Neutrophilic leukocytosis was rare (in 9 people, 8.33±0.43%), monocytosis was established in 22 cases (20.37±0.66%). It seems that the main role in antibody-dependent cytolysis is provided by cytotoxic lymphocytes, the content of which is, on average, 3 times higher than that of practically healthy individuals. Removal of cell decay products is provided by phagocytes and in this case mainly by monocytes; the deficit of actively phagocytic neutrophils in patients with AIT was established in 95.45±1.43% of cases, which is 10 times higher than in practically healthy individuals (9.30±0.69%). This defect can play a significant role in the further activation and prolongation of autosensisbilization.

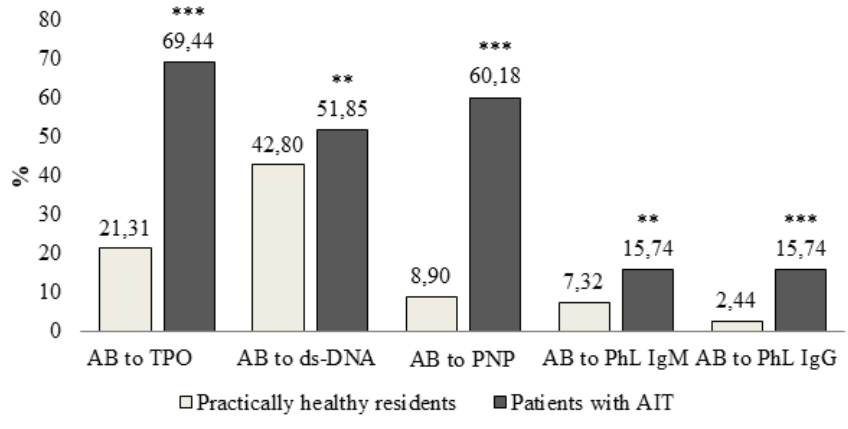
Autoimmune inflammation is accompanied by an increase in the blood levels of IL-6, TNF-α (26.67±1.71 and 18.36±0.63%, respectively). Thus, in some cases, the reaction of cytokines is systemic. IL-6 regulates the synthesis of acute phase proteins [17]. C-reactive protein induces TNF-α secretion through a signaling mechanism involving p38 MAP kinase, a Toll-like receptor [16]. The main manifestations of the biological activity of TNF-α are selective cytotoxicity with inhibition of the synthesis of the key enzyme of lipogenesis, lipoprotein kinase.

The range of autoantibodies in AIT is not limited to antibodies to TPO. Anti dsDNA concentrations exceeding the physiological limit (> 50 IU/ml) were found in 56 patients (51.85 ± 0.67%), anti RNP (>1.00 IU/ml) - in 65 patients (60.18±0.71%), antiphospholipids (aPL) of the IgM and IgG classes (> 10.00 IU/ml) were increased in 17 patients (15.74±0.37%). Antibodies to TPO exceeding the physiological level of the limit (>30 IU/ml) are set at 69.44±0.76% (75 people), Figure 1.

Comparative data on the content of phenotypes of lymphocytes in practically healthy people and patients with AIT (M ± m)

Cells, ×10⁹ Cells/l	Practically healthy residents	Patients with AIT	Reference limits of content
CD3+	0.74±0.02	0.98±0.03**	1.0–2.5
CD4+	0.37±0.02	0.53±0.02**	0.4–0.8
CD8+	0.33±0.02	1.01±0.56***	0.2–0.6
CD10+	0.32±0.02	0.27±0.02	0.05–0.6
CD3+CD16+CD56+	0.30±0.02	0.39±0.02	0.1–0.5
CD3– CD16+CD56+	0.42±0.03	0.28±0.02**	0.4–1.0
CD95+	0.26±0.02	0.46±0.01***	0.2–1.5
CD71+	0.34±0.02	0.41±0.02*	0.4–1.5

* p <0.5, ** p <0.01, *** p <0.001.



Frequency of registration of increased concentrations of antibodies in apparently healthy people and patients with AIT. *** – p <0,01, **** – p <0,001*

Conclusion. So, in patients with AIT, in addition to increased concentrations of antibodies to thyroid peroxidase, a high frequency of detecting increased levels of antibodies to DNA (ds-DNA) (52.3%), RNA (RNP) (60.3%), antiphospholipids (aPL) is recorded. classes IgM and IgG (16.17%). It seems that with autoimmune pathology, not only the concentration of autoantibodies increases, but also the spectrum of their specificity expands.

The inflammatory process in AIT is manifested by systemic reactions - thrombocytopenia (27%), increased levels of IL-6 and TNF-α (26 and 18%, respectively), lymphocytosis and monocytosis (15 and 20%, respectively). It is possible that platelet lysis initiates the development of the cytokine reaction and mononuclear cells.

Concentrations of T-lymphocytes (CD8 +) increase with AIT in fact 3 times with low activity of natural killer cells and phagocytic capacity of neutrophilic granulocytes. The clearance of cytolysis products is provided mainly by monocytes; deficiency of phagocytic ability of neutrophilic granulocytes can significantly prolong autosensitization. The development of cell-mediated cytotoxicity is formed at a low background level of activity (in 72%) of mature T-lymphocytes (CD3 +), natural killer cells (in 79%), as well as activated T-lymphocytes with a transferrin receptor (CD71 +) (in 70.37 %) and lymphocytes ready for proliferation (CD10 +) (in 96%), which is generally typical for residents of high latitudes.

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EFFICIENCY OF HOMEOBOX GENE EXPRESSION ASSESSMENT FOR PREDICTING OUTCOMES OF ASSSISTED REPRODUCTIVE TECHNOLOGY PROGRAMS

An analysis of the effect of *HOXA10* and *HOXA11* expression in the endometrial stroma of late reproductive women with tubal infertility factor on the outcomes of assisted reproductive technology (ART) programs was performed.

There was a tendency to a statistically significant decrease in the expression of *HOXA11* in the endometrial stroma during effective attempts to treat infertility.

Using the ROC (Receiver operator characteristic) method of analysis and calculations of the area under the ROC curve (AUC), it was found out that favorable levels of *HOXA11* expression for successful blastocyst implantation and live births in infertile women with their own and donor oocytes.

Keywords: *HOXA10*, *HOXA11*, expression, assisted reproductive technologies, implantation.

The potential role of *HOXA10* and *HOXA11* in the processes of implantation and functional regulation of tissues of the reproductive tract is recognized, however, the causes and consequences of expression features are actively discussed.

The ability to regulate the anatomical and functional identity of body segment structures by homeobox genes has been determined since the period of embryogenesis [10]. The relationship between the development of anomalies in *Hox/HOX* genetic mutations with a violation of not only organogenesis, but also the regulation of the encoding of transcription factors affecting the activity of "downstream" genes has been established.

Data on the *HOXA* genes and their probable role for implantation in women are insufficient and contradictory. The expression of *HOXA10* in the endometrium

of adult women occurs during the menstrual cycle [7], it is more pronounced in the functional layer compared to the basal one [18].

The role of *HOXA10* and *HOXA11* as moderators of activation or suppression of lower target genes [9] is based on the ability to regulate endometrial differentiation and proliferation by binding receptors to female sex hormones [4,18]. During implantation, the increased expression of *HOXA* mRNA genes is due to the transformation of stroma cells into decidual ones [15].

Abnormal expression of *HOX* genes is believed to be the cause of a violation of the implantation process, recurrent spontaneous abortions, conditions associated with infertility (endometriosis-associated, unclear genesis, polycystic ovary syndrome) [5,10,12,14].

There are reports that an increase in the expression of *HOXA11* in the endometrium is associated with an increase in the frequency of implantation [4,16,21]. Indicators of *HOXA11* proteins in infertile and fertile women, healthy and with gynecological diseases differ in various reports [10,11].

The mechanisms of endometrial receptivity disorders during the "implantation window" are connected to abnormal protein synthesis due to mutations and epigenetic abnormalities.

Conclusions about the aberrantly high expression of *HOXA10* mRNA in the fallopian tube mucosa during ectopic pregnancy were made by Unlu C. et al. (2016) [16]. There were no differences in the parameters of *HOXA10* and *HOXA11* proteins in the cells of the epithelium and stroma of the endometrium during the "implantation window" in the groups of women whose infertility was associated with low ovarian reserve, tubal-peritoneal factor, endometriosis, except for the sample with infertility of unclear genesis compared with fertile ones [3].

In other sources, the expression of *HOXA10* with H-score gradation in the groups with recurrent miscarriage and implantation failures was lower than in the control group, both in the glandular epithelium and the endometrial stroma [6]. The inconsistency and ambiguity of the data concerning the role of *HOXA10* and *HOXA11* in the regulation of endometrial receptivity, the assessment of the degree of its damage and the effectiveness of pregravid preparation determined the objective of our study.

The aim of the study was to evaluate the effect of *HOXA10* and *HOXA11* expression in the endometrial stroma of women of late reproductive age with tubal infertility factor on the outcomes of ART programs.

Material and methods. A prospective cohort study included 89 women of late reproductive age with tubal-peritoneal infertility who underwent ART programs at the Center of Obstetrics and Gynecology No. 1 LLC.

The sample included 68 women using their own oocytes, 21 – donor ones.

Inclusion criteria for the study: age 36-44, ovulatory cycle, normozoospermia or minor pathozoospermia of the husband (donor), embryos of good and excellent quality.

Exclusion criteria: infertility associated with the absence of ovulation; endometriosis, uterine fibroids of four cm or more, uterine factor of infertility, HIV infection, hepatitis B and C, severe pathozoospermia, systemic diseases; somatic diseases in the stage of exacerbation or decompensation.

Among women using their own oocytes, the first group consisted of 18 women with the onset of pregnancy, the second – 50 women with a negative result. At the second stage, these women were divided according to the live birth rate (the frequency of delivery of a live fetus(es) (take-home baby): the third group (n=14) – with a favorable outcome of ART programs, the fourth (n=54) – with a negative result.

In the sample using donor oocytes in ART programs (n=21), the following groups were distinguished: the first group consisted of 9 women with the onset of pregnancy, the second (n=12) – with a negative result. In order to analyze the frequency of live births, groups were formed: the third (n=6) – with the completion of ART programs with childbirth, the fourth (n=15) – with a negative result. The control group consisted of 20 healthy women (no chronic gynecological diseases) of reproductive age who applied for pregnancy planning.

In the cycles preceding the programs of assisted reproductive technologies, endometrial sampling was performed by aspiration biopsy using a Pipelle catheter during the expected "implantation window" (on the 17-25th day of the menstrual cycle, depending on the ultrasound monitoring data and on the 7th day after the peak of luteinizing hormone (LH)) (n = 89). Pathoanatomic examination of the material was carried out in accordance with the existing provisions.

An immunohistochemical study of endometrial biopsies was performed using standard sets of polyclonal antibodies from GeneTex, USA (rabbit polyclonal antibodies *HOXA10* and *HOXA11* receptor). Morphofunctional assessment of the endometrium was performed using the licensed Morphology 5.2 software. The results of the reaction of the *HOXA10* and *HOXA11* receptors were identified by calculating in percentage terms the relative density of stained endometrial stromal cells. Only clinically confirmed pregnancy (sonographic presence of the ovum) and live births were taken into account. The study was approved by the Ethics Committee of the Federal State Budgetary Educational Institution of Higher Education "South Ural State Medical University" of the Ministry of Health of Russia, it was conducted after the prior informed consent of the patients.

All statistical calculations were performed using the licensed statistical software package IBM SPSS Statistics v. 22 (IBM Corp., Armonk, NY, USA). The normality of the distribution of variables was checked taking into account the sample size using the Shapiro-Wilk criterion.

The results of the study are presented as a median with an interquartile range of Me (Q1-Q3). To compare groups by qualitative characteristics, Pearson's chi-square criterion or Fisher's exact criterion were used (in cases of the number of cells in which the expected frequency was> 0%). The Mann-Whitney U–test was used to determine statistically significant differences between the two independent groups. To identify the relationship between the signs, the Pearson and Spearman correlation coefficient was calculated, depending on the data distribution variant. To determine the threshold values (cut-off point), a ROC analysis was performed. To build a predictive model and estimate the OR (odds ratio), we used the method of multiple logistic regression, calculated 95% CI (confidence interval) for the OR. The significance level (p) when testing statistical hypotheses was taken to be $p<0.05$.

Research results and discussion. The duration of the menstrual cycle in the sample using own oocytes was 28 (28;28.5), donor – 28 (28;29) (p=0.771) without statistical differences (p>0.05). The endometrium of all patients (n=89) taken on the seventh day after confirmed ovulation, when stained with hematoxylin and eosin, corresponded to the stage of the secretion phase.

The pregnancy rate (PR) in the group of patients with their own oocytes in ART programs was 26.5% (n=18). The live birth rate in the group of patients with their own oocytes in ART programs was 21.7% (n=14).

In the group of women with the onset of pregnancy using their own oocytes in

Table 1

The nature of HOXA10 and HOXA11 expression in the sample using own oocytes. Me (Q1; Q3)

Gene	Groups				p
	I (n=18)	II (n=50)	III (n=14)	IV (n=54)	
HOXA10	6.2 (3.7;9.4)	7.2 (3.8;8.2)	7.6 (5.2;8.3)	6.0 (3.6;9.3)	p_{1-2}= 0.754 p_{3-4}= 0.623
HOXA11	5.1 (4.3;6.1)	7.4 (5.4;8.7)	5.0 (4.3;6.1)	7.1 (5.3;8.7)	p_{1-2}= **0.001** p_{3-4}= **0.006**

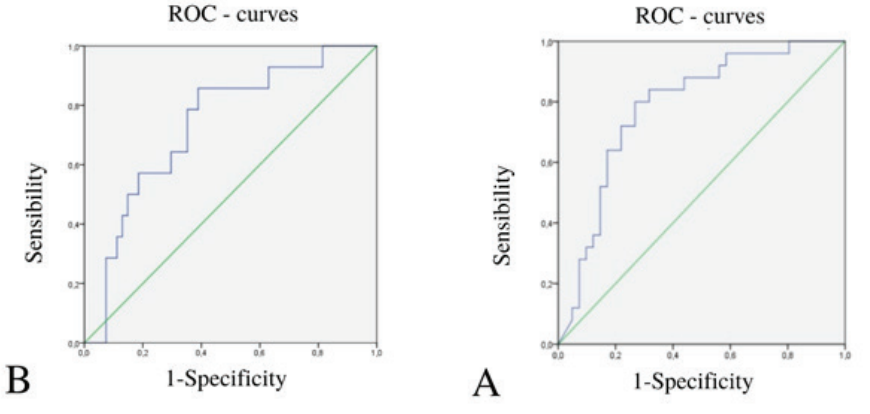

Fig. 1. ROC-curve of the relationship between HOXA11 expression in endometrial stroma cells and A. implantation and B. live birth in patients with their own oocytes in IVF/ICSI cycles

IVF/ICSI protocols, a tendency to a decrease in the expression of *HOXA10* and *HOXA11* was revealed (Table 1).

The level of *HOXA11* expression in stroma cells was statistically significantly lower in the group with pregnancy than in the group with ineffective attempts to treat infertility in ART programs (p=0.001).

The analysis of the frequency of live births in the sample showed the correlation of favorable outcomes of IVF/ICSI protocols with a lower *HOXA11* expression in the endometrial stroma (p=0.006). No such connection was found for *HOXA10* (p=0.623).

Using the method of ROC analysis and calculations of the area under the ROC curve (AUC), the prognostic significance of the expression of the *HOXA10* and *HOXA11* homeobox proteins in the endometrium was assessed in relation to the outcomes of ART programs (Figure 1).

The threshold value of *HOXA11* expression in endometrial stroma cells after successful implantation (group 1) at the cut-off point was equal to 6.1%. At a parameter value of ≤6.1%, the endometrium was characterized as favorable for implantation.

The sensitivity and specificity of the method were 80.0% and 73.0%, respectively. It was found that an increase in the expression of *HOXA11* in stromal cells by 1% reduces the probability of a decrease in implantation by 1.6 times.

The AUC values of *HOXA10* expression in the endometrium turned out to be statistically insignificant, which makes it impossible to calculate the prognostic coefficients of the onset of implantation (Table 2).

The importance of using *HOXA11* expression in endometrial stromal cells as a marker of the effectiveness of IVF/ICSI programs is shown.

In women with ART programs that ended in live fetal delivery (group 1C), the AUC of *HOXA 11* expression in the endometrial stroma was statistically significant (p=0.005). The value of the *HOXA 11* expression threshold parameter in the stromal cells of the endometrium, prognostically favorable for live birth, was equal to the same threshold parameter with a favorable prognosis for implantation and amounted to 6.1%. With an expression of less than 6.1%, the endometrium is characterized as favorable for the prognosis of live birth. The sensitivity and specificity of the method were 80% and 63%, respectively.

Table 2

Area under the ROC-curve (AUC) for the prognosis of implantation and live birth in patients with their own oocytes in IVF / ICSI cycles

Gene	AUC for the implantation prognosis	AUC for the live birth prognosis	*confidence interval (CI)95%*	*p*
HOXA10	0.525±0.074	0.462±0.074	0.380-0.670 0.316-0.608	p_1= 0.735 p_2= 0.666
HOXA11	0.767±0.064	0.744±0.071	0.642-0.892 0.567-0.849	p_1= **0.000** p_2= **0.005**

Table 3

Expression of HOXA10 and HOXA11 in women with different outcomes of ART programs using donor oocytes

Gene	Groups				p
	I (n=9)	II (n=12)	III (n=6)	IV(n=15)	
HOXA10	7.5 (3.7;7.9)	8.9 (3.5;10.8)	7.6 (6.6;8.5)	7.0 (3.6;9.7)	p_{1-2}= 0.320 p_{3-4}= 0.938
HOXA11	5.1 (4.1;5.8)	8.6 (6.6;10.0)	5.2 (4.8;5.8)	7.6 (5.9;9.2)	p_{1-2}= **0.001** p_{3-4}= **0.043**

Table 4

Area under the ROC-curve (AUC) of endometrial expression of HOXA10 and HOXA11 in women with donor oocytes in ART programs for the prognosis of implantation and live birth

Gene	AUC for the implantation prognosis	AUC for the live birth prognosis	*Confidence interval (CI) 95%*	*p*
HOXA10	0.630±0.130	0.489±0.125	0.243-0.734 0.375-0.884	p_1= 0.938 p_2= 0.320
HOXA11	0.917±0.067	0.789±0.103	0.785-1.000 0.586-0.991	p_1= **0.001** p_2= **0.043**

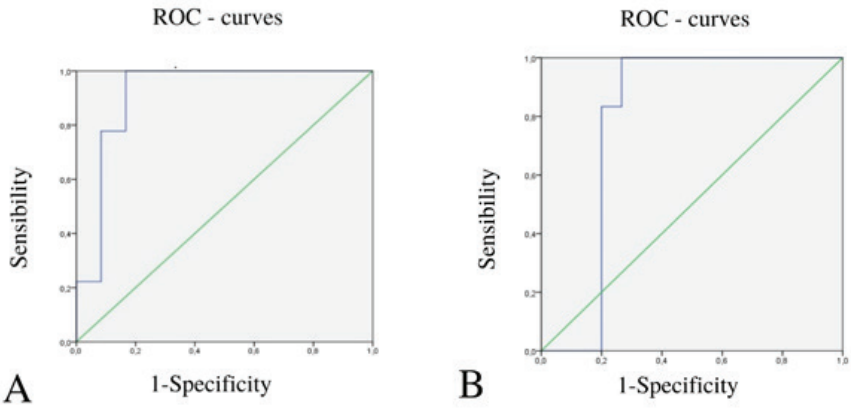

Fig. 2. ROC-curve of the relationship between HOXA11 expression in endometrial stroma cells and A. implantation B. live birth in patients with donor oocytes in ART programs

The pregnancy rate (PR) in the group of patients with donor oocytes in ART programs was 42.9% (n = 9). The live birth rate in women using donor oocytes was 30% (n = 6). Table 3 shows the expression of the studied immunohistochemical markers at different outcomes of ART programs in patients with donor oocytes.

The analysis of the positive outcomes of ART programs (pregnancy rate) using donor oocytes showed a lower expression of *HOXA10* and *HOXA11* in the endometrial stroma at the pregravid stage. The *HOXA11* index was statistically significantly lower compared to *HOXA10*.

Cases of pregnancy termination with live births also occurred predominantly in women with a lower *HOXA11* value (p = 0.043). The results of the ROC analysis are shown in Table 4.

The AUC of *HOXA11* expression in endometrial stromal cells is statistically significant for implantation and live birth in women with donor oocytes in ART programs.

The threshold value of *HOXA11* expression in endometrial stroma cells at the cut-off point, indicating a high probability of implantation in women with donor oocytes in ART programs, was 6.4%. The sensitivity and specificity of the method were 100.0% and 83.0%, respectively.

The probability of live birth in the group of women using donor oocytes increased at the threshold value of *HOXA11* expression in endometrial stroma cells at the cut-off point of 5.8% (p = 0.043). The sensitivity and specificity of the method were 83.0% and 80.0%, respectively.

Discussion. Low levels of *HOXA11* expression in stromal cells of the pregravid endometrium corresponded to a higher probability of implantation and live birth in a sample of women with their own and donor oocytes in IVF/ICSI programs. Calculations using ROC analysis confirmed the prognostic significance of *HOXA11*.

The low informative value of *HOXA10* as a marker of implantation can be interpreted from the standpoint of observations by other authors who noted a decrease in *HOXA10* mRNA in the middle phase of secretion in the endometrium of infertile women with hydrosalpinx, as opposed to fertile ones. Salpingectomy, on the contrary, led to a significant 15-fold increase in the expression of *HOXA10* in the glandular epithelium and endometrial stroma in comparison to the preoperative index [13].

The lack of involvement in the regulation of *HOXA10* protein implantation, identified by other authors [2], suggests the influence of the genotype on the "downstream" genes. Previous observations showed the absence of implantation during embryo transfer to mice with the *Hoxa10* genotype (-/-), with hemorrhage and tissue disorganization [1]. Probably, such changes are associated with insufficient decidualization of the endometrium.

The lowest *HOXA11* values in the group of women with live births with their own and donor oocytes in IVF/ICSI programs corresponded to the creation of optimal conditions for pregnancy, but the lack of similar observational experience in the literature indicates the need for further research.

Conclusions: The assessment of *HOXA11* expression in endometrial stromal cells is informative for predicting the probability of implantation and live birth in women of late reproductive age with tubal-peritoneal infertility in ART programs. For successful blastocyst implantation in women with their own and donor oocytes, favorable *HOXA11* expression levels are <6.1% and <6.4%, respectively; live births – <6.1% and <5.8%, respectively.

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DIAGNOSTIC AND TREATMENT METHODS

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COCHLEAR IMPLANTATION IN THE REPUBLIC SAKHA (YAKUTIA)

The article presents a statistical analysis of children's composition after cochlear implantation carried out in Otorhinolaryngology Department of Pediatric center of Republican Hospital No1 National Medical Center in the period from 2017 to 2019. The results of hearing and speech rehabilitation were obtained according to the scale for assessing the prospects of using children's cochlear implantation and categories of hearing perception were defined as well.

Keywords: cochlear implantation, otorhinolaryngology, audiology, rehabilitation, hearing, speech.

Introduction. The ability to perceive sound is one of the important features of the human body, which allows us to fully cognize the picture of the world around us. Hearing loss or congenital inability to hear in childhood patients is a serious burden not only in their socialization, but also in the learning process. [11] Attempts to restore hearing have been actively undertaken since the middle of the 20th century. [10]

The ability to restore hearing function in deaf people using direct electrical stimulation of the afferent fibers of the auditory nerve with a multichannel electrode system has become one of the most important achievements of medical science today. [4, 12] Cochlear implantation (CI) is the only method of treating patients with total deafness, which functionally provides intelligible speech perception. [7, 12] The regulatory document that controls the selection of candidates for surgery is the instructional material approved by the letter of the Ministry of Health of the Russian Federation of 15.06.2000 No. 2510 / 6642-32 "On the implementation of criteria for the selection of patients for cochlear implantation, methods of preoperative examination and prediction of effectiveness rehabilitation of implanted patients". The main selection criterion for CI is damage to the majority of hair cells. The selection of children is carried out in accordance with the division of patients into the pre-lingual and post-lingual categories, which have an important prognostic value. [5] For early diagnosis of hearing loss in the Russian Federation, universal audiological screening of newborns and children of the first year of life is carried out, including registration of otoacoustic emission and short-latency auditory evoked potentials. [1, 3] The optimal hearing and speech result in CI, in children with congenital deafness and hearing loss in the first year of life, can be achieved before the age of 3 years, the minimum recommended age is 6-12 months. [5, 8]

As part of the implementation of the decree of the Head of Sakha Republic (Yakutia) dated August 22, 2016 No. 1372 "On measures to improve high-tech types of medical care and innovative treatment methods in Sakha Republic (Yakutia)", as well as the signing of a Cooperation Agreement between the Ministry of Health of Sakha Republic (Yakutia) (Minister Okhlopkov M.E.) and Federal State Budgetary Institution 'National Medical Research Center otorhinolaryngology of the Federal Medical and Biological Agency' of Russia (director Daihes N.A.) in 2017 for the first time performed cochlear implantation operations in children aged 1 to 2 years. Since 2018, on the basis of Republican hospital No1 National center of medicine, a specialized day hospital has been put into operation to configure and replace the CI speech processors. [9, 13]

After the operation and connection of the implant, a telemetry session and speech processor (SP) settings are conducted in the Day Hospital of Republican hospital No1 National center of medicine at the appointed time. An integrated approach to hearing and speech rehabilitation is observed, in addition to classes with a teacher of speech rehabilitation, speech therapist, psychotherapist, children additionally study at home, according to the recommendations of specialists, with weekly video reports from their parents. [1, 2, 6]

The study of the obtained results of hearing and speech rehabilitation in children is currently very relevant.

Purpose of the study: to determine the effectiveness of hearing and speech rehabilitation of children after cochlear implantation, performed on the basis of the ENT department of Republican hospital No1 National center of medicine for the period 2017-2019.

Materials and methods. The study was carried out according to the medical records of an inpatient patient and data from the workflow automation system for medical institutions, based on the Day Hospital and ENT department of Republican hospital No1 National center of medicine from 2017 to 2019. The work is in accordance with the ethical principles of conducting scientific medical research with human participation. The parents of each patient signed an authorization

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agreement to conduct a study with the processing of medical and personal data.

We studied 23 medical records of the Day Hospital and ENT department with CT performed for the period 2017 - 2019. Diagnosis and coding of operations are set in accordance with ICD 10 and the Nomenclature of Medical Services. All patients met the selection criteria for CI. All patients were treated in accordance with the Clinical Recommendations of the Ministry of Health of the Russian Federation. All patients have implants of the Digisonic SP "Neurolec" model.

To assess the CI, the "Scale for assessing the prospects for the use of cochlear implantation in young children" was used. The assessment of the state of hearing was carried out according to the CAP scale (Assessment of the category of hearing perception). This scale allows you to determine the category of hearing ability and track the dynamics after the implantation, based on the reaction to sounds or partial / complete understanding of spoken language. Statistical processing of the obtained data was carried out using generally accepted methods of mathematical analysis, using the MS Office Excel 2019 program and the document management system for medical institutions MIS (Medical Informational System).

23 children were operated on in the ENT department in the period from 2017 to 2019. (in 2017 - 11 (47.8%) children, in 2018 - 6 (26%) children, in 2019 - 6 (26%) children)

At the time of the operation, 17 (73.9%) children were 1 to 3 years old, 1 child (4.3%) from 4 to 6 years old, 3 children (13%) from 7 to 13 years old, 18 years old - 2 children (8.6%). Among them there are 14 boys (60.8%), girls (39.1%).

Distribution by nationality: Yakuts - 17 (73.9%) children, Russians - 3 (13%) children, Evenki - 2 (8.6%) children, Tuvinians - 1 (4.3%) children.

Distribution of patients by place of residence: Yakutsk - 6 (26%) children, uluses - 17 (74%) children.

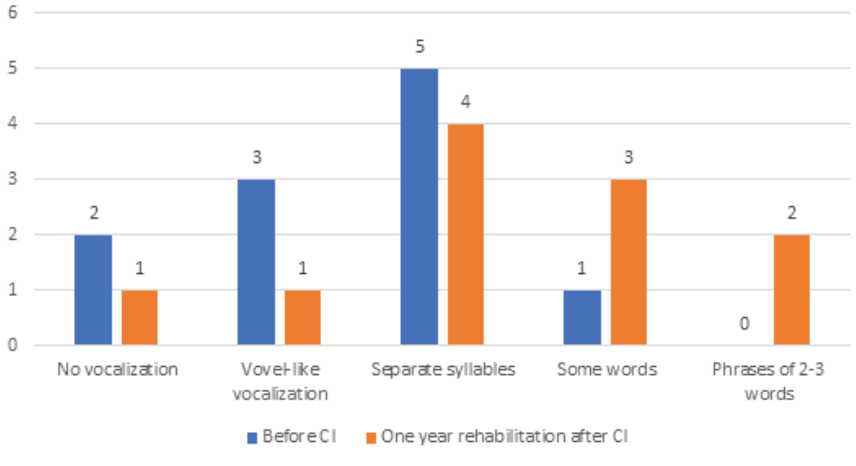
9 children (39.1%) had severe bilateral sensorineural hearing loss of the IV degree in both ears, bilateral deafness in 13 (56.5%) children, and 1 (4.3%) child had sensorineural hearing loss of IV degree for one ear and deafness to the other. (Figure 1)

According to the etiological factor, the following data were obtained: hereditary predisposition - 4 (17.3%) children (among them 1 child with a confirmed GJB2 gene mutation in 1 child (4.3%)), congenital anomaly in the development

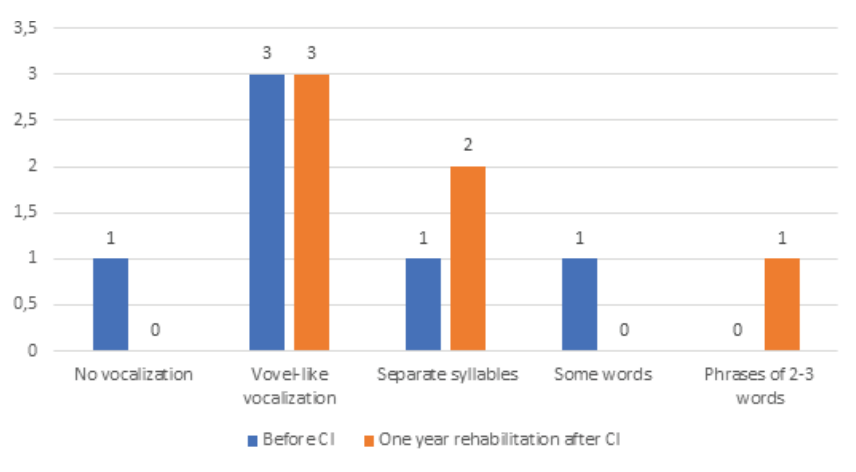
Assessment of the category of hearing perception (CAP) in patients with CI, abs. number (%)

Month	Points						
	0	1	2	3	4	5	6
in 2017							
0(first connection)		11(100)					
3		5(45.4)	5(45.4)	1(9.1)			
6		2(18.2)	5(45.4)	3(27.2)		1(9.1)	
9		1(9.1)	3(27.2)	4(36.4)		3(27.2)	
12			2(18.2)	6(54.5)		3(27.2)	
18			1(9.1)	4(36.4)	2(18.2)	4(36.4)	
24				2(18.2)	3(27.2)	5(45.4)	1(9.1)
in 2018							
0(first connection)		5(83.3)		1(16.7)			
3		2(33.3)	3(50)	1(16.7)			
6		1(16.7)	2(33.3)	2(33.3)	1(16.7)		
9		1(16.7)	1(16.7)	3(50)		1(16.7)	
in 2019							
0(first connection)		3(50)	1(16.7)	2(33.3)			

a



b



Dynamics of the patients with CI speech: a - in 2017, b – in 2018

of the snail - 1 (4.3%)) a child, suffered from meningitis - 2 (8.7%) children, taking ototoxic drugs - 2 (8.7%) children, with an unspecified etiology of the disease - 14 (60.8%) children. Concomitant diseases were observed in 6 (26%) patients. (Figure 2)

Some patients used super-powerful digital hearing aids before surgery - 7 (30.4%) children. Among them, with experience of using a hearing aid up to 6 months - 3 (42.8%) children, binaural - 4 (57.1%) children, monaural - 3 (42.8%) children.

Side of cochlear implantation: AD - 18 (78.2%) children, AS - 5 (21.7%) children, among them AD / AS - 1 child, the left side was operated at the Federal State Budgetary Institution 'National Medical Research Center otorhinolaryngology of the Federal Medical and Biological Agency' of Russia.

Results and discussion. Evaluation of patient selection according to the "Scale for evaluating the prospect of using cochlear implantation in young children" showed that in the study group of patients (23 children) in 19 (82.6%) cases the results were more than 14 points. For children with congenital deafness, a value of more than 14 points indicates that the use of CI is very promising. The remaining 4 (17.4%) cases include children with severe concomitant pathology.

The state of hearing in dynamics according to the CAP scale (Assessment of the category of hearing perception) in children is presented in three tables, for 2017 in Table 1, for 2018 in Table 2 and for 2019 in Table 3.

When assessing the state of speech of children operated in 2017 (11 patients) before cochlear implantation: no vocalization in 2 (18.1%) children, vowel-like vocalizations in 3 (27.2%) children, separate syllables in 5 (45.5 %) children, some words in 1 (9.2%) child. The results of rehabilitation one year after the CT: no vocalization in 1 child (9.1%) due to wearing a tracheostomy tube, vowel-like vocalizations in 1 child (9.1%), separate syllables in 4 children (36.7%), separate words in 3 children (27.2%), phrases of 2-3 words in 2 children (18.1%). (Fig. 3)

In patients operated in 2018 (6 patients), before cochlear implantation: no vocalization in 1 child (16.7%), vowel-like vocalizations in 3 children (27.3%), separate syllables in 1 child (16.7%), single words in 1 child (16.7%). The results of rehabilitation one year after the CT: vowel-like vocalizations in 3 children (50%), separate syllables in 2 children (33.3%), phrases of 2-3 words in 1 child (16.7). (Figure 4)

Conclusion. In the course of the study, according to the data of the clinical and audiological examination of children, there is a positive trend in the process of hearing and speech rehabilitation. After connecting the SP, children study the sounds surrounding them with great interest, and additionally study with their parents. After starting attending rehabilitation centers, they successfully integrate into society, communicate with peers and relatives. An integrated approach to the treatment of patients with severe sensorineural hearing loss and deafness, including CT and hearing and speech rehabilitation, both in specialized centers and at home, is the most effective method of treatment. The ultimate goal of cochlear implantation is to teach the child to understand the speech, to speak, the ability to socialize and fully integrate into society.

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COMBINED TWO-LEVEL SPINAL EPIDURAL ANESTHESIA WITH FIXATION OF EPIDURAL CATHETER IN SUBCUTANEOUS CANAL IN A LONG-LIVER PATIENT WITH A CLOSED HIP FRACTURE

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The article discusses a clinical case of combined two-level spinal-epidural anesthesia (CDSEA) with fixation of an epidural catheter (EC) in the subcutaneous canal in a long-liver patient with a closed hip fracture. Use of local anesthesia in the form of KDSEA, followed by long-term postoperative anesthesia in a long-liver patient with a high anesthetic risk and multiple comorbid pathology in the form of epidural analgesia with a reliable method of EC fixation in the subcutaneous canal during surgery for a fracture of the proximal femur contributed to its successful implementation, the absence of complications in the postoperative period and early activation of the patient.

Keywords: combined double-level spinal-epidural anaesthesia, subcutaneous tunnel, modified spinal needle.

Aim: to show the effectiveness of TCSEA in long-livers patients in the surgical treatment of fractures of the lower limb bones, as well as the effectiveness of the method developed by us for fixing EC in the subcutaneous canal, preventing its dislocation.

Introduction. Epidural anesthesia is firmly established in the practice of the anesthesiologist. One of the main advantages of this method is the ability to prolong anesthesia and provide postoperative pain relief. The Tuohy needle used for EC placement can also be used for EC in the subcutaneous "tunnel" [14].

The combination of spinal anesthesia with epidural anesthesia gives even more advantages, to be precise : we get a fast onset of high quality anesthesia, practically unlimited in time with the possibility of prolonging the blockade to several anatomical regions and minimal toxicity [13].

Also, with TCSEA, the incidence of post-puncture syndrome decreases to 1.3% [4]. The incidence of inadequate EA ranges from 6 to 8% [12]. The main reason is the displacement of the initially correctly established EC [15,16].

Dislocation and migration of EC from the epidural space can lead to inadequate anesthesia, unilateral anesthesia, perforation of the dura mater and total spinal block, intravascular injection of local anesthetic, termination of EA due to complete loss of EC [1].

Analyzing the literature data, we can talk about a high frequency of EC migration. So Grosby E. in a study in 1990 that included 211 patients who received EA for pain relief in labor,in 54% was noted the migration of catheters, while in 70% of this number the catheters completely left the epidural space [11]. Another study in 153 patients showed a 36% incidence of catheter displacement,at the same time, in 13.7% of cases, the catheters were displaced inward by 1-3 cm, and in 22.2% external migration of 1 cm or more was noted, and in 2%, complete loss of catheters [8] . Reliable fixation of EC reduces the risk of its migration and creates conditions for effective and high-quality EA. Reliable EC fixation can be ensured by the use of special fixation devices or EC fixation in the subcutaneous canal.

If adhesive stickers were used to fix EC, then the frequency of its migration was 75%, while migration of more than 2 cm was 20-25% [9]. Tunneling of EC in the subcutaneous canal is a reliable method of its fixation, in which EC migration was noted only in 10% of cases [10].

There are several ways to perform EC in the subcutaneous canal. For this, in the first method, an unmodified epidural needle is used, which is passed from the lateral position to the EC exit site [6]. In the second method, the epidural needle is modified by breaking off the needle cannula and is passed in this from the EC position in the lateral direction [2]. In the third method, a metal mandrel is drawn from the epidural needle from the EC position in the lateral direction, along which the epidural needle is passed to the EC position [3].

Further, with these three methods, EC is performed through the lumen of the epidural needle. The fourth method uses a three-component device, in which the outer cylinder diameter is 2.7 mm [7]. In our clinic, we have developed a new method for EC placement in the subcutaneous canal using a modified spinal needle, which was used to conduct spinal anesthesia in the complex TCSEA procedure .

This method showed the convenience of EC placement in the subcutaneous canal and the reliability of its fixation, which allows a long-term and high-quality postoperative EA, as well as the absence of EC dislocation and the absence of infectious complications.

Clinical case description. A 95-year-old patient, had a domestic injury on 03/21/2021, on the 03/22/2021, the ambulance team was taken to the admission department of the Kotovsk City Clinical Hospital, examined by a traumatologist on duty, hospitalized in the trauma department with a diagnosis: Closed fracture of the right hip neck with displacement of fragments. An instrumental examination was performed (X-ray examination of the thoracic viscera, X-ray of the right hip joint).

Radiologist's conclusion: Diffuse

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pneumosclerosis. Aorto-atherosclerosis. Transcervical fracture of the right femoral neck with a relatively pronounced displacement of the fragments. Electrocardiography was performed. Conclusion: Sinus rhythm 88 in 1 min. EOS is deviated sharply to the left. Incomplete Left bundle branch block.

According to the results of laboratory examination, an increased level of total bilirubin was revealed - 33.55 μmol / l, direct bilirubin - 17.21 μmol / l, platelets were reduced - 120 x 109 / l. Examined by a general practitioner and neurologist. Conclusion: ischemic heart disease. Atherosclerotic cardiosclerosis. Essential hypertension 3 St. , AH 2 St. , the risk score 4. CHF (Chronic Heart Failure) 2a (FC3). COPD 3 stg., Moderate severity, mixed type. Dilation of the cavities of the heart. Senile asthenia. Calcification of the valve apparatus: aortic stenosis, mitral valve insufficiency. CeVD (Cerebrovascular diseases) . CCI (chronic cerebral ischaemia) , Mild cognitive impairment.

Multisegmental osteochondrosis of the spine. Kyphoscoliosis of the thoracic region. Obliterating atherosclerosis of the arteries of the lower extremities. CKD Stg. 2(Chronic kidney diseases) . Hyperplasia of the prostate gland. Cysts of both kidneys. The patient is scheduled to undergo surgery: Closed reposition of the fracture of the right femoral neck, osteosynthesis with screws. Taking into account the patient's age, the presence of concomitant pathology, the anesthetic risk was set at 4 grade according to the ASA.

The council of physicians decided to perform a surgical intervention under local regional anesthesia by the TCSEA method with EC fixation under the skin of the lumbar region using a modified spinal needle. The operation was performed on the 2nd day after the patient was hospitalized. Features of the anesthetic: In the supine position in the operating room, the peripheral vein of the right upper extremity was catheterized.

Further, in the sitting position, TCSEA was performed with EC fixation in the subcutaneous canal using a modified spinal needle. The method was developed in our medical institution [5]. The essence of the method is that after the placement of the EC in the interval L3 - L4 (photo No. 1), spinal anesthesia is performed in the interval L2 - L3 with a G 26 needle (photo No. 2.) Hyperbaric solution of Bupivacaine 20 mg is introduced into the spinal canal.

The spinal needle is then modified: the needle pavilion is broken off. The EC is put on the proximal end of the needle (photo No. 3). EC G 20 is ideal for spinal needle G 26. The needle with EC on, is passed below the EC position under the skin of the lumbar region in the lateral direction (photo no. 4), forming a subcutaneous canal up to 70 mm long. The diameter of the subcutaneous canal is equal to the diameter of the EC G 20 - 0.9 mm. The channel is narrow and 70 mm long. This contributes to the firm fixation of the EC.

The greater the length of the inner part of the EC, the less the risk of infection of the epidural space and dislocation of the catheter. The length of the inner part of the EC is 160 mm (in the epidural space - 40 mm, from the ligamentum flavum to the skin exit - 40 mm, and in the subcutaneous canal - 80 mm). The EC also receives an additional bend at an angle of 90°, which also increases the reliability of its fixation. The time of EC placement in the subcutaneous canal was 5 minutes. After TCSEA, the patient is given an horizontal position. The onset time of spinal anesthesia was 10 minutes.

Anesthesia level - Th10. A good level of analgesia, sensory and motor block (Bromage -3 grade). Surgical intervention was performed - closed reduction of the fracture of the right femoral neck, osteosynthesis with screws. The duration of the operation was 1 hour 10 minutes. The patient underwent surgery satisfactorily, there were no hemodynamic and respirat ory disorders.

The volume of preoperative and intraoperative infusion was 900 ml. Diuresis during the operation was 120 ml. At the end of the surgery, the patient was transferred to the postoperative ward of the trauma department. After regression of spinal anesthesia, after the test dose, EA was started with a solution of Ropivacaine 2 mg / ml at a dose of 18 mg / h. EA was carried out for 4 days. The pain level was assessed by the VAS and was 2 cm (mild pain).

Changing of adhesive stickers and treatment with an antiseptic solution at the site of the epidural puncture and at the site of the EC outlet on the skin was carried out daily. No special fixing devices were used. Standard adhesive stickers were used. On the second day, the patient began to sit down in bed. On the third day, he began to sit up in bed with his legs down.

Four days after the operation, the EC was removed. The external dislocation of the EC at the time of extraction was 7 mm, which is assessed as an insignificant dislocation that does not affect the quality of anesthesia. Removal of EC went without technical difficulties. Treatment,

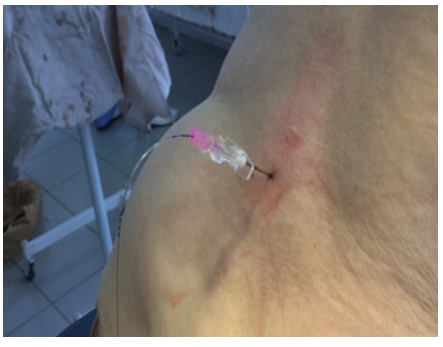

Fig. 1. Catheterization of the epidural space in the L3-L4 interval

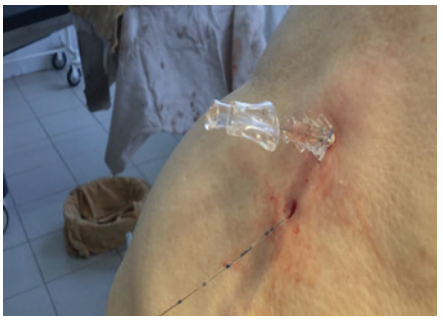

Рис. 2. Spinal anesthesia between L 2 - L3 with a G 26 needle

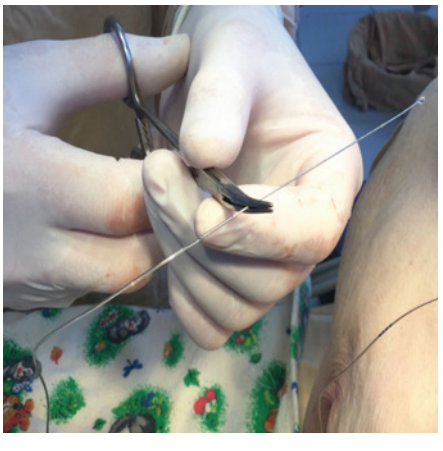

Рис. 3. The EC is put on the proximal end of the needle

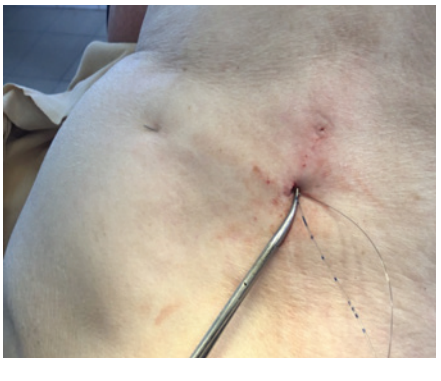

Рис. 4. The needle with the EC on it is held below the EC position under the skin of the lumbar region

activation and rehabilitation took place as planned. On the 15th day, the patient was discharged for outpatient treatment by a traumatologist.

Conclusion. The use of regional methods of anesthesia in long-livers patients during surgical treatment of fractures of the bones of the lower extremities significantly reduces the risk of developing perioperative complications of the cardiovascular and respiratory systems. Reliable fixation of EC in the subcutaneous canal, performed using a modified spinal needle, prevents EC dislocation from the epidural space, which contributes to high-quality postoperative analgesia.

Reducing the level of pain in the postoperative period contributes to the early activation of patients, reducing the risk of postoperative complications, especially in long-lived patients.

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DURATION OF DRUG FIXATION AT THE EDGE OF RESECTION DURING TARGETED CHEMOEMBOLIZATION

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УДК 616.61-006.694

The aim: to determine the duration of drug presence in the parenchyma, introduced into the edge of resection and fixed with a hemostatic suture.

Materials and methods. The present study is based on the results of 13 experimental operations on patients with malignant neoplasms of the renal parenchyma. A dynamic assessment of the content of contrast agents at the edge of the resection, after their introduction through the renal artery, was performed.

Results and discussion. Transarterial injection of a contrast agent into the renal parenchyma and its subsequent fixation at the edge of resection with a hemostatic suture, maintains a high concentration of the drug for a week after surgery Conclusion. The presence of high concentrations of the targeted drug at the margin of the kidney resection in the early postoperative period will create an additional barrier of anti-contraction protection.

Keywords: Kidney cancer, balloon targeted chemoembolization.

Introduction. In the Russian Federation in 2018, kidney cancer was diagnosed for the first time in 23,157 people; the mortality rate in the first year of life after diagnosis was 14.5%. In the structure of cancer incidence, kidney cancer is 4.7% [3].

In the tumor tissue, which has an increased mitotic potential, angiogenesis constantly occurs to meet the metabolic needs of the growing tumor. In this regard, in the new millennium, drugs have become widespread - inhibitors of vascular growth factors that block angiogenesis [5].

Despite the success of targeted therapy, the studies of the last decade demonstrate insufficient effectiveness of the treatment, which is due to the multifactorial nature of the causes of oncoangiogenesis [1, 2, 6]. Also, one cannot ignore the toxicity of targeted drugs, the low sensitivity of certain forms of cancer to inhibitors of angiogenesis, as well as the high likelihood of relapses against the background of targeted therapy [7, 11]. Bevacizumab, a humanized anti-VEGF monoclonal antibody, was the first drug approved by the Food and Drug Administration (FDA) for the treatment of metastatic colon, ovarian, kidney, non-squamous cell lung cancer, and glioblastoma multiforme [9, 10]. However, it has not shown clinical significance when used as monotherapy, with the exception of glioblastoma [8].

Dissatisfaction with the results of targeted therapy prompted the search for new areas of application of antiangiogenic drugs based on the mechanism of their action. An example of such an innovative approach was the method of balloon chemoembolization and resection of malignant tumors of parenchymal organs, in essence of which the authors received a patent for invention of the Russian Federation No. 2711549 [4]. The essence of the method consists in the transarterial injection of an angiogenesis inhibitor into the parenchyma segment of the organ with a tumor through the coaxial canal of a balloon catheter installed and inflated in the segmental branch of the artery to provide local and reversible intraoperative ischemia of the segment. After the injection of the targeted drug, tumor resection is performed, followed by suturing the parenchymal wound with a hemostatic suture and immediate removal of the embolizing balloon catheter. As is known, as a result of ischemia, tumor tissue secretes vascular endothelial growth factor (VEGF). The introduction of the antiangiogenic drug Bevazuzimab directly at the time of tumor ischemia inactivates the synthesized VEGF, depriving the prospects of continued growth or recurrence of a malignant tumor.

It is also impossible not to take into account the high margin of vitality of malignant cells - the oncocytes remaining in the margin of the organ resection under conditions of ischemia continue to release vascular growth factor, which can provoke tumor recurrence or activation of possible metastatic foci. Our study is aimed at determining the duration of fixation of the preparation at the edge of the resection.

Materials and research methods. In the urology department of GAU Republican Hospital No. 1 - National Center of Medicine in 2018-2019, kidney resections were performed in 13 patients with a diagnosis of renal cell carcinoma T1 N0 M0. In 10 patients, the histological report showed clear cell carcinoma, in 3 operated patients papillary variant of kidney cancer was revealed. All patients gave written consent to participate in the experimental study.

In 8 patients, the tumor was localized in the left kidney, in 5 - right-sided lesion. The sizes of the formations ranged from 18 to 56 mm. According to computed tomography data, the mean score on the morphometric scale R.E.N.A.L. was estimated at 5.6 points. Surgical support was performed by lumbotomy access, in a lateral position, with isolation and clamping of the renal artery. No additional arteries feeding the kidney were found in any case. After resection and removal of the preparation, U- and Z-shaped hemostatic

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sutures were applied to the wound edges, the duration of thermal ischemia did not exceed 20 minutes.

Modeling of targeted balloon chemoembolization was performed in the following way. After isolation of the renal pedicle, the renal artery was clamped with a Satinsky vascular forceps. Thus, the kidney was completely excluded from the main blood flow. Distal to the clamping, closer to the kidney, the artery was punctured with an infusion cannula of the "needle-butterfly" type to a depth of 3-4 mm, and a solution of contrast agent was injected through it. To quantify the densitometric density of the contrast fixed in the renal parenchyma by computed tomography, Ultravist was used in an amount of 20 ml, diluted in 50 ml of saline in 7 patients. To qualitatively determine the duration of fixation of the substance at the edge of resection in 6 patients by magnetic resonance imaging, a preparation containing gadolinium - Gadovist 7.5 ml, also diluted in 50 ml of saline was used. Immediately after the introduction of the marker, an atypical resection of the formation within the healthy tissue was performed, followed by suturing of the parenchymal wound. 7 patients were examined by computed tomography on the day of surgery and on the 6th day of the postoperative period on a 64-slice General Electric Optima 660 apparatus without the use of contrast enhancement. A qualitative assessment of the degree of saturation with the inserted marker of the resection margin was performed in 6 patients on the 2nd and 6th days by means of magnetic resonance imaging on a Siemens Magnetom Avanto tomograph. The studies were carried out in coronal and axial projections with 3 mm sections, according to the T1 vibe protocol and with a tension of 1.5 Tesla.

Results and discussion. In response to acute hypoxia during surgical removal of the tumor, malignant cells secrete special substances that prevent the development of hypoxia. There are several varieties of them, the most active of which is vascular endothelial growth factor (VEGF). Its production occurs immediately in response to hypoxia of tumor tissue, acting on the corresponding receptors, vascular growth factor triggers a cascade of reactions that activate neoangiogenesis. In the presence of metastatic foci or perico-multiple oncological process in the body, the effect of VEGF on them is a prognostically unfavorable factor.

Based on the understanding of this mechanism, a new method is proposed that combines the antiangiogenic effect with the surgical method of eliminating the oncological focus. Transarterial administration of an angiogenesis blocker, which has a direct inhibitory effect on vascular growth factor at the very moment of active production of the latter, eliminates one of the main mechanisms for the further spread of the malignant process.

To assess the amount of the substance injected into the parenchyma and fixed at the edge of the resection with a hemostatic suture, an Ultravist iodine-containing contrast was used. We compared the densitometric density of the kidney parenchyma with the injected contrast and the density of the contralletral healthy kidney in dynamics 6 days after the operation.

Figure 1 shows a CT scan of a patient with a tumor of the left kidney that underwent resection within a healthy tissue with a preliminary intra-arterial injection of Ultravist. On the day of surgery, 4 hours later, this patient underwent computed tomography. A snapshot of the tomogram is shown in Figure 2, where the following are marked: a - the area of the resection edge with the injected contrast agent; b - an area of healthy kidney tissue outside the field of surgical manipulation; c - parenchyma of the contralateral organ for control measurement.

On the 6th day after the operation, a similar computed tomography (Fig. No. 3) was performed to determine the densitometric density (DMP) of the renal parenchyma at the previous points. Analysis of PMD indicators was performed using statistical packages SPSS (Windows version 7.5.2). The significance of differences between quantitative indicators was assessed using the Student's t test for normally distributed values. Differences were considered significant at $p < 0.03$.

The obtained values of densitometric density are presented in table No. 1. For a dynamic assessment of the degree of saturation with a contrast agent, the DMP index of the area of the opposite kidney not exposed to surgery was taken as 100%, which made it possible to assess the content of the marker in the resection margin relative to the intact parenchyma in dynamics.

The introduction of a contrast agent into the edge of the resection increases its densitometric density by 3 times compared to the intact tissue - the DMP values at the edge of the resection are 222.51% higher than the density of a healthy kidney. Restoration of blood flow after performing the main surgical technique washes out the contrast agent from the kidney tissue not stitched with a suture - the DMP of the parenchyma of the operated kidney outside the field of surgical manipulation is 25.83% higher than the DMP of a healthy kidney. 6 days

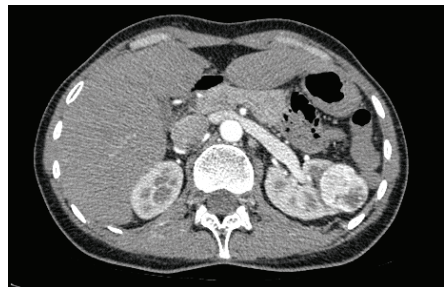


Fig. 1. Tumor of the left kidney.

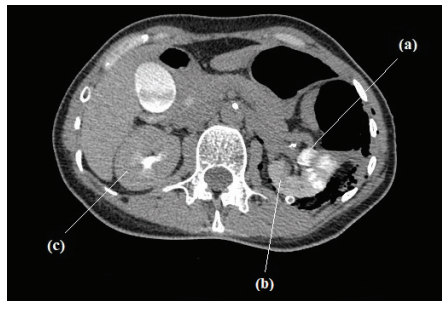


Fig. 2. Computer tomography image right after surgery. a - kidney resection area with a fixation of a contrast agent; b - zone of intact parenchyma, not exposed to surgical intervention; c - an area of the parenchyma of the contralateral kidney for control measurement.

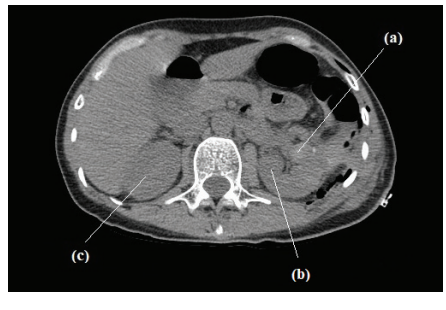
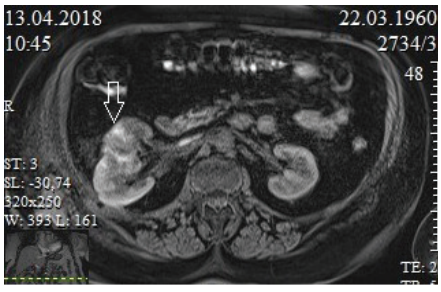


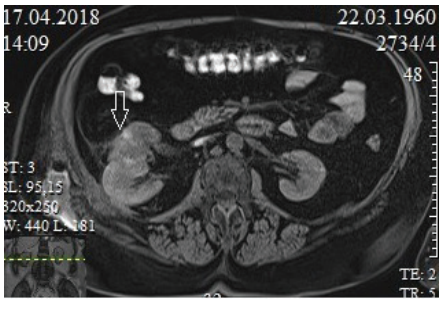
Fig. 3. Computed tomogram on the 6th day after surgery

Densitometric density of various parts of the kidney over time (*p=0,021*)

	0 day		6-th day	
	D.M.P.	%	D.M.P.	%
Parenchyma zone of the contralateral kidney (c)	75,5	100	32,5	100
Zone of intact renal parenchyma (b)	95	125,83	33,5	103,07
Resection and hemostasis area (a)	243,5	322,51	71	218,46



a



b

Fig. 4. MRT on the 2nd day, 6th day

after the operation, the measurement of the densitometric density showed a high density of the resection edge sutured with a hemostatic suture - 118.46% higher than the density of the unoperated organ, while the DMP of the intact parenchyma of the operated kidney is higher than the healthy organ by only 3.07%.

The presented data clearly demonstrate that the substance introduced into the arterial bed of the kidney and fixed in the tissues with a hemostatic suture is fixed in them for a long period of time. The coefficient of elimination of the substance was 1.47. This means that 6 days after the operation, more than half of the substance introduced transarterially into the resection margin remains in the tissue.

For a qualitative assessment of the degree of fixation of the substance at the edge of the resection, magnetic resonance imaging was performed in patients who underwent kidney resection on the 2nd and 6th days after the operation according to the method described above, with the difference that instead of Ultravist, Gadovist in volume 7 was introduced into the renal artery. , 5 ml. This study is based on the registration of a magnetic resonance signal from kidney tissues with an injected paramagnet. The study was carried out on a Siemens Magnetom Avanto magnetic resonance imager with 1.5 Tesla strength according to the T1-vibe protocol with a slice thickness of 3 mm in coronary and axial projections.

The obtained tomograms clearly show the areas of accumulation of paramagnet by the kidney tissue in the resection zone (Fig. No. 4 and Fig. No. 5). In dynamics, 6 days after the operation, the presence of residual contrasting at the edge of the resection is noted, which indicates its insignificant elimination. The leaching of the substance occurs mainly by diffusion, at a very low rate, as indicated by a decrease in the concentration of the substance introduced into the kidney parenchyma by no more than half during the first week.

Conclusion. The performed study of prolongation of the injected substance convincingly proved the presence of high concentrations of the marker at the edge of resection of the parenchymal organ during the first week of the postoperative period. This gives reason to hope that the targeted drug Bevatsimab, injected into the segment affected by the malignant tumor, will also maintain a high concentration at the resection margin for a sufficient time to provide additional anti-angiogenic protection after surgical resection of the malignant neoplasm.

Reference

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DIAGNOSIS AND TREATMENT OF GASTRODUODENAL ULCER BLEED-ING

Gastroduodenal ulcer bleeding remains an urgent problem in emergency surgery. This litera-ture review presents modern data on gastroduodenal bleeding of ulcerative etiology. The review considers the issues of epidemiology and etiology, as well as diagnosis and treatment of ulcera-tive gastroduodenal bleeding.

Keywords: peptic ulcer bleeding, peptic ulcer bleeding relapse, conservative hemostasis, endoscopic hemostasis.

Acute bleeding from the upper gastrointestinal tract remains one of the most important and complex problems in urgent surgery. Despite a large number of studies and publications, patient's treatment strategy of diagnosing and therapy remains the subject of discussion. The absence of standardized therapeutic and diagnostic algorithms creates a complexity in case management of this particular patient population.

Materials and methods. The analysis of articles in Russian and English languages, investigating ulcerative gastroduodenal bleeding, with a limited publication date of 10 years have been conducted. Articles exceeding a 10-year period were admitted in case of similar articles absence or scientifically valuable research. The literature search was made on the bases of scientific e-libraries, such as PubMed, eLIBRARY, Cyber Leninka, Google Scholar and Cochrane Library.

Epidemiology. Gastric and duodenal ulcers are the major causes of gastrointestinal bleeding. According to Russian authors, the frequency of gastroduodenal bleeding of ulcerative etiology in the bleeding general structure accounts for 56.1% - 59% and it remains the primary cause of gastrointestinal bleeding. Despite significant ulcerative gastroduodenal bleeding diagnosis and treatment progress, unadjusted mortality rate remains extremely high reaching 20.3%. Several factors like elderly and senile age, co-morbidity, recurrent bleeding with repeated endoscopic hemostasis and surgical treatment aggravates the patienthood. In such a way, the mortality rate of the specified patient's category increases up to 53% [3].

The challenge of health services delivery to patients with gastroduodenal bleeding outside the Russian Federation is no less urgent. In the post-Soviet area, the ulcerative hemorrhages fraction in the structure of gastroduodenal bleeding amounts to 72.8%, while in the structure of emergency surgical care it comes up to 6.3% with a mortality rate reaching 4.4% [1]. In Western Europe and North America, these rates range from 26% to 50.6% with a mortality rate of up to 13.8% [30]. The ratio of male and female in the world is approximately the same (69.9% -75% and 25% -30.1%, respectively) [2].

However, recently, we can trace a decrease in the number of ulcerative bleeding and an increase in the frequency of bleeding of non-ulcer etiology in the general structure of hemorrhages. This fact may be associated with improved diagnosis and differential diagnostics as a result of the widespread introduction of esophagogastroduodenoscopy (EGDS).

Etiology. The major etiological factors are Helicobacter pylori infection and NSAIDs.

The discovery of H. pylori in 1982 has changed the understanding of the etiology of peptic ulcer disease [26]. The relation of H. pylori infection to the development of ulcerative gastroduodenal bleeding has made adjustments to the diagnosis and treatment of the disease. However, neither domestic nor international recommendations on ulcerative gastroduodenal bleeding give clear instructions for clinicians as regards testing for H. pylori infection detection in acute hemorrhage conditions [4, 17, 22].

According to a 2006 meta-analysis, it was found that the sensitivity of H. pylori tests based on endoscopy (biopsy for rapid urease testing, histology, and seeding) is low in acute stages of ulcerative bleeding. The reasons explaining this event are not clear. Feces antigen analysis is less accurate and has many false-positive results, probably due to cross-reactions with blood components in the lumen of the gastrointestinal tract, in such a way serological test cannot be recommended as the first diagnostic test for H. pylori infection in hemorrhagic conditions. In this connection, the best possible test is the breathing helicobacter urease test [16].

It is common knowledge that Nonsteroidal anti-inflammatory drugs and antiaggregants increase the risk of ulcerative gastroduodenal bleeding development; however, on numerous occasions they are proposed due to the concurrent vascular diseases. At the same time in the cases of ulcerative bleeding drug withdrawal duration as well as the timing of the antiplatelet therapy resumption remain controversial. Currently, if possible, it is applicable not to stop taking aspirin, although if the withdrawal is necessary, the duration of aspirin withdrawal should not exceed 3 days in the cases of ulcerative gastroduodenal bleeding.

Recently, there has been an increase in ulcerative gastroduodenal bleeding caused by idiopathic ulcers not related to H. pylori infection and the use of NSAIDs, and can reach approximately 20% of all cases. The main etiological factor of such ulcers is stress. After the earthquake in Japan in 2011, the number of gastroduodenal ulcer bleeding cases increased by 2.2 times, which also indicates the vital role of stress in the development of peptic ulcer disease.

Therefore, peptic ulcer disease is a polyetiological, multifactorial disease that has many development causes which require hard research.

Clinical manifestation. Normally, clinical implications of upper gastrointestinal bleeding are manifestative for clinicians. Core symptoms signalizing the bleeding are haematemesis or coffee-ground vomit and melaena. However, if there are no such symptoms, the bleeding diagnosis can be cumbrous. According to the statistics, in almost half of

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upper gastrointestinal tract (GIT) bleeding cases vomiting does not occur, and in 5% of cases there are no pathognomonic symptoms at all. This can lead to delayed initiation of treatment [2].

Endoscopic diagnostics in hematochezia remains a significant dilemma: it is bleeding from the intestine or accelerated transit of blood from the source of the upper gastrointestinal tract. What to do first, colonoscopy or EGDS? Hematochezia can cause hemorrhages from the upper gastrointestinal tract, if the intensity of bleeding is high enough and the digestive enzymes are not enough to convert hemoglobin to hematin hydrochloric acid, occurring in up to 14% of cases [14]. So, in order to determine the therapeutic and diagnostic tactics, it is necessary to conduct prospective studies, although it seems reasonable to perform an EGDS before an endoscopy of the lower gastrointestinal tract.

Diagnosis and endoscopic imaging. Endoscopic imaging is the core diagnostic method for gastroduodenal bleeding. Since gastric lavage is not always effective enough, there is a reasonable ground to use prokinetic therapy as a part of preparation for gastroduodenal ulcer bleeding endoscopic examination.

Recent studies have shown that intravenous erythromycin prior to emergency esophagogastroduodenoscopy improves the gastrointestinal mucosa imaging, reduces the number of repeated examinations and the duration of patients hospitalization [32]. However erythromycin as prokinetic is not yet widespread in national clinics.

The alternative to erythromycin would be the prescription to use metoclopramide 30 minutes prior to the endoscopic examination in patients with upper gastrointestinal bleeding signs. At the same time, there are studies the results of which, on the contrary, show the ineffectiveness of metoclopramide in comparison with placebo [13]. A number of authors point out higher efficiency of erythromycin, so it requires further study with a larger number of participants.

Conservative therapy. One of the first recommendations in the ulcerative gastroduodenal bleeding treatment is hunger; nevertheless, there are no criteria for the duration of the fasting diet, as well as the time for the resumption of oral or enteral alimentation. According to the study results, patients who underwent endoscopic hemostasis for ulcerative gastroduodenal bleeding or those who have a high risk of recurrence should refrain from oral or enteral nutrition for 48 hours. In the case of a low recurrent bleeding risk, nutrition can be resumed immediately after endoscopic examination [19]. At the same time, the study carried out by M. Khoshbaten points out that oral feeding did not significantly affect the water-electrolyte balance and treatment results in patients with ulcerative gastroduodenal bleeding who underwent endoscopic hemostasis, but it reduced the length of hospitalization [23].

"The gold standard" of the ulcerative gastroduodenal bleeding medical therapy is the usage of Proton-pump inhibitor (PPI).

The primary mechanism of PPI prescription is Hong Kong protocol, which represents bolus IPP dosing followed by intravenous infusion within 72 hours. However, the debating point is the switch from intravenous PPIs to oral ones. On this basis a metaanalysis incorporating six random investigations was performed during the period from 2006 to 2011. In total, 615 patients were randomly assigned to receive PPIs orally (n=302) or intravenous (n=313). According to the results of the analysis, there was no significant difference between oral and intravenous PPIs in terms of recurrent bleeding, average blood transfusion volume, surgical interference demand and all-cause mortality rate. Hospitalization duration was significantly reduced in patients using oral PPIs. In addition, it was emphasized that oral PPIs demonstrated efficiency similar to intravenous PPIs in patients with bleeding ulcerative etiology, but the results were pooled from open-label studies with a limited sample size.

Blood transfusion. The hemoglobin threshold level for red blood cells transfusion in patients with gastrointestinal bleeding is controversial. The general clinical principles are used in order to restore the volume of blood circulation, although it seems that an individual approach to transfusion is inevitable. 2014 Clinical guidelines for ulcerative gastroduodenal bleeding stress that blood transfusion is indicated when hemoglobin level is less than 90 GM/DL [4].

In order to find the better approach scientist conducted a randomized study with two groups of patients: the first one to apply restrictive strategy (blood transfusion with hemoglobin values less than 70 GM/DL) and the second one to apply liberal strategy (blood transfusion with hemoglobin less than 90 GM/DL). A restrictive strategy of maintaining a hemoglobin level of 70-90 GM/DL was as safe and as effective as the traditional goal of reaching a hemoglobin level of 90-110 GM/DL. In patients with bleeding ulcers, there were fewer differences between the two strategies, although all valuable clinical outcomes were better with the restrictive strategy (mortality 3% versus 5%, recurrent bleeding 10% versus 17%, surgery 2% versus 6%) [35].

Similar results are highlighted in the conducted meta-analysis that combined the results of four studies [36]. In particular, included studies did not specifically examine gastroduodenal ulcer bleeding, but also they had different methodologies, inclusion and exclusion criteria. Nevertheless, the results of the study support the restrictive strategy: there was a significant decrease in mortality and the duration of hospitalization, and the percentage of recurrent bleeding was slightly lower. The exact optimal strategy for blood transfusion is unclear and should always be individualized for each patient.

In addition, the analysis of four published and one unpublished randomized controlled studies, which included 1965 people, showed that the amount of transfused red blood cell mass in the restrictive blood transfusion strategy group was less than in the liberal strategy group. The restrictive strategy was associated with a lower risk of bleeding recurrence and death regardless of all causes [28].

Endoscopic hemostasis. The key method of patients with ulcerative gastroduodenal bleeding treatment is endoscopic hemostasis.

Currently, the methods of endoscopic hemostasis are divided into two types: mechanical and thermal. A meta-analysis carried out in 2009 comprising 75 studies evaluating endoscopic hemostasis methods illustrated that both thermal and mechanical hemostasis methods are effective [25].

Injection hemostasis is the most common method. Injection hemostasis as monotherapy is significantly inferior to combined hemostasis in the form of the adrenaline solution injection combined with other methods of hemostasis [25]. In various medical institutions, adrenaline solution, ethanol, ethoxysclerol, isotonic sodium chloride solution, etc. are preferred as a drug for injectable hemostasis. This choice is justified by the secondary pharmacological effect. However, according to the meta-analysis carried out in 2003 by M. Bardou and co-authors, which included 38 studies, none of the drugs used for injection hemostasis have advantages over others [10].

Existing thermal methods of hemostasis are divided into contact and non-contact methods. The applied methods of contact hemostasis include electrocoagulation and the use of heater probes. Talking about methods of electrocoagula-

tion, the preference is given to bipolar and multipolar probes. Electrocoagulation devices deliver energy in a fixed circuit, heating tissues up to 1000 C, then their action is stopped, limiting the depth of tissue damage, thereby reducing the risk of perforation. The methods of bipolar and multipolar coagulation are easier to use, since they have a local effect, without requirement of patient's grounding. Heating probes maintain a constant temperature of approximately 2500 C for a given time, supplying the required amount of energy, which can cause uncontrolled tissue damage depth and lead to a high risk of an organ paries perforation reaching 3% of cases [24].

Argon plasma coagulation (APC) is a non-contact method of thermal endogemostasis. Since the first description and early experience of using this method, it has become widespread in clinical practice [18]. The unquestioned advantage of this method is the small depth of the coagulation scab (up to 3 mm), which makes it possible to use argon plasma coagulation for bleeding from deep ulcers and with a thin organ paries, since the probability of perforation is much lower than with contact methods of hemostasis. Injection of epinephrine in combination with APC has shown the same efficiency and safety as injection of epinephrine in combination with a heating probe in the process of patients with ulcerative gastroduodenal bleeding treatment.

Recently developed in Japan, the soft coagulation clip forceps method is increasingly applied in the cases of ulcerative bleeding. Thus, soft coagulation turned out to be more effective than coagulation with a thermal probe in achieving endoscopic hemostasis, making it possible to achieve hemostasis in 96% of cases. The use of clip forceps in terms of efficiency and safety is not inferior to APC and is no less successful than clipping in the treatment of patients with ulcerative gastroduodenal bleeding [8]. One of the soft coagulation method's advantages is the reduction of the required time to achieve hemostasis [8].

One of the most fully studied method of mechanical hemostasis is clipping with metal clips. When properly used and positioned, the clips can cause overall hemostasis similar to the surgical deligation of a bleeding vessel. They do not cause significant tissue damage and do not interfere with the healing of ulcerative defects.

Taking into consideration that clips are metal, people also need to remember about their compatibility with magnetic resonance imaging (MRI). Although all commercially available clips are marked as incompatible with MRI, a study on biological models of pigs showed that MRI can be performed with all available types of clips, with the exception of Tri-Clip, since they separated from the stomach tissue during the experiment, which means they should be considered incompatible with MRI [15].

Combined endoscopic hemostasis is usually the procedure of choice. A 2014 Cochrane review conducted a meta-analysis of 19 randomized controlled trials with 2033 patients, which pointed out that combined hemostasis in the form of an injection of epinephrine and a second method of hemostasis is more effective in comparison with injection hemostasis with epinephrine only. This combination reduces the risk of rebleeding, the need for surgery, and mortality [34]. In a meta-analysis published in 2016, including 2888 patients, the effectiveness of various endoscopic hemostasis methods was studied. In the end, only clipping and combined hemostasis, including adrenaline injection and thermal exposure, were the most effective [9].

Local hemostatic means represent the new method of endoscopic hemostasis. Currently, there are three types of powder available: Hemospray, EndoClot and Ankaferd Blood-Stopper. For instance, Hemospray's combined technical and clinical success rate was 88.5% in humans and 81.8% in pig models studied. Bleeding recurrence was observed in 38 patients within 72 hours after treatment (16.2%) and in three pigs' models (27.3%). No side effects have been associated with the use of Hemospray [11].

There are also other methods of endoscopic hemostasis known in medical practice, such as OVESCO clips, Endoloops, bandage dressing, etc., which require further study in order to assess the possibility and effectiveness of their use in clinical practice.

Roentgen-endovascular treatment methods. Patients with recurrent ulcerative gastroduodenal bleeding, in cases when endoscopic hemostasis is unsuccessful, represent a serious problem. Percutaneous translumbar angiographic embolization can be an alternative to surgical measure.

In patients with bleeding ulcers after unsuccessful endoscopic hemostasis, X-ray endovascular treatments reduce the need for surgery without increasing overall mortality with fewer complications, but the reccurence rate may be higher (up to 34.4%) [37]. A study evaluating five-year experience showed that percutaneous transcatheter embolization reduced the frequency of rebleeding to 3.4%, and the need for surgical treatment to 10.3% [21].

Operative therapy. The indication for surgical treatment is ongoing bleeding with the ineffectiveness of other hemostasis methods. Operational activity in ulcerative gastroduodenal bleeding reaches 16-33%, and postoperative mortality is up to 32.5% [6]. In case of ulcerous gastroduodenal bleeding, laparotomy should not be finished in all patients with retroclusion of a bleeding vessel, since this tactic is complicated, as according to the statistics, up to 50% of cases end up by recurrent bleeding due to progressive necrosis in the area of the ulcer and arrosion of the bleeding vessel [6]. In critical cases when the risk of surgery is extremely high, repeated endoscopic hemostasis or X-ray endovascular treatment can be considered as an alternative.

Bleeding recurrence. Bleeding recurrence sharply worsens the prognosis of the disease. All mortality causes were significantly lower in patients who underwent only one procedure of endoscopic hemostasis (3%) compared to patients whose cases required more than one endoscopic hemostasis (6%), X-ray endovascular treatment (9%) or surgery (14%) [29].

The etiology of peptic ulcer disease complicated by bleeding is also a significant factor: after hemostasis, idiopathic ulcers showed a higher percentage of bleeding recurrence in comparison with ulcers associated with H. pylori infection and those caused by taking NSAIDs (30% versus 7.4% and 2.7%, respectively) [12].

Previously, the Forrest type of ulcer played great importance in predicting bleeding recurrence. However, according to a recent research, the risk of rebleeding for Forrest 1b ulcers is less than for Forrest 2a and 2b ulcers, and may not require high-dose PPI therapy after successful endoscopic hemostasis [20], which casts doubt on this approach.

Many scales have been created in an attempt to predict the risk of recurrent bleeding and the outcome of a patient's treatment. The most frequently used, especially in foreign practice, are the Glasgow-Blatchford and Rockall scales. A research described in the article 'Comparison of Glasgow-Blatchford score and full Rockall score systems to predict clinical out-comes in patients with upper gastrointestinal bleeding' was carried out in order to analyze the data of the scale. Thus, the Glasgow-Blatchford scale performed better in predicting bleeding recurrence [27]. Another international study pointed out in the article 'Comparison of

risk scoring systems for patients presenting with upper gastrointestinal bleeding: international multicentre prospective study' indicates that the Glasgow-Blatchford scale is the most accurate for predicting the need for interference, but all available prediction scales have low predictive accuracy for other criteria, including endoscopic therapy and mortality rate, therefore their clinical value for high-risk patient management is somewhat limited [31]. Domestic scales for the prognosis of recurrent bleeding have also been developed. The first one is the system for predicting recurrent bleeding by M.M. Vinokurov and the second ones are the systems developed at the Department of Faculty Surgery of the RUDN University which include the system for predicting recurrent bleeding (SPRK) and an improved system for predicting recurrent bleeding II (SPRK II). According to the results of the study carried out by domestic authors, the most optimal for use in clinical practice is SPRK II [5]. Despite the large number of relapse prediction scales, further work is needed to improve the systems.

Reoccurring endoscopic hemostasis. Endoscopic hemostasis is the basic method for stopping the bleeding in patients with ulcerous gastroduodenal bleeding, but none of the methods allows to achieve the final hemostasis without recurrent bleeding in all cases, which means that the choice of the hemostasis method in case of repeated bleeding is relevant. In accordance with clinical guidelines, emergency surgery is prescribed in patients with ongoing bleeding with ineffective or impossible endoscopic hemostasis, or with relapse [4]. However, at the same time these recommendations point out the possibility of repeated endoscopic hemostasis in case of relapse or the use of endovascular methods. Repeated endoscopic hemostasis, in case of recurrent bleeding, is considered effective in up to 97% of cases, avoiding surgical intervention in up to 84% of recurrence cases [7]. Performing repeated endoscopic hemostasis, as well as the ability to refuse patients surgical treatment, can reliably reduce mortality by 2-3 times [7, 29].

Repeated endoscopic hemostasis should be an alternative to surgical treatment for recurrent ulcer bleeding; at least a second attempt to achieve endohemostasis should be taken, especially in patients with severe comorbidity.

Conclusion. In conclusion, ulcerative gastroduodenal bleeding still remains a vital problem in urgent surgery and is accompanied by high mortality. So, to create a unified diagnostic and treatment algorithm for gastroduodenal bleeding of ulcerative etiology requires a great amount of research.

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HEALTH ORGANIZATION

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ANALYSIS OF PATIENT ROUTING AT THE HOSPITAL STAGE TO THE NEUROLOGICAL DEPARTMENT OF THE CENTRE FOR NEURODEGENERATIVE DISEASES OF THE CLINIC OF YAKUTSK SCIENTIFIC CENTRE FOR COMPLEX MEDICAL PROBEMS

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The article analyzes the routing of patients with neurodegenerative diseases from medical organizations of the Ministry of Health of the Republic of Sakha (Yakutia) to the neurological department of the Center for Neurodegenerative Diseases (CND) of the Clinic of the Yakutsk Scientific Center for Complex Medical Problems (YSC CMP). A comparative analysis of directed and refined diagnoses was carried out. Diseases that cause difficulties in their diagnosis have been identified.

Keywords: levels of medical care, patient routing, specialized medical care, neurodegenerative diseases.

Introduction. In the constituent entities of the Russian Federation (RF), a three-tier system of medical care has been formed. Based on the Order of the Ministry of Health of Russia dated December 29, 2012 No. 1706 (as amended on February 13, 2013) in the constituent entities of the Russian Federation, structural transformations for the provision of specialized care should be carried out on the basis of standards of medical care and procedures for its provision, phased provision of specialized medical care, with routing of referral of patients in medical organizations at 3 levels of medical care (MC). In stationary conditions, the provision of medical care is possible on the basis of optimizing the structure of the hospital bed fund. Thus, the routing of patients at all levels is a phased provision of medical care, the succession of their stages. The creation of patient routing according to various profiles increases the availability and quality of specialized medical care for the population. [7]

Thanks to this Order of the Ministry of Health of the Russian Federation, it became possible in the regions to develop routing schemes for patients to receive specialized emergency and routine medical care [1,6, 9, 8]. The routing should reflect the detailed movement of patients to medical organizations at each level. In the Republic of Sakha (Yakutia), the epidemiological situation of neurodegenerative diseases has been studied only for individual diseases. The obtained epidemiological indicators indicate that the percentage of neurodegenerative diseases (NDD) among all diseases of the nervous system is relatively high [5]. The most studied are type 1 spinocerebellar ataxia (SCA), oculopharyngeal myodystrophy (OPMD) [11], Charcot-Marie-Tooth disease (CMT) [2], Parkinson's disease (PD) [12], amyotrophic lateral sclerosis (ALS) [3]. Yakutia is the territory of the greatest distribution of SCA 1 in the world - 34.4 cases per 100 thousand population [13]. The situation with Alzheimer's disease, which ranks 1st in the world among NDDs [14], as well as for various genetic and inherited diseases of the nervous system common in Yakutia, remains unexplored. Given the age-dependent nature of NDD, the incidence of cases in Russia, as well as in the world, is steadily increasing and creates a medico-social problem for the health care and social protection authorities, since the aging of society is rapidly increasing [4]. In this regard, the organization of optimal routing of persons with a suspected NDD creates conditions for timely diagnosis and the appointment of adequate treatment, and for patients with a specified diagnosis, it becomes possible to receive high-quality specialized rehabilitation treatment, which largely forms the prognosis and course of the disease. Thus, taking into account the epidemiological situation and the lack of a round-the-clock hospital in the republic for patients with NDD, and based on the Order of the Ministry of Health of Russia [7], the order of the Ministry of Health of the RS (Y) No. 01-07 / 184 dated 14.02.2019 "On the order of routing of neurological patients suffering from neurodegenerative diseases at the outpatient and hospital stages "[7]. This Order of the Ministry of Health of the Republic of Sakha (Yakutia) made it possible to open a round-the-clock neurological hospital for this category of patients as part of the Center for Neurodegenerative Diseases on the basis of the Clinic of the YSC KMP and make it possible to receive specialized care for patients with NDD. The neurological department was opened on 01.04.2019. for 30 beds, of which 15 beds are intended for patients with neurodegenerative pathology and 15 beds for patients with other diseases of the nervous system, dorsopathies and cerebrovascular diseases, with the exception of acute cerebrovascular accidents.

Aim of the study: to analyze the hospital stage of routing neurodegenerative diseases in the Republic of Sakha (Yakutia) to the neurological department of the Clinic of the YSC KMP and to identify their spectrum by nosology.

Materials and methods. The materials for the study were:

1. Databases of patients with SCA 1 type, Parkinson's disease, dementia and muscular dystonias * of the neurological department of the YSC KMP Clinic for 2019 **;

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2. Reporting data of district neurologists for 2019;

3. Regulatory documents of the Ministry of Health of the Russian Federation, the Ministry of Health of the Chelyabinsk and Sverdlovsk regions and the Republic of Sakha (Yakutia) on the procedures for routing patients;

4. A comprehensive program for the opening of the Center for Neurodegenerative Diseases on the basis of the Clinic of the YSC KMP;

* The largest number of patients in the neurological department were patients with SCA1 type, primary dementia, Parkinson's disease and muscular dystonias. The rest of the diseases from the NDD group were diagnosed in isolated cases.

** Taking into account the epidemiological situation in the Russian Federation in connection with the new coronavirus infection in 2020-2021, and the limitation of the volume of specialized care during this period, the routing period for 2019 was studied.

Research methods:

1. Retrospective analysis of databases of patients with SCA type 1, Parkinson's disease, dementia and muscular dystonia;

2. Analysis of the reported data of district neurologists;

3. Statistical method.

The retrospective analysis included a database study (DB) of patients with type 1 SCA, Parkinson's disease, primary dementia, and muscular dystonia. The database contains personal and demographic data of patients, the results of neuroimaging: Magnetic resonance imaging (MRI) of the brain and spinal cord, electroencephalography (EEG), in patients with SCA type 1 - electroneuromyography (ENMG) and spirography (SPG) to confirm the developing anterior limb lesion in advanced and terminal stage of the disease in patients with respiratory failure. When studying the database of primary dementia, in addition, neuropsychological scales were studied: the Montreal Cognitive Scale (MoCA) and the Brief Mental Status Assessment Scale (MMSE). Annual reports of regional neurologists of the republic for 2019, for information on hospitalization in the neurological department of the Clinic of the YSC KMP, referral and clarified diagnoses as a result of hospitalization. Statistical processing of the material was carried out on a personal computer using the Statistica version 12 software package. During the statistical processing of the research results, the proportions were calculated according to the frequency table. The proportions were compared using a contingency table with the calculation of Pearson's $\chi 2$ criterion.

The critical value of the significance level (p) was taken equal to 5%.

Study inclusion criteria:

1. Patients who underwent examination and rehabilitation treatment in the round-the-clock hospital of the neurological department of the Clinic of the YSC KMP for the period from 01.04.2019 - 01.01.2020;

2. Patients with established diagnoses of SCA type 1, Parkinson's disease, primary dementia (BA, LVD, DTL, PNP) and muscular dystonias.

Exclusion criteria:

1. Patients who underwent examination and rehabilitation treatment in the round-the-clock hospital of the neurological department of the Clinic of the YSC KMP for 2020-2021;

2. The presence of a causal relationship between the development of cognitive disorders and cerebrovascular disease;

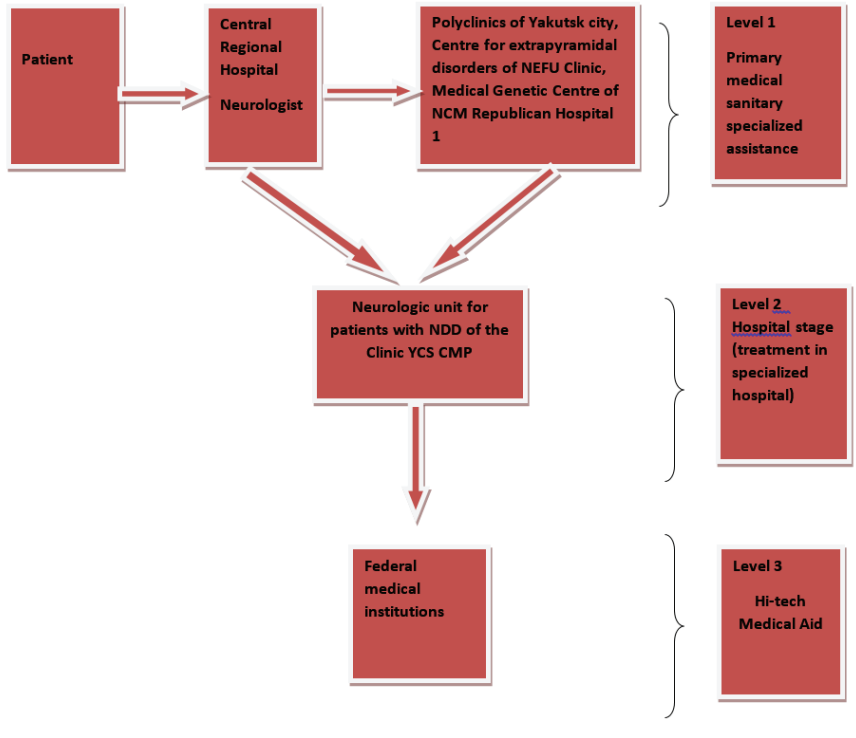
3. Patients with other neurodegenerative diseases.

In total, in 2019, 444 patients were hospitalized in the neurological department, 281 patients with diseases of the nervous system, including 210 patients with SCA type 1, PD, MD and PD.

Results and discussion. Hospitalization in the neurological department for patients with neurodegenerative pathology is one of the stages in the provision of specialized medical care and refers to the 2nd level of medical care [10]. The opening of this department took place on 01.04.2019. on the basis of the Clinic of the Yakutsk Scientific Center for Complex Medical Problems by re-profiling unprofitable beds of the cardiological and gynecological departments. The severe course of diseases of patients with NDD is an undoubted obstacle to visiting polyclinics, and the lack of round-the-clock hospitals for rehabilitation or rehabilitation treatment and examination deprives them of receiving these types of MP. Therefore, the predominant and comfortable type of MP for such patients is treatment in a round-the-clock hospital. Timely verification of the diagnosis and the appointment of adequate restorative treatment at the same time determines the prognosis of the disease and improves the quality of life of patients.

From Figure it follows that the patient can be admitted to the neurological department for patients with neurodegenerative diseases both from the central regional hospital and from the polyclinics of the city of Yakutsk. Admission to the department is carried out in a planned manner after a preliminary consultation of a neurologist with the head of the neurological department, which makes it possible to cover patients with specialized care from all uluses of the republic.

Specialized medical care was



Routing scheme for patients with neurodegenerative diseases at the hospital stage

Discrepancies between directional and specified clinical diagnoses (%)

The main diagnosis (n=210)	Divergence of directional and refined diagnoses. %
Spinocerebellar ataxia of type I (n=68)	0
Parkinson's disease (n=55)	1.8
Muscle dystonia (n=51)	5.88
Neurodegenerative disease plus (n=3)	100
Alzheimer's disease (n=21)	90.48
Dementia with Levi's corpuscles (n=3)	100
Frontotemporal degeneration (n=6)	-«-
Progressive supranuclear paralysis (n=3)	-«-

provided to patients who were sent from all the uluses of the republic, but were significantly more often sent from the Arctic (39%) and central (33%) zones, compared with the southern (16%) and Vilyuisk (12%) ($\chi2=36.95$; $p<0.0001$) regions.

The total number of hospitalized patients in the neurological department for 2019 made up 444 cases, of which 281 cases were associated with diseases of the nervous system (BNS)(63,3%), 40 (9%) patients with dorsopathies and 123 (27.7%) with cerebrovascular diseases.

Of all the diseases of the nervous system, neurodegenerative diseases prevailed, which accounted for 75%, and other diseases of the nervous system accounted for only 25%.

The main share of VAT falls on type 1 SCA (n=68) - 24%, Parkinson's disease (n= 55) - 20%, muscular dystonia (n=51) - 51% and primary dementia (n=32) - 11%. The share of other NDZ (n =75) is 27%.

The table shows that there were no discrepancies in the directional and refined diagnoses in patients with SCA1, which can be explained by the fact that the diagnosis was previously confirmed by molecular genetic diagnostics in all patients. A low percentage of the discrepancy in the diagnosis was also found in Parkinson's disease (1.8%) and muscle dystonia (94.1%), which can be explained by the fact that these patients were examined on an outpatient basis earlier at the Center for Extrapyramidal Disorders and Botulinum Therapy at the Clinic of the North-Eastern Federal University. At the same time, doctors have great difficulties in diagnosing primary dementias (Alzheimer's disease, dementia with Levi's corpuscles, frontotemporal degeneration) and neurodegenerative disease accompanied by dementia-progressive supranuclear paralysis. In addition, NDZ with atypical additional neurological symptoms also cause difficulties, which can be explained by the rarity of these clinical cases and uncharacteristic symptoms.

Most often, patients were referred with a diagnosis of dyscirculatory encephalopathy, which was not confirmed in 55% of cases, with a diagnosis of unspecified NDZ - in 21% of cases, Parkinson's disease in 16% of cases, essential tremor in 5% of cases and cervical osteochondrosis-in 3% of cases.

The list of established clinical diagnoses has expanded; in comparison with the directional diagnoses, it includes BA (50%), DTL (8%), LVD (13%) and PNP (8%).

With a directional diagnosis of dyscirculatory encephalopathy, Alzheimer's disease, dementia with Levi's corpuscles and muscle dystonia were established in 61% (n=14) of cases, and frontotemporal degeneration in 17% (n=4) of cases.

In the vast majority of cases (82%), the directional diagnoses coincided significantly more with the final ones ($\chi2=171.01$; $p<0.0001$), and the differences in diagnoses were 18%.

Thus, despite the large number of coincidences of directional and refined diagnoses, the greatest difficulties in conducting a differential diagnosis in neurologists are caused by primary dementia, and patients are more often diagnosed with dyscirculatory encephalopathy.

Conclusion. Analysis of the hospital stage 2 of the routing level of patients with neurodegenerative pathology confirmed that:

1. The provision of specialized care is an urgent problem in the Republic of Sakha (Yakutia) and the creation of a regional routing procedure for this category of patients to provide them with specialized care in the republic has become a timely step on the part of the FGBNU YSC KMP and the Ministry of Health of the Republic of Sakha (Yakutia);

2. An analysis of the routing of the hospital stage of patients with neurodegenerative pathology showed that it is necessary to train neurologists during the improvement cycles in neurodegenerative pathology;

3. The opening of a neurological department for patients of the republic on the basis of the Clinic of a federal scientific institution is an example of the consolidation of a scientific medical institution and regional health care.

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HEALTHY LIFESTYLE. PREVENTION

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PREDICTION OF NEGATIVE CLINICAL OUTCOME OF CRITICAL CONDITION USING THE APACHE-II, SOFA, NRS-2002 SCALES

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During the first 24 hrs of patient's admission to ICU, it is essential to perform a negative outcome screening, which can be done using acute physiology severity scales - APACHE-II and SOFA. It is known that these scales do not include nutritional insufficiency assessment, which itself affects survivability of critically ill patients. The model that uses three scales: assessment of pathophysiological changes - APACHE-II, intensity of multiple organ failure – SOFA, and assessment of nutritional insufficiency risk - NRS-2002, reliably improves the accuracy of the negative outcome prognosis in an ICU patient compared to their individual application.

Keywords: APACHE II, SOFA, NRS-2002, critically ill patients, predictors, mortality.

Introduction. Evaluation of patient's condition severity in an intensive care unit (ICU) is an essential task in the work of a resuscitator. Identification of patients with a high risk of developing a negative outcome at early stages of intensive care provides an opportunity for timely adjustment of the diagnostic and treatment process. Different comprehensive prognostic scales are used to address this issue. The most popular scales are APACHE-II that reflects pathophysiological changes in the patient's organism at admission and serves to predict a disease outcome, and SOFA that allows tracing the dynamics of the multiple organ dysfunction syndrome [1,2]. Numerous studies have proven that critically ill patients with nutritional insufficiency (NI) stay longer in ICU and hospital, and demonstrate a higher mortality rate [3]. The drawbacks of these scales include the fact that they do not consider patient's protein-energy metabolism and nutritional status – the risk of NI. One of the most convenient and frequently used, in the world practice, scale assessing the risk of NI is Nutritional Risk Screening 2002 (NRS-2002), which is recommended by the European Society for Clinical Nutrition and Metabolism (ESPEN) [4]. It can be applied to all inpatients including those treated in ICU [4]. The NRS-2002 score helps identifying NI patients and serves a lethal outcome predictor in critically ill patients [5]. The meta-analysis assessing usefulness of NRS 2002 as a predictor of postoperative outcomes in the abdominal surgery included 11 studies in total. Postoperative complications developed much more frequently in the risk-group patients (the odds ratio (OR) - 3.13, p <0.00001). Mortality was also significantly higher in patients having a higher risk score according to NRS 2002 (OR – 3.61, p <0.009) [6].

Absence, in the available literature, of information on how the prognostic value of APACHE-II and SOFA scores will change if the risk of developing NI assessed by the NRS-2002 score will be taken into account, makes our study relevant.

Purpose of the study: to assess the informative value of the model predicting a negative outcome in ICU patients through combined application of APACHE-II, SOFA, and NRS-2002 scores.

Materials and methods. A prospective single-center study was carried out in ICU of JSC Neftyanik Hospital, Tyumen, in 2012-2017. The inclusion criteria were over 24hrs. in ICU and age between 18 and 80 years. The exclusion criteria were coma and/or impossibility to get answers to questions, shock, moribund state, age older than 80 years of age, pregnancy; moderately ill patients staying in ICU for less than 24 hrs. During the first 24 hours

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in ICU, patients were assessed according to APACHE-II, SOFA, and NRS-2002. Questioning was carried out by two resuscitators. The study included 176 patients (89 women and 87 men); of them, 110 subjects (62.5%) were surgical patients and 66 (37.5 %) - therapeutic. The causes for admission to ICU were as follows: an acute surgical pathology - peritonitis (n=34), acute pancreatitis (n=24), intestinal obstruction (n=16), urosepsis (n=9), suppurative-septic diseases of different locations (n=6), gastrointestinal hemorrhage (n=15), thrombosis of major vessels (n=3), pneumothorax (n=1), mediastinitis (n=1); and therapeutic diseases – chronic cardiac insufficiency (n=19), pneumonia (n=17), liver cirrhosis (n=10), delirium (n=4), chronic obstructive lung disease (n=2), anaphylactic shock (n=1), dyscirculatory encephalopathy (n=5), leucosis (n=4), epilepsy (n=1), acute renal failure (n=2), acute exposure to alcohol surrogates (n=2). The patients were split into two groups: the first group included the deceased patients (n=60), the second – the survived patients (n=116). Blood serum C-reactive protein (CRP) was used as a criterion of systemic inflammatory response intensity. The recorded demographic information included gender and age; body mass index (BMI) was calculated, too. Patients' characterization is shown in table 1.

The raw data obtained were processed using the SPSS software package. Normality of distribution was checked by the Shapiro-Wilk test. The findings are given as the mean and mean square deviation M±σ or as the median and quartiles Me, [Q25; Q75]. The prognostic value of scores was assessed using the logit regression technique. To establish the separation power, ROC analysis was undertaken. Nigel Kirk's determination coefficient was calculated. The null hypothesis was discarded at p<.05.

Results and discussion. In our study we observed statistically significant inter-group differences (table 1) for APACHE-II, SOFA, and NRS-2002 scales. The score according to all three scales was higher in patients of the first group, in which BMI and blood plasma CRP were higher, too. No statistically significant differences were noted in the therapeutic group while among the surgical patients the number of survived patients was statistically significantly greater. One can see from table 2 that the patients of the first group stayed in hospital statistically significantly longer and stayed on mechanical lung ventilation (MLV) for a longer period of time.

Logit regression identified indices in-

Table 1

Clinical and Laboratory Characteristics of Patients in the Compared Groups

Index	Group I (n=60)	Group II (n=116)	p
Age, (years)	62.7±18.8	59±16.4	0.16[d]
Gender, (male, %)	64.4	35.6	0.05[f]
Body Mass Index, (kg/m$^{2)}$	24 [21.4;27.7]	26 [23.3;30.2]	0.009[e]
APACHE-II[a], (score)	15 [12;20]	9 [5;13]	<0.001[e]
SOFA[b], (score)	4 [3;7]	2 [1;3.75]	<0.001[e]
NRS-2002[c], (score)	5 [4;6]	3 [2;5]	<0.001[e]
C-reactive protein, (mg/L)	94.1 [44.2;181.5]	60.4 [11;166]	0.045[e]
Therapeutic patients, %	51.5	48.5	0.35[f]
Surgical patients, %	23.6	78.4	<0.001[f]

Note: a – Acute Physiology and Chronic Health Evaluation; b – Sequential Organ Failure Assessment; c – Nutritional Risk Screening 2002, d – Student's t-test, e – Kruskall-Wallis h-test, f – Pearson's chi-squared test.

Table 2

Duration of Treatment in ICU and Duration of Mechanical Lung Ventilation in the Compared Groups

Parameter	Group I (n=56)	Group II (n=120)	p
Bed-days in ICU, days	5.6±4.7	4.6±4.2	0.3
Bed-days in hospital, days	5 [3;9]	10 [7;15.75]	<0.001
Bed-days on MLV, days	3 [1;4]	1 [0;1]	<0.001

Table 3

Predictive Value of Scales and Some Indices in respect of the Risk of Lethal Outcome (Logit Regression)

Index	Odds Ratio (OR)	95% CI	p=
Age, (years)	1.01	0.99-1.03	0.16
Body Mass Index, (kg/m$^{2)}$	0.92	0.87-0.98	0.055
APACHE II, (score)	1.2	1.12-1.27	<0.001
SOFA, (score)	1.4	1.2-1.6	<0.001
NRS-2002, (score)	1.7	1.38-2.21	<0.001
C-reactive protein, (mg/L)	1	0.99-1	0.18

Note. Table 3, 5 CI - confidence interval.

Table 4

ROC Analysis of the Predictive Value of APACHE-II, SOFA, and NRS-2002

Index	AUC	p	COV	Sensitivity, %	Specificity, %
APACHE-II, score	81.5	<0.001	>13.5	74	74.5
SOFA, score	79.7	<0.001	>2.5	71.7	72.6
NRS-2002, score	73.7	<0.001	>3.5	89.1	50.9

Note. AUC-area under curve, COV-cut-off value.

dependently influencing the risk of lethal outcome – APACHE-II, SOFA, and NRS-2002 scores (table 3). Age, CRP and BMI did not display a separation power in respect of the risk of lethal outcome in ICU patients (table 3)

To analyze the quality of models, ROC analysis was carried out; its results are given in table 4.

The largest area under the ROC curve was found in APACHE-II scale (table 4). All models were statistically significant (p<0.001). APACHE-II demonstrates a very good quality of the model while SOFA and NRS-2002 – just good. The best correlation of sensitivity and specificity was found in APACHE-II. When the score is APACHE-II>13.5 or SOFA>2.5, or NRS-2002>3.5, a high risk of lethal outcome development is surmised (table 4). To establish the model featuring the best predictive value, combinations of scores according to all three scales were made (table 5).

The resultant models were statistically significant (p<0.001). Based on regression coefficients' values, APACHE-II, SOFA, and NRS-2002 are directly related to the lethal outcome probability. The best area under the ROC curve was observed in the model that accounted scores of all three scales together – APACHE-II, SOFA, and NRS-2002 (p<0.001). According to Nigel Kirk's determination coefficient, the model takes into consideration 40% of factors determining the lethal outcome probability. Its diagnostic efficacy amounted to 74%, its sensitivity being 83.6% and specificity - 55%. The advantage of this study is that when the scores of all three scales are used, it improves the accuracy of prognosis that can be calculated by a binary logit regression formula.

Table 5

ROC Analysis of the Predictive Value of Models of Combined Application of APACHE-II, SOFA, and NRS-2002 Scales

Index	AUCd	Sensitivity, %	Specificity, %	95% CI	p
APACHE II+SOFA	84.6	82.6	74.5	0.78-0.91	<0.001
APACHE II+ NRS-2002	83.3	78.3	74.5	0.77-0.89	<0.001
SOFA+NRS-2002	84.6	78.3	71.3	0.78-0.9	<0.001
APACHE II+ SOFA+ NRS-2002	86.4	84.8	70.8	0.80-0.92	<0.001

Note. AUC – area under curve, CI – confidence interval.

$$p = \frac{1}{(1+e^{-z})} \times 100,$$

where p is the probability of occurrence of an outcome, units; z is the logit function power exponent, e – is the Euler's number (≈2.718).

$$z = -4{,}3 + 0{,}97 \times X_{Apache\ II} + \\ + 0{,}2 \times X_{Sofa} + 0{,}41 \times X_{NRS-2002},$$

where X is independent indices (scores).

Conclusion. Each of the APACHE-II, SOFA, and NRS-2002 scales separately is an independent predictor of a negative outcome in critically ill patients, and their scores can be used as a prognostic criterion. Their combined use improves the accuracy of the negative outcome prognosis in ICU patients.

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PREDICTON OF REPRODUCTIVE LOSSES IN THE FIRST TRIMESTER IN WOMEN WITH CHRONIC PYELONEPHRITIS AND ANEMIA

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After a comprehensive examination of a sample of pregnant women with anemia and chronic pyelonephritis, the risk factors for belonging to groups with a high infectious risk in the first trimester were identified in this sample. The risk of reproductive losses is determined by angiopathy of uterine vessels, supplemented by a disruption of homeostasis and violation of the microbiome of the urogenital tract in the absence of pregravid recovery and prevention of placental insufficiency.

Keywords: iron deficiency anemia, anemia of chronic diseases, chronic pyelonephritis, reproductive losses, risk factors.

The period of embryogenesis, early fetogenesis and placenta formation is actively studied as important for pregnancy outcomes and the most vulnerable to various factors.

Predicting gestational complications in women with anemia and chronic pyelonephritis (CP) is impossible in the absence of clear ideas about the features of the uterine-fetal interaction at the time of cytotrophoblast invasion into the walls of the spiral arteries. The inability to fully remodel the spiral arteries of the uterus into vessels with low vascular resistance leads to abnormal initiation of the villous trophoblast and impaired placental perfusion [8].

The issues of pathophysiological adaptation of the embryo / fetus in women with anemia and CP have been poorly studied from the standpoint of identifying groups with a high infectious risk and predicting adverse pregnancy outcomes from miscarriage to the birth of an intrauterine infected child [4,13,16,20].

An increase in obstetric and perinatal complications is associated with iron deficiency (ID) on the background of infectious and inflammatory diseases [1,9].

Understanding the mechanisms of chronic placental ischemia and its consequences in pregnant women with extragenital diseases (EGD) is connected to the possibility of early diagnosis of potential fetal problems [6,15].

The issues of the course of pregnancy in women with EGD are inherently related to the clarification of the characteristics of the urogenital microbiome, immune and metabolic reserves of the body, the presence / activity of infectious and inflammatory processes [7,27].

The influence of the timing of ID manifestation and the severity of anemia on the outcomes of pregnancy is debated [11]. The development of anemia in the third trimester is associated with a lower frequency of prematurity and low weight [22]. There is practically no data in the literature on the effect of anemia on the probability of reproductive losses (RL) in the first trimester. The expansion of ideas about the possibilities of a real influence on the formation of an early placenta and the preservation of the physiological foundations of the embryo-placental interaction is achievable by identifying predictors of critical trophic and metabolic disorders and the possibility of real overcoming the termination of pregnancy in the early stages. The effect of the timing of the start of therapeutic and preventive measures on the course of pregnancy in women with CP and anemia is also not specified.

The aim of the study was to determine risk factors and prognostic criteria for termination of pregnancy in women with a high infectious risk caused by extragenital diseases (anemia, chronic pyelonephritis).

Material and methods. Women with CP (n = 320) and anemia (n = 308) were retrospectively divided into groups depending on pregnancy outcomes: with progression and termination in the first trimester (by the type of undeveloped pregnancy or miscarriage) (with CP n = 135 and n = 185 respectively, and anemia n = 62 and n = 246, respectively).

We identified pregnant women with iron deficiency anemia (IDA) (n = 108) and anemia chronic diseases (n = 200).

Inclusion criteria: singleton progressive pregnancy; the woman's informed consent for the use of biological material for scientific purposes, extragenital diseases found before pregnancy and confirmed by specialists (CP, anemia).

Exclusion criteria: multiple pregnancies and those resulting from assisted reproductive technologies; severe somatic diseases in the decompensation stage, precancerous and oncological diseases; stillbirths; chromosomal abnormalities and congenital malformations of the fetus.

Methods of research: laboratory (general analysis of blood, urine, assessment of iron metabolism (serum, ferritin, transferrin, total iron-binding capacity (TIBC) of serum), microbiological (smear microscopy for the degree of purity (Gram stain), bacteriological inoculation of cervical discharge for flora and sensitivity to antibiotics, polymerase chain reaction (PCR study), molecular genetic research (Femoflor). Microscopy and culture examination of urine were performed as part of screening, in the presence of bacteria or dysuria in the urinary sediment, pain in the lumbar area on a set of standard media (5% blood agar, Endo medium, Sabouraud, MRS-arap), with incubation in aerobic conditions at 37°C for 18 hours.

Sonography of the fetus and placenta with dopplerometry was performed on expert class devices Voluson E8, Tochiba Aplio XG.

The obtained endometrial tissue samples were fixed with 10% formalin solution for 24 h, embedded in paraffin, sections were prepared with a thickness of 6 microns, and stained with hematoxylin and eosin.

The level of production of placental proteins (pregnancy-associated protein (PAPP-A), placental lactogen) was assessed.

Immunoreactivity was studied based

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on the results of ELISA-detected Probably of pathology, an enzyme-linked immunosorbent assay of the number and affinity of individual embryotropic autoantibodies interacting with embryogenesis regulatory proteins (Biopharm – test LLC).

The degree of reliability and approbation of the results of the work. The statistical analysis was carried out using the IBM SPSS Statistics 23 program, parametric analysis methods in accordance with the results of checking the compared masses for the normality of distribution, descriptive statistics (arithmetic mean (M), mean error of the mean (m), Student's t-test, odds ratio (OR), confidence interval (CI, 95%).

The analysis of intergroup differences in qualitative characteristics was carried out using the criterion χ2, less than five – the exact two-sided Fischer test. The significance level (p) when testing statistical hypotheses was taken to be p≤0.05.

The construction of a predictive model for calculating the risk was performed using the binary logistic regression method according to the formula:

$$P = \frac{1}{1+e^{-z}}, z = a0 + a1x1 + \\ + a2x2 + a3x3 + \ldots + anxn,$$

where p is the probability of the outcome, x1… xn are the values of the predictors in a nominal, ordinal or quantitative scale, a1… an are the regression coefficients, using Wald statistics. The effectiveness (the proportion of correctly predicted cases of the presence and absence of the studied pathology), sensitivity (the presence of pathology), specificity (the absence of pathology), the prognostic value of a negative result (PVNR) and a positive one (PVPR) were determined, ROC analysis (receiver operating characteristic) of the error curve was done. The Area Under Curve (AUC) under the ROC curve was calculated.

Results and discussion. The effectiveness of pregravid rehabilitation of women with EGD is proved by a lower incidence of infectious and inflammatory diseases during pregnancy: anemia chronic diseases in comparison with IDA (28,6% vs 59.0%, p=0.03), exacerbation of CP one and a half times more often than cases of remission of the disease (39,4% vs 60.7%, p=0.02).

Treatment and preventive measures during the first wave of placentation were taken in every fifth pregnant woman with CP (21,9%), IDA – 4.5 times more often than with anemia chronic diseases (p=0.005).

According to the calculations of logistic regression, markers that determine the belonging of pregnant women with anemia and CP to the group with a high infectious risk were: the absence of pregravid preparation (χ2 = 8.6; p = 0.003), the total absence of the pregravid stage and the early prevention of placental insufficiency (χ2 = 12 , 3; p = 0.000) (Table 1).

The effectiveness of the predictive model for identifying patients with a high infectious risk in the sample with EGD (CP and anemia) is reflected by the data: regression coefficient B – 1.22; Wald statistic χ2 – 57.9; p – 0.00; Exp B – 3.4, Nagelkirk exponent – 0.39.

The area under the curve AUC-0.82 allows us to regard it as reliable. The value at the "cut-off" point – 0.5 – shows the positive predictive significance of the model when the value is exceeded. Model accuracy assessment is presented as the following: sensitivity – 95.9%; specificity – accurately predicted – 40.0%. The diagnostic efficiency of the logistic model, determined by the proportion of all correct predictions, is 83.2%. PVRP (the prognostic value of a positive result – 95.9%, PVNR (the prognostic value of a negative result) – 40.0%.

Such conclusions allow us to assert that proper pregnancy management in women with EGD (CP, anemia) begins not from early stages, but from pregravid recovery.

According to the logistic regression data, belonging to the group with a high infectious risk was determined by the fact of CP exacerbation, with an increase in temperature on the background of inflammatory criteria (leukocyturia, proteinuria, hematuria), mainly in the absence of timely antibacterial therapy for the detection of asymptomatic bacteriuria (AB).

CP exacerbation occurred in 7.5% of women in the first trimester (χ2=8,759; p=0.003), with bacteriuria – in all pregnant women.

The probability of developing unfavorable obstetric outcomes with the lack of rational management tactics for pregnant women with CP was confirmed by other authors. The expediency of treating urinary tract infections [26], the prevalence of which in pregnant women reaches 10%, is explained by the risk of CP exacerbation [17]. Asymptomatic bacteriuria (AB) requires treatment to avoid the risk of developing acute pyelonephritis in 25-30% [14]. The formation of the focus of inflammation in persistent infection is determined by the ability of uropathogenic strains to synthesize virulence factors and damage of kidney tissues [24].

According to the logistic regression data, moderate anemia of pregnant women is among the features that presuppose belonging to a high infectious risk group.

Moderate anemia was detected in 12 women (37.5%) from the group with a high infectious risk (n = 32), with reproductive losses in the first trimester. In the sample with prolonged pregnancy, the disease was noted in 14.5% of women without a high infectious potential.

In total, moderate anemia in the first trimester of pregnancy was identified in 39.8% of women.

The effect of moderate anemia on pregnancy outcomes is explained by a number of factors: decreased bioavailability of iron and a violation of its transfer through the placenta to the fetus [25]. Programming the rate of fetal development in ID is explained by the deterioration of vascularization on the background of a violation of the primordial trophoblast initiation and the formation of primary placental insufficiency [21]. Timely and adequate therapy of anemia, taking into account its genesis, is most significant due to the correlation of ID with chronic infectious and inflammatory diseases of the genitourinary system.

According to the calculations of the logistic model, the cumulation of miscarriage risks with EGD is determined in the presence of the features presented in Table 2.

The high prognostic significance of the model is proved by the following criteria: sensitivity – 91.8%, specificity – 46.0%, diagnostic efficiency – 81.4%, PVPR – 91.8%, PVNR – 46.0%. The area under the AUC curve – 0.94, "cut-off" – 0.5 – shows a high predictive value of the model when the indicator is exceeded.

Pregnant women with CP and anemia in the group with RL were distinguished by increased microbial contamination of the genital tract in comparison with a favorable pregnancy result: bacterial vaginosis (38.5% versus 25.9%, p = 0.002) and infection of the cervical canal with E. coli (24.4% against 13.2%, p = 0.001), Str. haemolyticus (14.2% versus 5.1%, p = 0.0006), Str. epidermalis (11.4% versus 5.6%, p = 0.03).

"Disruptions" of local infectious protection in women with abortion due to CP and anemia lead to a high level of bacterial contamination of the cervical canal: in CP – Mycoplasma genitalium (23.2% versus 12.2%, p = 0.005) and Ureaplasma urealyticum (32, 4% versus 14.2%, p = 0.005) – 2 and 2.3 times more often than in anemia. Women with reproductive losses (RL) on the anemia background were distinguished by a high frequency of Gardnerella vaginalis (27.2% versus

Table 1

Factors determining the belonging of pregnant women with CP and anemia to the group of a high infectious risk

Factors	Regression coefficient B	Wald statistic, χ^2	Value, p	Exp B
Multiple intrauterine surgeries	1.126	7.024	0.008	3.085
Absence of pregravid preparation	1.596	8.604	0.003	4.936
Absence of pregravid preparation / early prevention of placental insufficiency	1.517	12.307	0.000	4.558
Exacerbation of chronic pyelonephritis	1.496	8.759	0.003	4.465
Moderate pregnancy anemia	1.788	18.080	0.000	5.976
Constant	-1.231	10.070	0.002	0.292

Table 2

Risk factors for miscarriage in the first trimester of pregnancy on the background of a high infectious risk in women with CP and anemia

Factors	Regression coefficient B	Wald statistic, χ^2	Value, p	Exp B
Relapses of bacterial vaginosis, contamination of the cervical canal	1.5	8.4	0.004	4.7
Contamination of the cervical canal, urine	0.9	3.9	0.049	2.5
Hyporeactivity	1.4	4.9	0.03	3.9
pulsation index in the uterine arteries >1,5 at 6-8 weeks	1.7	9.1	0.003	5.3
Placental lactogen, mg / l <1.4 (min 1.5 times less than the initial)	1.1	4.3	0.04	2.9
PAPP-A>620, mU / l (min 1.3 times more than the initial)	1.9	8.4	0.004	6.8
Constant	-0.7	5.2	0.02	0.5

16.8%, p = 0.01) – almost 1.5 times more often than with CP.

Asymptomatic bacteriuria (AB) in women with abortion was detected 2.6 times more often than with progression (19.4% versus 7.4%) due to Escherichia coli (45.8%), Klebsiella pneumonia (15.3%), Proteus mirabilis and Staphylococcus spp. (9.7% each), which confirmed the need for repeated bacteriological examination of urine during pregnancy (Mancia G. et al., 2013; Tan M.Y. et al., 2018).

Thus, the probability of pregnancy termination in the first trimester increased in the presence of recurrent bacterial vaginosis (BV) and contamination of the cervical canal (p = 0.004, χ2 = 8.4), simultaneous persistence of infections in the cervical canal and urine (p = 0.0049, χ2 = 3.9).

The frequency of reduced production of embryotropic autoantibodies in groups with reproductive losses (RL) on the EGD background was detected twice as often as during pregnancy prolongation (62.9% versus 30.4%, p = 0.03, χ2 = 4.9).

Recurrences of BV and microbial contamination of the cervical canal were indicators of an immunodeficiency state associated with the risk of RL in a sample with CP and anemia.

A change in the microbial community with the replacement of lactobacilli with Gardnerella vaginalis and anaerobic representatives acts as a trigger for an inflammatory reaction and an innate immune response of the vaginal epithelium.

In vitro studies have shown that G. vaginalis can weaken the barrier function of the epithelium through direct tissue damage and inflammation [29].

The high frequency of BV relapses is connected to the inability of antimicrobial drugs to eliminate vaginosis-associated infections [32]. BV can result in a 1.5-2 times increased risk of infection with C. trachomatis, N. gonorrhoeae, T. vaginalis [12]. The ascent of vaginosis-associated infections into the upper genital tract is accompanied by the probability of colonization of the placenta and the development of inflammatory diseases of the pelvic organs, infection of the fetus and placenta, and premature birth [28].

Simultaneous infection of the cervical canal and urine in pregnant women with anemia and CP indicates an initial decrease in nonspecific resistance, long-term persistence of pathogenic pathogens in the body of pregnant women and an increase in the frequency of intrauterine infection, which, in turn, causes adaptation breakdowns [2].

Interestingly, viable G. vaginalis bacteria can be absorbed by vaginal epithelial cells with the participation of active reorganization of the epithelial cytoskeleton, and that activates factors that promote the attachment of other pathogenic bacteria, for example, E. coli [23].

Microbial balance violations in the loci of the urogenital sphere are accompanied by changes in the immune system and homeostasis mechanisms, the deepening of which with the onset of pregnancy poses a threat of its termination.

The fact of a significant combination of infectious and inflammatory processes in the kidneys and vaginal dysbiosis in pregnant women with CP and anemia is consistent with the data on the violation of the vaginal biotope in 70-80% of pregnant women with CP [10,31].

The role of BV and persistent infectious and inflammatory process in the urinary tract as triggers of ovum infection is confirmed in the literature, along with the conclusion about the need for proper recovery of women before conceiving a child [3,5].

The analysis of the causes of suboptimal trophoblast invasion with a change in the immunomodulatory function of the placenta from the early stages of pregnancy made it possible to establish a number of regularities.

Abnormal vascular indices (pulsation index (PI) in the uterine arteries (> 1.5 at 6-8 weeks) ($\chi 2 = 9.1$, $p = 0.003$)) acted as markers of placental ischemia and were more informative in predicting miscarriage in combination with biochemical tests for evaluating placental function [18,19,30].

The co-factors of an early placental dysfunction associated with the probability of pregnancy termination were: low production of placental lactogen (<1.4 mg / ml) (min 1.5 times less than the initial) ($\chi 2 = 4.3$, $p = 0.04$), excessive PAPP-A (> 620 mU / l) (min 1.3 times more than the initial) ($\chi 2 = 8.4$, $p = 0.004$).

Obviously, chronic infectious and inflammatory processes in pregnant women with anemia and CP, especially on the background of changes in the microbiome of the urogenital tract, contribute to the abnormal development of the placenta with a violation in uterine-fetal interaction at the molecular-cellular level. The analysis of risk factors in women with EGD (CP and anemia) indicates a significant connection of the peculiarities of the course of pregnancy with the adaptive resources of the female body.

Pathomorphological examination of the abortion material (miscarriages and non-developing pregnancy) evacuated during uterine emptying of women with CP and anemia showed abnormal development of syncytiotrophoblast with tissue dystrophy, especially with leukocyte infiltration: samples with immaturity of villous trophoblast and endometritis / deciduitis (30.1%); incomplete gravidar transformation of the endometrium and inflammatory changes (22.4%); signs of endometritis in general (65.8%).

The persistence of the inflammatory process in the endometrium, which subsequently determines the violation of the uteroplacental-fetal hemodynamics during the waves of cytotrophoblast invasion, proves to be the basis for a comprehensive examination of women with pregnancy termination on the CP and anemia background in the first trimester.

Thus, pregnant women with anemia and CP are distinguished by dysregulation of the activity of the "placenta-fetus-kidney" complex associated with angiopathy of the uterine vessels [7,8], with aggravated disruption of homeostasis and the microbiome of the urogenital tract in the absence of pregravid recovery and / or prevention of early placental insufficiency due to the "crisis" of angiogenesis of the early placenta.

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ACTUAL TOPIC

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INCIDENCE OF MALIGNANT NEOPLASMS IN THE POPULATION OF THE TERRITORIES OF THE REPUBLIC OF SAKHA (YAKUTIA) FOR THE PERIOD 2010-2019

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The article presents data on the study of the current situation of the incidence of malignant neoplasms in the population of the territories of the Republic of Sakha (Yakutia), assessments of its relationship with the amount of pollutant emissions and ethnicity. It has been revealed that the age-standardized incidence rate of cancer of all localizations (C00-96) for the period 2010-2019 in the Republic of Sakha (Yakutia) is lower than in the whole of the Russian Federation, both in men and women, and has a similar growth trend. Higher average long-term incidence rates of cancer of all localizations (C00-96) have been observed in men and women living in the city of the republican significance Yakutsk and in industrial zones, and the lowest rates have been revealed for agricultural zones. No statistically significant correlations have been found between atmospheric pollution from stationary sources and the incidence of cancer, while a negative association with the number of indigenous people living in the municipality has been revealed. It is assumed that the increase in the incidence of cancer is associated with improvements in early detection of cancer that is common for both the Republic of Sakha (Yakutia), and the Russian Federation, and with the variety of physical and chemical factors harmful to humans in municipalities.

Keywords: malignant neoplasms, cancer incidence, standardized indicators, zoning of territories, environmental factors.

Introduction. Despite advances in technological development of early diagnosis and treatment methods, oncological diseases continue to grow steadily. According to the World Health Organization (WHO), the global cancer burden and cancer deaths are expected to increase to 54 million and to 16 million per year, correspondingly by 2050 [5]. As reported by the Global Burden of Disease Cancer Collaboration, in 2007-2017, the global cancer burden increased by 33%, and the cancer DALYs, being in the 6th place earlier, reached the 2nd place in the ranking, following cardiovascular diseases [4]. However, an introduction of updated and more effective methods of early cancer diagnosis, treatment and prevention contribute to a decrease in mortality rates and an increase in the life expectancy in high-income countries. Thus, in the United States from 1991 to 2017, a continuous decrease of the cancer death rates led to an overall decrease in mortality by 29% [3].

The Republic of Sakha (Yakutia) is the largest subject of the Russian Federation (3 million sq. km) with the population of 972 000 people, as of 2020, (the population density is 0.31 people per sq. km). According to the 2010 All-Russian Population Census, representatives of 126 peoples live in the Sakha Republic (Yakutia), with the largest groups represented by the Yakuts (45.5%), Russians (41.2%), and Ukrainians (3.6%). In accordance with the administrative-territorial division, 34 municipal districts and 2 urban districts make the Sakha Republic (Yakutia), significantly differing in their population density, climatic and socio-economic living conditions, ethnic and age composition. These differences significantly affect the rates of population morbidity and mortality. Although Yakutia is not among Russian regions with highest incidence of malignant neoplasms (MNO), recently, there has been a steady rise in the cancer burden. According to Rosstat, there is 31.3% increase in cancer incidence in 2010- 2019 (from 213.8 to 280.7 per 100.000 population), while more significant dynamics has been found among females (33.9%), as compared with males (28.3%).

Aim of the study: to characterize the current state of the malignant neoplasm burden among the population of the Sakha Republic (Yakutia), to assess cancer associations with the amount of pollutant emissions and ethnicity.

Materials and research methods

Data from the State Medical Statistics on cancer in 2010- 2019, published by the P. Herzen Moscow Oncological Research Institute - a branch of the National Medical Research Radiological Centre of the Ministry of Health of the Russian Federation, and data from the State Budgetary Institution of the Republic of Sakha

(Yakutia) "Yakutsk Republican Oncological Dispensary" (forms 7, 35) have been used in the study.

The malignant neoplasm incidence rates in the Sakha Republic (Yakutia) and the Russian Federation have been calculated based on the age-standardized rates (SR) per 100 000 population (world standard population) for the period between 2010 and 2019, as well as average long-term values for the entire observation period with 95 % confidence interval.

The system of zoning into 5 social-territorial zones, proposed by M.A. Tyrylgin [2] has been applied for zoning the territory of the Sakha Republic (Yakutia). Standardized indicators of the malignant neoplasms incidence for all localizations (C00-96) have been calculated by the method of direct standardization. The age structure of the population of the Sakha Republic (Yakutia) differs from the structure of the European population and the standard of the structure of the world population due to the positive natural growth and low life expectancy. In this regard, the age structure of the population of the Sakha Republic (Yakutia), according to the 2010 All-Russian Population Census, has been adopted as a standard.

Data from the 2010 All-Russian Population Census have been used to describe the ethnic composition of the territories. Indicators of pollutant emissions from stationary sources into the atmosphere (tons) have been selected to assess the degree of environmental pollution. The average level of the indicator for the period between 2005 and 2019 for each municipality (MO) has been calculated. The grouping of municipalities with similar age-standardized rates (SR) of cancer incidence rates of all localizations (C00-96) in 2015-2019 has been conducted using the method of two-stage cluster analysis (IBM SPSS Statistics 22). The search for possible links between incidence rates, environmental pollution, and an ethnic composition has been undertaken using the Spearman correlation and private correlation analyses.

Results and discussion. An analysis of mean values of age-standardized incidence rates for a 10-year period with the boundaries of 95% CI showed that, in general, the incidence of malignant neoplasms in the Sakha Republic (Yakutia) was lower than the average for the Russian Federation, both among males (by 10.4%) and females (by 13.6%). Since 2010 there has been an increase in the cancer incidence by 3.9 and 11.3%, respectively (in the Russian Federation - by 2.6 and 12.2%, respectively) among males and females in the Republic of Sakha (Yakutia), as well as in the Russian Federation, as a whole, with the cancer burden 32.1% higher among males (in the Russian Federation - by 27.2%), as compared with females (Fig. 1).

Comparison of the SP incidence of malignant neoplasms of all localizations (C00-96) per 100,000 population in the socio-territorial zones of the Republic of Sakha (Yakutia) in 2015-2019 (Fig. 2) has shown higher incidence rates of malignant neoplasms in men and women living in Yakutsk, the capital of Sakha (295.1 and 240.0), and the industrial zone (284.2 and 225.8), while the lowest rates have been observed in the agricultural zone (216.7 and 171.9 per 100,000 of the corresponding population). The levels of malignant neoplasm incidence rates in men and women in Yakutsk are 1.4 times higher than those in the agricultural zone. In all social-territorial zones, the cancer incidence in men is 1.2-1.3 times higher than in women. Differences between the indicators of mixed and industrial, agricultural and industrial, Arctic and agricultural zones are statistically significant ($p < 0.05$).

The analysis of prevalence of cancer incidence SP (C00-96) in 35 municipalities of the Republic of Sakha (Yakutia) has showed significant differences, which remain with time interval extension. Thus, in 2015-2019, the SP incidence of cancer among men varied from 170 to 351, among women from 116 to 287 per 100,000 of population.

The existing differences require detailed analysis to find the reasons for their occurrence. In the course of the cluster analysis of SP incidence rates, three clusters were formed (Tables 1 and 2):

- among the male population, cluster 1 with lower rates of cancer incidence included 14 (9 agricultural, 3 Arctic and 2 mixed zones); cluster 2 - 8 municipalities (3 Arctic, 2 industrial, 2 mixed and 1 agricultural zones), and cluster 3 - 13 municipalities (3 industrial, 5 Arctic, 1 agricultural and 3 mixed zones, and Yakutsk);

- among the female population, cluster 1 includes 12 municipalities (2 Arctic, 7 agricultural and 3 mixed zones); cluster 2 - 10 municipalities (4 Arctic, 3 agricultural and 3 mixed zones), cluster 3 - 13 municipalities (5 industrial, 5 Arctic, 1 agricultural and 1 mixed zones, and Yakutsk).

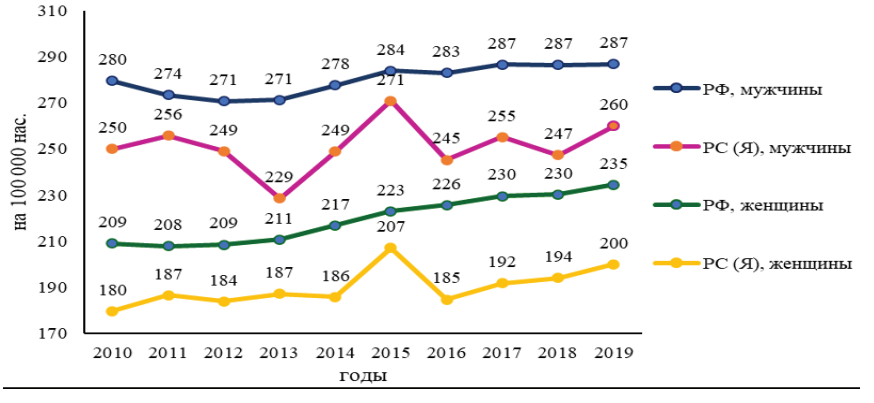

Fig. 1. Comparison of standardized cancer incidence rates for men and women in the Russian Federation and the Republic of Sakha (Yakutia) for 2010-2019, all localizations (C00-96), per 100,000 of the corresponding population (world population standard)

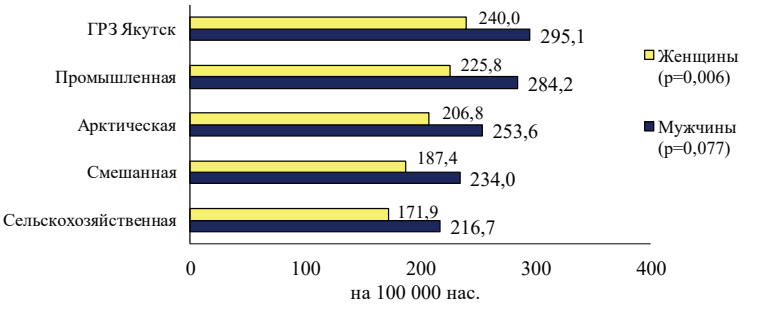

Fig. 2. Comparison of the standardized indicators of the incidence of cancer of all localizations (C00-96) in men and women in social-territorial zones of the Republic of Sakha (Yakutia) for 2015-2019 (the standard is the population of the Republic of Sakha (Yakutia)) in comparison of mixed and industrial zone p=0.016, rural and industrial p=0.001, Arctic and rural areas p=0.017

Significant differences between the male and female population in the levels of indicators, both between, and within municipalities affect the distribution of municipalities into clusters. Nevertheless, the prevalence of municipalities of the Arctic and industrial zones of the Sakha republic in cluster 3, and the prevalence of agricultural zones in cluster 1 is observed among both men, and women.

The above data suggest possible effects of unfavorable environmental factors and the ethnic composition of the population on cancer incidence rates. Moreover, both of these factors can be inter-related due to a higher share of the non-indigenous population in industrial regions (uluses) of Sakha. According to the Census, the share of the indigenous population, including the Yakuts and indigenous peoples of the North, vary in municipalities from 4 to 99.4%.

Correlation analysis according to Spearman revealed that the amount of pollutant emissions into the atmosphere from stationary sources has a moderate negative correlation with the share of the indigenous population in municipalities (r=-0.59, p <0.001), which correlates with a smaller number of indigenous people living in industrial uluses.

A search for potential correlations between levels of morbidity and environmental pollution, i.e. between the variables "pollution emissions" and the SP of the cancer incidence has not revealed any statistically significant correlations (r=0.25, p=0.193). A negative correlation has been found between the variables “a share of the indigenous population” and the SP incidence of cancer (C00-96) both among males (r = -0.42, p = 0.012), and females (r = -0.43, p = 0.011). Application of the method of private correlation analysis with “the share of the indigenous population” as a “control” variable making it possible to alleviate effects of the factor “ethnicity”, has not revealed any statistically significant links between the variables “ pollutant emissions” and the SP of cancer incidence (r = 0.18, p = 0.311).

Conclusions. The study of the current situation of morbidity from malignant neoplasms of all localizations (C00-96) among the population of the Republic of Sakha (Yakutia) has revealed the following:

–standardized incidence rates of malignant neoplasms of all localizations (C00-96) among males and females, according to the world population standard, in the Republic of Sakha (Yakutia) are lower as compared with the Russian Federation. However, in 2010-2019, a similar trend towards an increase in the cancer incidence among the male and female population has been observed in the Republic of Sakha (Yakutia), as in the whole of the Russian Federation, while the cancer incidence among men is higher as compared with women;

- within social-territorial zones of the Republic of Sakha (Yakutia), higher average long-term indicators of cancer incidence of all localizations (C00-96), standardized by the age composition of the Republic of Sakha (Yakutia), have been observed in men and women living in Yakutsk and in industrial zones, while the lowest indicators have been found for agricultural areas. And, the difference between values of average long-term indicators (2015-2019) in industrial and agricultural zones is 1.4 times;

- despite significant differences in levels of SP incidence of cancer (C00-96)

Table 1

Distribution of municipalities of the RS (Y) by clusters according to levels of SP incidence of cancer (C00-96) in 2015-2019, males

Municipality	SP per 100000 population	Social-territorial zone
Cluster 1 n=14 196 (95% CI 185-206)		
Tattinsky ulus	169.8	Agricultural
Kobyaisky ulus (region)	170.1	Mixed
Bulunsky ulus (region)	174.7	Arctic
Olekminsky region	177.7	Mixed
Suntarsky ulus (region)	181.0	Agricultural
Anabarsky National (Dolgan-Evenki) ulus (region)	191.1	Arctic
Verkhnevilyusky ulus (region)	194.3	Agricultural
Ust-Aldansky ulus (region)	204.7	Agricultural
Amginsky ulus (region)	205.2	Agricultural
Oleneksky Evenkiysky National region	206.3	Arctic
Vilyuskyi ulus (region)	210.5	Agricultural
Namsky ulus	215.1	Agricultural
Gorny ulus	217.2	Agricultural
Churapchinsky ulus (region)	220.1	Agricultural
Cluster 2 n=8 241 (95% CI 235-248)		
Lensky region	233.6	Industrial
Oimaykonsky ulus (region)	234.0	Industrial
Momsky region	236.5	Arctic
Allaikhovky ulus (region)	237.7	Arctic
Nyurbinsky region	240.0	Agricultural
Abyisky (region)	244.8	Arctic
Verkhnekolymsky ulus (region)	250.0	Mixed
Verkhoyansky region	254.1	Mixed
Cluster 3 n=13 292 (95% CI 277-306)		
Ust-Maysky ulus (region)	264.8	Mixed
Ust-Yansky ulus (region)	268.2	Arctic
Khangalassky улус	270.3	Mixed
Tomponsky region	273.3	Mixed
Aldansky region	278.4	Industrial
Even-Bytantaisky National ulus (region)	281.3	Arctic
Megino-Kangalassky ulus	288.2	Agricultural
Yakutsk (city of the republican significance) (CRS)	295.1	CRS
Mirninsky region	303.9	Industrial
Nizhnekolymsky region	304.0	Arctic
Neryungrinsky region	305.8	Industrial
Srednekolymsky ulus (region)	306.8	Arctic
Zhigansky National Evenkisky region	351.3	Arctic

Note: Data are presented as mean and 95% CI.

Table 2

Distribution of MD of the RS (Y) by clusters depending on the level of SP incidence of cancer (C00-96) in 2015-2019, females

Municipality	SP per 100000 population	Social-territorial zone
Cluster 1 n=12 154 (95% CI 142-166)		
Even-Bytantaisky National ulus (region)	116.0	Arctic
Amginsky ulus (region)	127.8	Agricultural
Gorny ulus	141.8	Agricultural
Kobyaisky ulus (region))	142.2	Mixed
Churapchinsky ulus (region)	149.0	Agricultural
Verkhnekolymsky ulus (region)	157.0	Mixed
Vilyuskyi ulus (region)	162.1	Agricultural
Suntarsky ulus (region)	165.6	Agricultural
Tomponsky region	166.5	Mixed
Nyurbinsky region	168.3	Agricultural
Namsky ulus (region)	170.5	Agricultural
Abyisky (region)	177.7	Arctic
Cluster 2 n=10 195 (95% CI 188-203)		
Allaikhovky ulus (region)	180.7	Arctic
Verkhoyansky region	182.2	Mixed
Megino-Kangalassky ulus (region)	187.7	Agricultural
Olekminsky region	191.8	Mixed
Tattinsky ulus (region)	191.9	Agricultural
Nizhnekolymsky region	192.3	Arctic
Ust-Aldansky ulus (region)	200.9	Agricultural
Khangalassky улус (region)	206.0	Mixed
Bulunsky ulus (region)	208.5	Arctic
Momsky region	210.5	Arctic
Cluster 3 n=13 232 (95% CI 217-246)		
Verkhnevilyusky ulus (region)	213.5	Agricultural
Mirninsky region	213.5	Industrial
Lensky region	214.3	Industrial
Ust-Yansky ulus (region)	214.3	Arctic
Srednekolymsky ulus (region)	214.8	Arctic
Zhigansky National Evenkisky region	216.1	Arctic
Neryungrinsky region	217.1	Industrial
Ust-Maysky ulus (region	224.2	Mixed
Yakutsk (city of the republican significance) (CRS)	240.0	CRS
Oimaykonsky ulus (region)	246.3	Industrial
Oleneksky Evenkiysky National region	248.2	Arctic
Aldansky region	258.5	Industrial
Anabarsky National (Dolgan-Evenki) ulus (region)	289.6	Arctic

in 35 municipalities of the Republic of Sakha (Yakutia), remaining with extension of time intervals, and, respectively, in the three clusters formed according to the intensity of the indicators among males and females, the highest rates in the Arctic and in industrial zones, and the lowest rates in agricultural zones are common for all the clusters;

- no statistically significant associations have been established between atmospheric pollution from stationary sources and cancer incidence.

Thus, the search for causes of morbidity in individual localizations of malignant neoplasms, and indicators of environmental pollution to assess impacts of environmental factors on development of malignant neoplasms in the Republic of Sakha (Yakutia) should be continued. The increase in the cancer incidence could be due to early cancer detection, common for the Republic of Sakha (Yakutia) and the Russian Federation, associated with modernization of the oncological service within the framework of the National Project "Health", and differences in physical and chemical factors in the Sakha Republic municipalities. A correlation between the proportion of the indigenous population and the cancer incidence requires additional research, including genetic study.

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LONG-TERM SYMPTOMS OF COVID-19 IN RESIDENTS OF YAKUTSK

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УДК 61.616-06

The analysis of the main symptoms of residents of the city of Yakutsk aged 20 to 72 years was conducted, who had a new coronavirus infection COVID-19 3, 6, 9, 12 months ago.

According to the CT protocol from the anamnesis, the severity of the course of the disease has a direct relationship with age, BMI and gender. Almost half of the surveyed indicate a health disorder after suffering from COVID-19. The subjects with hypertension, chronic kidney disease, diabetes mellitus, chronic respiratory tract disease, and ischemic heart disease most often indicated fatigue. With fatigue, the vital capacity of the lungs was lower by 16-17%, a significant decrease in the activity of the enzymes of energy metabolism lactate dehydrogenase and creatine kinase in men was revealed.

Keywords: COVID-19, long-term effects, symptoms.

The long-term health effects of SARS-CoV-2 from COVID-19 remain unclear. According to literary sources, some complain of symptoms for 3 or more weeks, while others suffer for months [4]. In the majority of patients, 100 days after the diagnosis of COVID-19, computed tomography (CT) revealed persistent lung pathologies, and one third of patients had dyspnea [2]. A study in Wuhan, China of 736 survivors of an acute infection with SARS-CoV-2 (COVID-19) showed that after 6 months they were mainly worried about fatigue or muscle weakness, sleep problems, anxiety and depression [1]. Исследователи, анализируя литературу, отметили, что физическое состояние, наблюдаемое у перенесших вирус SARS-CoV, было ниже, чем у здоровых людей, а через 1-2 года наблюдалось неполное восстановление физических функций. Учитывая сходство патологии и клинических проявлений, вызванных SARS-CoV и SARS-CoV-2, пациенты с COVID-19, вероятно, будут иметь аналогичные нарушения физических функций [9]. Weakness has been associated with decreased physical endurance caused by cardiopulmonary dysfunction. In addition, SARS-CoV-2 can cause long-term effects in the central nervous system (CNS), gastrointestinal tract, liver and kidneys [6]. In this regard, scientific research is needed on the impact of the long-term consequences of COVID-19 on human health in order to organize adequate measures for full rehabilitation.

The aim of the study is to assess the long-term consequences of COVID-19 in residents of Yakutsk who had the disease from 3 to 12 months ago.

Material and research methods. The study involved 164 residents of Yakutsk, aged 20 to 72 years old, who had had COVID-19 from 3 to 12 months back. Of these, 96 women (58.18%), men - 68 (41.46%). The average age was 51.07 ± 0.97 (Me-51.5; CI: 49.15-52.98), for men - 50.41 ± 1.51 (Me-51; CI: 47.38-53, 44), women - 51.54 ± 1.26 (Me-53; CI: 49.02-54.05). According to the duration of the transferred COVID-19, all the subjects were divided into 4 groups: up to 3, up to 6, up to 9, up to 12 months ago (Table 1).

In assessing the health status of the patients examined at the Clinic of the Yakutsk Scientific Center for Complex Medical Problems, a questionnaire was used, including questions about the presence of complaints after suffering COVID-19 and diseases of the cardiovascular, nervous, musculoskeletal, and respiratory systems; biomedical research: reception of specialists (cardiologist, neurologist, rheumatologist, therapist), determina-

Table 1

Patient groups by date after undergoing covid-19, abs. number/%

Sex	Periodic term, months				Total
	Up to 3	Up to 6	Up to 9	Up to 12	
Everything	15/9.2	77/47.2	48/29.4	23/14.1	163/100
Men	6/8.8	32/47.1	18/26.5	12/17.6	68/41.46
Women	9/9.5	45/47.4	30/31.6	11/11.6	95/58.18

Table 2

CT indicators depending on age and gender, abs. number/%

CT	Sex	Age groups, years						Total
		20-29	30-39	40-49	50-59	60-69	70-79	
CT 0	Men	1/10	4/40	2/20	3/30	0/0	0/0	10
	Women	0/0	4/23.5	4/23.5	5/29.4	4/23.4	0/0	17
CT 1	Men	1/5.3	5/26.3	5/26.3	4/21.1	4/21.1	0/0	19
	Women	1/2.4	9/22	12/29.3	11/26.8	7/17.1	1/2.4	41
CT 2	Men	0/0	2/13.3	2/13.3	4/26.7	5/33.3	2/13.3	15
	Women	1/2.4	9/22	12/29.3	11/26.8	7/17.1	1/2.4	27
CT 3	Men	0/0	3/15	5/25	7/35	4/20	1/5.0	20
	Women	0/0	1/16.7	0/0	1/16.7	2/33.3	2/33.3	6
CT 4	Men	0/0	0/0	0/0	1/25	2/50	1/25	4
	Women	1/50	0/0	0/0	0/0	1/50	0/0	2
	Everything	5	28	35	42	41	10	161

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tion of hematological, biochemical and immunological parameters, anthropometry, spirometry, ECG. The study was approved by the local committee on biomedical ethics at the Federal State Budgetary Scientific Institution YSC KMP No. 52 dated March 24, 2021 and was conducted subject to the voluntary informed consent of the participants. Determination of biochemical parameters of blood serum was carried out on a biochemical analyzer "LABIO 200" (China) using reagents from the firm " Analyticon" (Germany).

Statistical processing was performed using the SPSS 11.5 for Windows software package. The normal distribution of quantitative indicators was determined by the Kolmogorov-Smirnov test. Descriptive analysis data are presented in the tables as Me (median), Q1 and Q3 (quartiles 25% and 75%).

The significance of differences was assessed using Student's t-test and ANOVA for independent samples with normal distribution and Mann-Whitney test for abnormal distribution. The probability of the validity of the null hypothesis was taken at p <0.05. Correlation analysis was performed using the Pearson and Spearman method, where r is the correlation coefficient, p is the significance of the result.

Results and discussion. Taking into account the data of the protocols of CT of the lungs during illness and discharge reports of patients with COVID-19, all patients were divided according to the degree of lung involvement into 5 groups: CT0 (zero) - no signs of viral pneumonia; KT1 (light) - the presence of a zone of compaction of the type of "frosted glass", the involvement of less than 25% of the volume of the lungs; KT2 (moderate) - the presence of a zone of compaction of the type of "frosted glass", the involvement of 25 to 50% of the lung volume; KT3 (heavy) - zones of compaction of the “frosted glass” type, involving from 50 to 75% of the lung volume. Increase in the volume of the lesion by 50% in 24-48 hours against the background of respiratory disorders during dynamic observation; CT4 (critical) - diffuse compaction of the lung tissue of the "ground glass" type and consolidation in combination with reticular changes. Involvement of more than 75% of the lung volume. Hydrothorax [3].

Correlation analysis showed that the degree of lung involvement had an inverse relationship with gender (r = 0.238; p <0.002): CT0-CT2 is more common in women, and severe and critical lung damage, CT3 and CT4, is more common in men (Table 2).

Table 3

The main complaints of the surveyed after the transferred COVID-19, abs. number/%

Complaints	Total	%	Sex	Term, months			
				Up to 3	Up to 6	Up to 9	Up to 12
Fatigue	68	43	Men	2/8,7	11/47,8	5/21,7	5/21,7
			Women	7/15,6	18/40,0	14/31,1	6/13,3
Deterioration of hair condition	51	33,1	Men	1/8,3	8/66,7	3/25,0	0,0
			Women	4/10,3	22/56,4	11/28,2	2/5,1
Decreased working capacity	50	32,7	Men	2/8,3	13/54,2	5/20,8	4/16,7
			Women	4/15,4	8/30,8	10/38,5	4/15,4
Dyspnea	44	28,2	Men	1/6,3	9/56,3	5/31,3	1/6,3
			Men	2/7,1	12/42,9	10/35,7	4/14,3
Debility	42	26,6	Women	2/13,3	8/53,3	2/13,3	3/20,0
			Men	4/14,8	14/51,9	6/22,2	3/11,1
Sweating	40	25,5	Women	2/12,5	9/56,3	4/25,0	1/6,3
			Men	2/8,3	12/50,0	8/33,3	2/8,3

Table 4

Frequency of complaints based on severity of COVID-19, abs. number/%

Complaints	Пол	The degree of lung damage accordin to the results of CT SCAN				
		CT 0	CT 1	CT 2	CT 3	CT 4
Fatigue	Men	5/21.7	7/30.45	2/8.7	6/26.1	3/13
	Women	9/20	19/42.2	15/33.3	1/2.2	1/2.2
Deterioration of hair condition	Men	2/16.7	4/33.3	3/25	2/16.7	1/8.3
	Women	5/12.8	18/46.2	10/25.6	5/12.8	1/2.6
Decreased working capacity	Men	4/16.7	5/20.8	6/25	6/25	3/12.5
	Women	10/15.2	18/27.3	15/22.7	19/28.8	1/3.8
Dyspnea	Men	1/6.3	5/31.3	4/25	4/25	2/12.5
	Women	4/14.3	13/46.4	8/28.6	2/7.1	1/3.6
Debility	Men	3/20	5/33.3	2/13.3	2/13.3	3/20
	Women	4/14.8	13/48.1	7/25.9	2/7.4	1/3.7
Sweating	Men	1/6.3	5/31.3	5/31.3	3/18.8	2/12.5
	Women	4/16.7	6/25	10/41.7	3/12.5	1/4.2

In addition, the degree of lung damage during COVID-19 disease had a direct relationship with age (r = 0.307; p <0.000) and BMI (r = 0.286; p <0.000).

In the examined subjects, a mild form of lung lesion with CT1 is typical for age groups from 20-29 to 40-49 years, in older groups, moderate lung damage with CT2 is more common. Severe lung damage with CT3 was more common in the 70-79-year-old group. The critical form with CT4 was among the subjects in the age groups from 50-59 to 70-79 years (Fig. 1).

Severe lung injury CT3 was most often in patients with overweight and obesity of the 1st degree, and critical lung injury with CT4 was most often in overweight patients (Fig. 1). An analysis of the questionnaires of the surveyed showed that 40.9% noted a health disorder after suffering a new coronavirus infection. The most common complaints were fatigue, deterioration of hair condition (loss), decreased performance, shortness of breath, weakness and sweating. Table 3 shows the frequency of occurrence of the main complaints depending on the post-covid period. Most of the complaints were among the subjects who were sick with COVID -19 4-6 and 7-9 months ago, and more often in women. Among 24 people

Table 5

Enzyme activity in men and women with complaints of fatigue

Enzyme, U/L	Sex	Fatigue		No fatigue		p
		Me	Q1 – Q3	Me	Q1 – Q3	
LDH	Everything	381.60	348.00-430.50	395.50	355.50-435.50	0.123
	Men	376.00	345.00-425.000	400.00	346.75-442.50	**0.049**
	Women	381.50	346.75-442.50	377.00	338.00-428.25	0.480
CK	Everything	74.50	57.25 – 102.75	88.00	68.00-121.75	**0.050**
	Men	79.00	64.00-128.00	120.00	101.00-185.25	**0.025**
	Women	72.00	55.50-90.00	70.00	54.50-86.25	0.489
ALP	Everything	195.00	165.25-247.75	220.00	169.5-255.5	0.820
	Men	206.00	189.00-249.00	219.00	181.00-255.750	0.682
	Women	182.50	146.75-245.250	230.00	161.25-261.25	0.862
GGT	Everything	30.50	21.00-44.00	32.00	21.75-51.00	0.533
	Men	36.00	21.00-43.00	40.00	27.75-55.75	0.243
	Women	29.00	20.75-44.25	26.00	18.75-52.75	0.786
ALT	Everything	21.50	14.25-32.50	22.00	15.00-39.00	0.959
	Men	25.00	17.00-50.00	40.50	21.00-47.00	0.646
	Women	21.00	13.00-27.00	26.6	13.75-52.75	0.990
AST	Everything	24.00	20.00-26.75	25.00	21.00-31.00	0.282
	Men	23.00	21.00-28.00	25.00	22.75-33.00	0.502
	Women	25.00	20.00-26.25	23.00	19.00-31.00	0.376

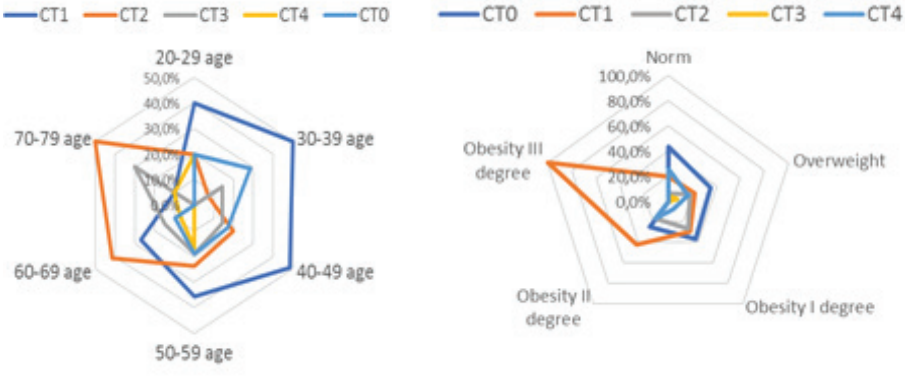

Fig. 1. CT indicators depending on age and BMI

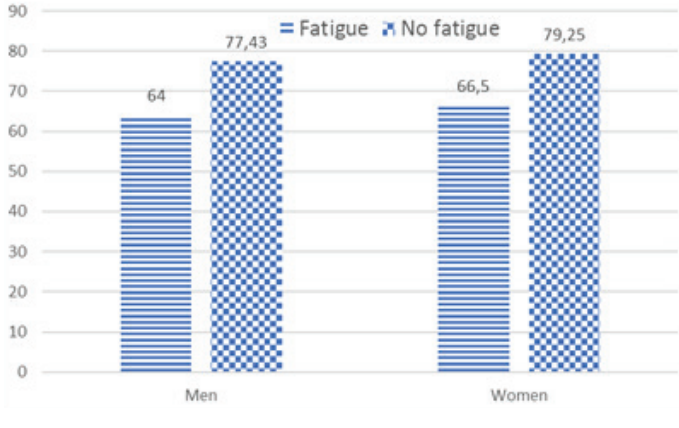

Fig. 2. Lung vital capacity during inhalation (%) in the presence of complaints of fatigue

who underwent a new coronavirus infection for 10-12 months ago, symptoms of fatigue persisted in 11 people (45%), decreased efficiency - in 8 (33%). Other symptoms to a lesser extent also persist for up to a year, except for hair loss in men.

The main complaints, depending on the severity of the course of COVID-19, are shown in Table. 4. Fatigue, deterioration of hair condition, shortness of breath and weakness are most of all complaints in women with CT1 and CT2. Decreased working capacity is observed in all groups and most often in the CT3 group. Among those who have CT0, without lung damage, there is also a sufficient number of complaints of fatigue and decreased performance.

Among the subjects with complaints of fatigue, the vital capacity of the lungs during inspiration in men was lower by 17.35%, in women - by 16.09% (Fig. 2). Among the subjects with complaints of fatigue, 29 people (43.9%) have hypertension, 22 (36.7) have chronic kidney disease, 18 (31.0) have diabetes mellitus, 15 (24.6) have chronic respiratory tract disease, 9 people (15.8%) suffer from ischemic heart disease. In addition, fatigue is associated with the incidence of pathology of the musculoskeletal system ($r = 0.227$; $p < 0.015$).

Analysis of the data on the activity of enzymes of energy metabolism, depending on the presence of complaints of fatigue, showed that the activity of lactate dehydrogenase and creatine kinase was significantly lower in men (Table 5).

The activity of creatine kinase in the subjects decreased after 5-8 months after the transferred infection, further restoration of the enzyme activity was observed (Fig. 3).

Long-term symptoms of COVID-19, such as fatigue, decreased performance, are noted in a few sources [5,8]. Many authors call for further studies of the long-term effects of the novel coronavirus infection caused by SARS-CoV-2, not only among patients recovering from severe acute illness, but also among patients with mild to moderate disease [7].

Conclusion. The severity of the course of COVID-19, according to the CT scan during the disease, has a direct relationship with age, BMI and gender: the new coronavirus infection is more difficult for men of age.

40.9% of the surveyed indicated health problems after the COVID-19 disease. The main symptoms are fatigue, deterioration of hair condition (loss), decreased performance, shortness of breath, weakness and sweating. Fatigue after 12 months after the disease

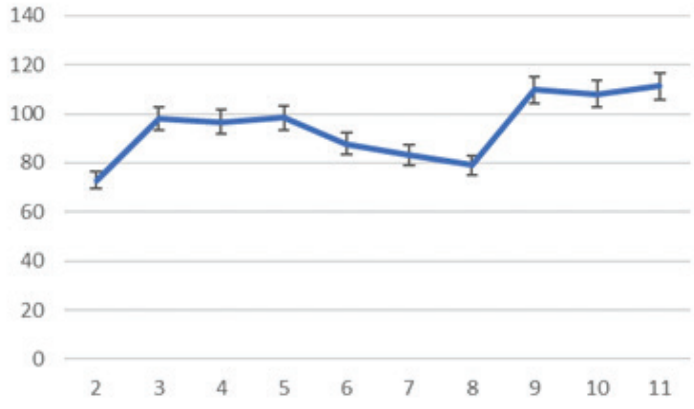

Fig. 3. CK activity depending on the term after the transferred COVID-19, months

COVID-19 remained in 11 people (45%), decreased efficiency - in 8 people (33%). Most often, fatigue is indicated by those surveyed with hypertension, chronic kidney disease, diabetes mellitus, chronic respiratory tract disease, ischemic heart disease. In subjects with complaints of fatigue, the vital capacity of the lungs was lower by 17%. The activity of enzymes of energy metabolism lactate dehydrogenase and creatine kinase is reduced in men with complaints of fatigue. In-depth research is needed to find the causes of persistent long-term symptoms after suffering COVID-19.

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CORONAVIRUS INFECTION (COVID-19) IN CHILDREN OF THE REPUBLIC OF SAKHA (YAKUTIA)

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УДК 616.988

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The preliminary epidemiological data on coronavirus infection COVID-19 in children in the Republic of Sakha (Yakutia) is presented in the article. Retrospective analysis of 431 cases of coronavirus infection in children who were hospitalized in the Clinical Hospital of Children's Infectious Diseases during the period from March 2020 to December 2020 was conducted. Peaks of the epidemic process were identified in May and November 2020. The COVID-19 virus was identified in 88% of cases, in 54 cases the diagnosis was made based on clinical and epidemiological data. The highest risk group in terms of developing pneumonia are children and teenagers 10-18 years old. Considering of the epidemic process and the peculiarities of the implementation of coronavirus infection, the alertness of pediatricians in the field should be formed.

Keywords: coronavirus infection (COVID-19), pneumonia, children, Yakutia.

Introduction. Information about a new coronavirus infection first appeared in Wuhan City, Hubei Province, People's Republic of China, in December 2019. [5, 6]. A large-scale epidemic spread in China, the World Health Organization outlined the outbreak as an emergency situation and announced the beginning of the pandemic in March 2020 [7]. In Moscow and St. Petersburg the date of registration of the first cases is March 2, 2020 and March 7, 2020, respectively. In Yakutsk (Republic of Sakha (Yakutia)) the first patient with COVID-19 was registered on March 18, 20 [1]. Today the entire world community is focused on fighting with this threat. Different aspects of the epidemic process are being studied to develop effective countermeasures.

Up to the present time, the researches have appeared on the analysis of the prevalence, diagnosis, clinic, and treatment of coronavirus infection in children. For example, a meta-analysis including

data from 2874 children with COVID-19 showed that the most frequent symptoms of infection were fever 48.5% (95% CI: 41.4-55.6%) and cough 40.6% (95% CI: 33.9-47.5%). Asymptomatic infection was observed in 27.7% (95% CI: 19.7-36.4%) of cases. A severe course was observed in 1.1% of cases (out of 1933 patients). Unilateral (29.4%, 95% CI: 24.8-34.3%) and bilateral lesions (24.7%, 95% CI: 18.2-31.6%) were found with almost equal frequency. The symptom of "frosted glass" was observed in 32.9% (95% CI: 25.3%-40.9%) of cases [4]. Domestic authors have also shown that COVID-19 in children has a number of specific features, in particular the disease is often asymptomatic or with a subtle clinical picture. The outcome of the disease in children is usually favorable [2, 3].

Methodology. Retrospective analysis of medical records of children hospitalized with the diagnosis "Coronavirus infection" was conducted from March to December 2020. The work was performed on the basis of the State Budgetary Institution of the Republic of Sakha (Yakutia) "Children's Infectious Clinical Hospital", Yakutsk, where all children with suspected COVID-19 living in the Republic of Sakha (Yakutia) were hospitalized according to patient routing. All children underwent PCR study for coronavirus infection. A computed tomography scan was performed when indicated.

Statistical calculations were performed with IBM SPSS Statistics 17 software (IBM®, USA). Mann-Whitney, Pearson $\chi 2$ criteria were used to compare groups. Quantitative variables were presented as mean and standard deviation in M (SD) format. The critical value of the significance level for statistical hypothesis testing was assumed to be 5%.

Results. 431 children between the ages of 0 and 18 (191 girls and 240 boys) received inpatient treatment with a diagnosis of coronavirus infection at the Children's Infectious Clinical Hospital of the Republic of Sakha from March to December 2020. 405 children of them were residents of Yakutsk and its suburbs, 25 were children from the districts of the Republic of Sakha (Yakutia) and 2 children were from other regions of the Russian Federation.

As shown in Picture 1, the maximum incidence peaks were observed in May-June and October-November 2020.

The analysis of the age structure of the hospitalized (Pic. 2) showed a slightly higher proportion of older children (53.6% of cases in children 7 years and older).

In 377 cases out of 431 (87.5%), the COVID-19 virus was identified, and in 54 cases the virus was not identified, but COVID-19 was diagnosed clinically or epidemiologically (Table 1). The frequency of virus identification was independent of patient age.

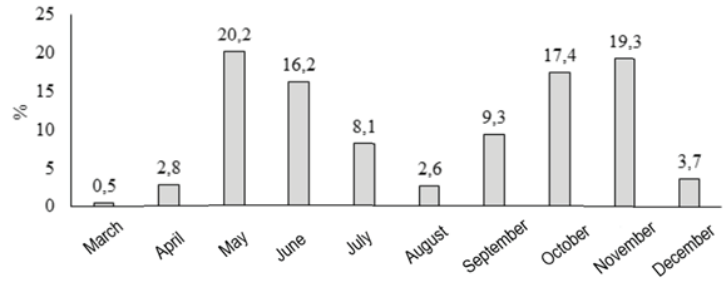

Fig.1. Distribution of patients with COVID-19 by months in 2020

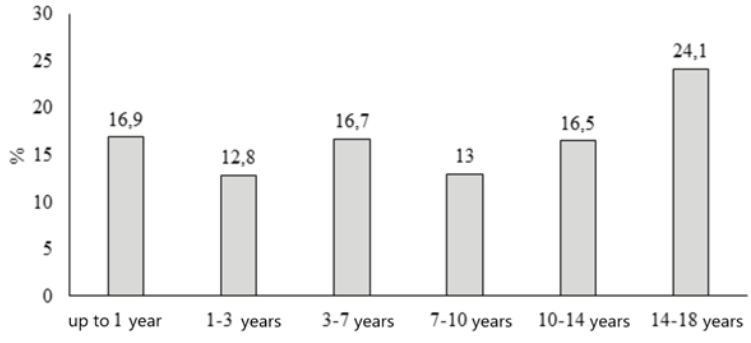

Fig. 2. Age structure of children with COVID-19 in the Republic of Sakha (Yakutia)

In 142 children (32.9%) pneumonia was diagnosed, in some cases it was accompanied by pleurisy. And the most frequently confirmed pneumonia was detected in children 10-14 years (45.1%), 14-18 years (43.3%). In 289 cases there were other manifestations of infection. Pneumonia was statistically significantly more frequent in older children (p=0.005). COVID-19 virus was identified in 90% of pneumonia cases.

On average, children spent 11.4 (4) bed-days in the hospital. The length of stay in case of pneumonia was 12.7 (4.0) days versus 10.8 (3.9) days for other conditions (p<0.001). In 310 cases patients were discharged with recovery, in 119 with improvement, and in 2 cases with no change. In these 2 cases there was a transfer to another medical institutions.

Conclusion. A seasonal increase in the incidence of coronavirus infection in children in the Republic of Sakha (Yakutia) was observed in May and November 2020. During the study period, mainly urban children were hospitalized. The proportion of rural children was 5% (25 children). COVID-19 virus was identified in 88% of cases of clinical picture of new coronavirus infection. Pneumonia was detected in 44% of children aged 10-18 years. The virus was detected in 90% of pneumonia cases. The outcome of the disease was favorable; no cases of death or severe complications were recorded.

Frequency of COVID-19 virus detection and development of pneumonia among children in the Republic of Sakha (Yakutia)*

Age	Virus detected, n (%)	Pneumonia, n (%)
Up to 1 year	65 (89.0)	20 (27.4)
1-3 years	49 (89.1)	13 (23.6)
3-7 years	64 (88.9)	17 (23.6)
7-10 years	44 (78.6)	15 (26.8)
10-14 years	62 (87.3)	32 (45.1)
14-18 years	93 (89.4)	45 (43.3)
All ages	377 (87.5)	142 (32.9)
p	0.436	0.005

Note: * - the data is presented as n (%), where n is the absolute number of observations: p-achieved level of significance in comparing age groups (Pearson's criterion χ^2).

Given the peculiarities of the epidemic process, the high proportion of coronavirus infection in the form of pneumonia in elder children requires the vigilance of pediatricians in the development of the 3rd wave of the pandemic.

The work was performed within the research theme "Monitoring of the health of children in the Republic of Sakha (Yakutia)" (State Registration Number: 0120-128-07-98), within the Government Order of the Ministry of Science and Education of the Russian Federation (FSRG-2020-0016).

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HEART RATE VARIABILITY IN PATIENTS WITH MODERATE AND SEVERE COVID-19

The study of heart rate variability (HRV) in COVID-19 is of undoubted interest, as it allows one to judge about disturbances in the work of the cardiovascular system, as well as about shifts in the state of autonomic regulation of physiological functions. Both of these systems are targets for viral damage.

The aim. To investigate how HRV changes in seriously ill COVID-19 depending on the severity of the disease, as well as to determine the prognostic role of ROC analysis in predicting the outcome of the pathological process. Materials and methods. observations were carried out on 29 patients of moderate severity (age 58.7 ± 6.5 years), and 55 seriously ill (59.4 ± 9.2 years) COVID-19. The control group consisted of 69 people (mean age 62.5 ± 9.6). HRV was studied using the Rehovot Dynamic Light Scattering apparatus (Israel) and using an original algorithmic approach. Statistical processing was performed using the R language version 3.6.2. To assess the relationship between the studied indicators, the Pearson correlation method was applied. To calculate the threshold values for survival and mortality, which have predictive value, an ROC analysis was performed. Results. In patients with COVID-19, HRV parameters significantly decreased. There were no significant differences in the studied values between groups of different severity. The data obtained indicate a violation of the activity of both the sympathetic and parasympathetic divisions of the autonomic nervous system. ROC analysis of HRV did not provide a predictive model for COVID-19 with a high probability of an outcome. Conclusion. In patients with moderate and severe COVID-19, the main indicators of HRV are significantly reduced. Using ROC analysis of HRV, no significant predictors of favorable and fatal outcomes were found in patients with COVID-19.

Keywords: COVID-19, microcirculation, heart rate variability.

It is known that the method of studying heart rate variability (HRV) is subtly responsive to the action of stress stimuli, as well as to any changes in the body of a healthy and sick person. At the same time, HRV in different courses of COVID-19 has practically not been studied. Elucidation of this issue is extremely important not only for theory, but also for practical medicine, because it quite accurately allows us to judge changes in the work of both the cardiovascular system and regulatory mechanisms that ensure a balance in the activity of the sympathetic and parasympathetic divisions of the autonomic nervous system. (ANS) under stress, as well as a wide variety of pathological conditions [22, 26].

The list of target organs affected by the SARS-Cov-2 virus is not limited to the respiratory tract. Alteration of the cardiovascular and central nervous systems is essential in the pathogenesis of the new coronavirus infection. It has now been established that cardiovascular manifestations in patients with COVID-19 include myocardial damage, arrhythmias, cardiac arrest, heart failure, and bleeding disorders [7,8]. They have a fairly widespread character and a protracted course. Moreover, the mechanism of heart damage in patients with COVID-19 includes direct damage to myocardial cells mediated by angiotensin-converting enzyme 2 (ACE2) receptors, as well as systemic inflammation, which causes indirect damage to myocytes [21]. Moreover, patients with COVID-19, whose disease is fatal, always suffer from cardiopulmonary complications [25].

A. Izcovich et al. [19], having analyzed 207 studies by various authors, identified the main factors that provide valuable predictive information about mortality and / or severe disease in patients with COVID-19. Among them, a significant part was associated with lesions of the cardiovascular system, cerebrovascular diseases, chronic obstructive pulmonary disease, cardiac arrhythmia, arterial hypertension, diabetes, dyslipidemia, respiratory failure, etc. Moreover, a decrease in HRV often correlates with an increase in the concentration of C-reactive protein (CRB). There is no doubt that HRV in COVID-19 is primarily influenced by the inflammatory process caused in response to viral invasion. According to the

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authors, "... a short-term intermittent HRV analysis in patients with COVID-19 can provide potential value for the prognosis of the disease."

The mechanisms of heart damage in COVID-19 are not well established, but likely include increased stress due to respiratory failure and hypoxemia, direct myocardial damage caused by SARS-CoV-2, and indirect damage from a systemic inflammatory response. But most likely, the complex of all the listed factors influences the function of the heart [5].

Considering the presented data, it seems promising to study HRV shifts in patients with moderate and severe COVID-19.

Clinical characteristics of patients and research methods.

Our observations were carried out on 29 moderately severe patients (age 58.7 ± 6.5 years) and 55 seriously ill patients (59.4 ± 9.2 years) who received treatment at the 1st City Clinical Hospital of Chita, redesigned for the treatment of patients COVID-19, in 2020. The control group consisted of 69 people (mean age 62.5 ± 9.6 years). The groups did not differ in the frequency of concomitant diseases (diabetes mellitus, hypertension, arthritis and arthrosis, metabolic syndrome). Exclusion criteria: the presence of oncopathology, acute coronary syndrome, non-coronary myocardial damage, strokes, encephalitis, taking antiarrhythmic and cardiotropic drugs, cardiac arrhythmias, unstable hemodynamics. All our activities were carried out with the consent of the subjects and complied with ethical standards developed on the basis of the Helsinki Declaration of the World Association "Ethical Principles for Conducting Scientific Medical Research with Human Participation" as amended in 2008 and the "Rules of Clinical Practice in the Russian Federation" approved by the order of the Ministry of Health of the Russian Federation dated June 19, 2003 No. 266. The studies were approved by the decision of the local ethical committee of the Chita State Medical Academy of the Ministry of Health of the Russian Federation (protocol No. 102 dated 05/15/2020).

Standard therapy was carried out in accordance with the current version of the temporary guidelines of the Ministry of Health of the Russian Federation. "Prevention, diagnosis and treatment of new coronavirus infection COVID-19" and local treatment protocols. Antiviral and antibacterial therapy was used. Low molecular weight heparins (LMWH) were prescribed at a dose of 1 mg / kg 2 times a day or continuous intravenous infusion of unfractionated heparin (UFH) with a starting rate of 1000 U / h with subsequent correction of the injection rate based on the coagulogram parameters. Respiratory therapy included inhalation of humidified oxygen, non-invasive mechanical ventilation. Symptomatic therapy consisted of glycemic correction, administration of antipyretic drugs, nutritional support, and maintenance of water and electrolyte balance.

HRV was studied using the mDLS apparatus (Dynamic Light Scattering, Rehovot, Israel) and using an original algorithmic approach. For this purpose, a technique has been developed for the spectral decomposition of the signal into frequency components associated with hemodynamic sources of different shear rates of blood layers. To assess the characteristics of HRV, the method of photoplethysmography (PPG) was used [10, 11]. Information on the variability of RR intervals was extracted from the pulse component of the mDLS signal, and HRV indicators were calculated [10]. The measurements were made for 5 minutes.

The following time indicators were used: HR (Heart Rate) - heart rate (HR) [beats / min]; SDNN (Standard Deviation of RR intervals) - standard deviation of all RR intervals [msec] (reflects all long-term components and circadian rhythms responsible for variability); SDSD - standard deviation of differences between adjacent intervals (represents variability in short time intervals); RMSSD (Root Mean Square of the Successive Differences) - the square root of the root mean square of the differences in RR intervals [msec] (refers to changes in the short term and reflects deviations in the tone of the autonomic nervous system, which are predominantly vagus-mediated); MAD (Median Absolute Deviation) is the absolute deviation of the median (used to assess the quality of the signal under study by the presence of outliers, ie, extreme values) [6, 14].

Frequency analysis is presented by indices: PWR - total power of oscillations in all frequency ranges; LF - power in the low frequency range (0.04-0.15 Hz), due to the activity of the sympathetic division and reflects the delay time of the baroreflex loop; HF - power in the high frequency range (0.16-0.5 Hz) is associated with respiratory movements and is mainly due to vagal activity; PWR is the sum of the low-frequency LF and high-frequency components of the HF; LF / HF - power ratio (reflects the overall sympatho-vagus balance).

Statistical processing was performed using the R language version 3.6.2. To assess the mutual relationship between the studied indicators, the Pearson correlation method was used (the choice of the method was due to the distribution of groups reliably close to normal). The quantitative characteristics of the traits were represented by the median (Me, Q2 - the second quartile), the first and third quartiles (Q1 and Q3, respectively). To calculate the threshold values of mortality that have predictive value, an ROC analysis was carried out [1]. When construct-

Heart rate variability in patients with moderate and severe COVID-19

Test item	Control. n=69	Patients with moderate COVID-19. n=29	Patients with severe COVID-19.	p1	p2	p3
HR	86.6 [75-98.5]	79.4 [73.7-86.6]	83.1 [69.3-93.4]	0.33	0.44	0.74
SDNN	145 [94.9-164]	70 [36.9-95.2]	59.8 [41.6-104]	< 0.0001	< 0.0001	0.57
SDSD	113 [82.1-123]	71.3 [22.8-90.3]	57.1 [34.3-96.8]	< 0.0001	< 0.0001	0.34
RMSSD	168 [105-189]	97.5 [29.9-122]	78.9 [42.3-140]	< 0.0001	< 0.0001	0.38
MAD	72.5 [32.5-125]	35 [15-50]	25 [20-35]	0.0004	< 0.0001	0.41
PWR	6910 [3280-11100]	1140 [417-3140]	992 [563-1990]	< 0.0001	< 0.0001	0.77
LF	2260 [866-3810]	302 [126-456]	229 [102-496]	< 0.0001	< 0.0001	0.72
HF	3780 [1660-5710]	666 [98.6-1970]	577 [166-1070]	< 0.0001	< 0.0001	0.83
LF/HF	0.61 [0.463-0.795]	0.524 [0.395-1.08]	0.521 [0.352-0.983]	0.95	0.71	0.95

Note. Data representation Me [P25-P75]. Comparison of groups according to the "Wilcoxon rank sum test" criterion. p1 - control and moderate patients; p2 - control and severe patients; p3 - moderate and severe patients. Correction for multiple comparison "Hommel (1988)".

ing the ROC-curve there is a change in the value (threshold) of the investigated factor, when deciding on the possible lethality and at a given threshold, the sensitivity and specificity of prediction are calculated from the experimental data. The test cycle starts at 0% sensitivity and 100% specificity, ends at 100% sensitivity, 0% specificity. Predictive accuracy was assessed using an expert scale of indicators [17, 28]; the area under the ROC curve (Area Under Curve - AUC), which can be taken as follows: 90-100% - excellent; 80-90% is good; 70-80% is acceptable; 60-70% - weak; 50-60% - unsatisfactory [4, 15.].

Results. As follows from the indicators given in the table (Table 1), the heart rate in patients with COVID-19 did not differ significantly from the norm. At the same time, SDNN, reflecting all long-term components and circadian rhythms during the recording, was significantly reduced in both the group of moderate and severe patients. The data obtained indicate that subtle adaptive responses aimed at maintaining homeostasis in the activity of the cardiovascular system are impaired in patients with COVID-19. This opinion is confirmed by a decrease in patients with COVID-19 in both study groups by almost 2 times in SDSD, RMSSD and the absolute deviation of the median - MAD. At the same time, no significant differences in the values of SDSD, RMSSD and MAD between the groups of patients with COVID-19 of different severity were found.

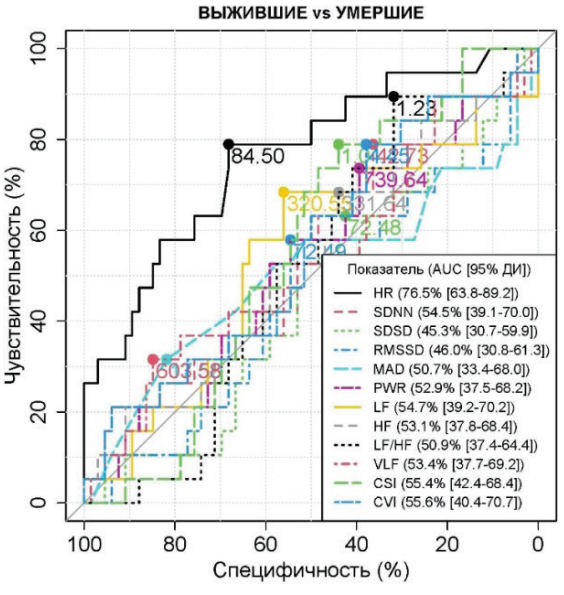
The data presented by us on changes in HRV in patients with COVID-19 undoubtedly reflect shifts in the balance of the sympathetic and parasympathetic divisions of the autonomic nervous system (ANS). This, in particular, is evidenced by a significant decrease in the LF index in both studied groups, due to the activity of the sympathetic division of the ANS. The data obtained indicate that patients with COVID-19 have a distinct violation of sympathetic innervation.

But at the same time, in such patients, there is a decrease in the HF index, associated with the function of the respiratory system and mainly due to vagal activity. Consequently, in patients with moderate and severe form of COVID-19, the activity of both sympathetic and parasympathetic divisions of the ANS is impaired. Imbalances in their balance are also indicated by a decrease in patients with moderate severity of the nonlinear sympathetic CSI index and a decrease in both degrees of severity of the course of COVID-19 in the nonlinear parasympathetic CVI index.

Apparently, these shifts in COVID-19 are complex, and, first of all, can be caused by those disorders that arise in the central nervous system itself against the background of the direct or indirect action of the virus. In particular, J Matschke et al. [23] showed that in more than half of the patients who died from COVID-19, the virus is found in the brain tissue. At the same time, traces of SARS-CoV-2 were detected in the cells of the brain stem and neurons extending from it. The most common finding is pronounced neuroinflammatory changes in the brain stem. Apparently, the virus can enter the central nervous system through the vagus nerve. In all patients, to one degree or another, the authors observed astrogliosis - an abnormal proliferation of astrocytes.

According to N Krishna et al. [20], in addition to respiratory and systemic damage to many organs and systems of the body, COVID-19 is characterized by a wide range of neurological manifestations (encephalitis, meningitis, myelitis, acute disseminated encephalomyelitis, metabolic and acute hemorrhagic necrotizing encephalopathy, cerebrovascular diseases, Guillain-Barré syndrome cranialis, vegetative dystonia and myopathy). These facts alone may indicate that the revealed changes in the balance of the sympathetic and parasympathetic divisions of the ANS may be organic in nature [9].

An essential feature of the course of the new coronavirus infection, which differs from other acute respiratory diseases, is its protracted course [12]. In the case of severe forms involving the lower respiratory tract, pathological processes can last for several weeks [16]. In these conditions of the protracted course of COVID-19 with periods of progression and relief of systemic inflammation, the body's defense systems are trying to adapt. However, in the case of a discrepancy between the regulatory capabilities of the prevalence of cell alteration, a breakdown of protective programs and adaptation processes is possible [24]. In this case, there is a decompensation of the disease and the formation of multiple organ failure. It was found that adaptation disorders are more often recorded in conditions of a low functional reserve of protective systems against the background of immunopathies and metabolic disorders of various origins [24].



The survivors vs the dead
Sensitivity (%)
Specificity (%)

The use of ROC analysis of HRV did not allow us to identify predictors of the outcome of COVID-19. Of all the studied indicators, only HR corresponded to the required significance (76.5% [63.8-89.2]), which should be regarded as an acceptable result (Fig. 1). Perhaps the low predictive value of HRV is due to the fact that the studies were carried out once.

Currently, there is no doubt that COVID-19 is a systemic disease that, in severe stages, affects all organs and systems of our body, without exception, and leads to the development of multiple organ failure. Systemic endothelial dysfunction, which develops in COVID-19, and is accompanied by a "cytokine storm", largely determines the disorders that occur in the hemostatic system [3]. It should be noted that increased aggregation of blood cells to the damaged endothelium, pronounced expression of tissue factor on the endothelium of blood vessels, heart, alveocytes and other organs and tissues, impaired fibrinolytic activity of blood, accompanied by an increase in the concentration of D-dimer, a drop in the level of natural anticoagulants, ultimately leads to the development of thrombotic micro- and macroangiopathy, which cannot but affect the damage and dysfunction of the receptor apparatus [13, 27] and, therefore, play a significant role in the imbalance of the ANS activity.

Finally, another assumption suggests itself. Of course, the nervous system cannot stand aside with such significant changes in the body that arise as a re-

sult of cell contamination with the SARS-CoV-2 virus [9]. Already with moderate forms of the course of COVID-19, both the sympathetic and parasympathetic divisions of the ANS are working at the limit. But at the same time, as our data show, the homeostatic balance will change, due to which they move to a completely new stage of interaction, which partly allows the body to adapt to significantly changed conditions of existence. It is possible that these shifts are of a temporary compensatory nature. At the same time, in severe patients, these protective adaptive shifts to ensure homeostatic reactions are clearly not enough, which ultimately leads to an imbalance in the ANS activity, thrombosis and multiple organ failure, often ending in death.

It is very likely that the deviations in the balance of sympathetic and parasympathetic influences revealed in this study, as well as a decrease in their general activity, may be early predictors of depletion of the central link in the regulation of the functioning of defense mechanisms. And the subsequently recorded pronounced deviations of the immune-inflammatory response, changes in the hemostasis and fibrinolysis systems, disturbances in ventilation-perfusion coupling in the lungs have a character dependent on the state of global dysregulation of adaptive programs [2].

Finally, it should be noted that the ROC analysis of HRV indicators failed to identify predictors of the severity of COVID-19. It is possible that failures in this direction are due to the fact that the analysis was carried out once.

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ARCTIC MEDICINE

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SEARCH FOR THE ASSOCIATION OF THE A1166C POLYMORPHIC MARKER OF THE *AGTR1* (RS5186) GENE WITH ESSENTIAL HYPERTENSION IN INDIGENOUS ETHNIC GROUPS OF YAKUTIA

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The aim of case-control study was to assess the possible association between angiotensin II type 1 receptor *(A1166C)* gene polymorphism and essential hypertension in the indigenous population of Yakutia (Yakuts-56, Chukchi-34, Yukagirs-77, Evens-184). 168 subjects with the essential hypertension (cases) were compared to 179 normotensive subjects (controls). The frequency of the C allele was 17.9 and 19.4% among cases and controls, respectively (p = 0.600). No association was found between the angiotensin II type 1 receptor *(A1166C)* gene polymorphism and essential hypertension in the study population.

Keywords: essential hypertension, genotype, *AGTR1* gene, A1166C, rs5186, Yakutia, risk factors.

Arterial hypertension (AH) is a common risk factor for chronic noncommunicable diseases, contributing significantly to the risk of mortality and disability from its complications. Essential hypertension makes up to 95% of all cases of hypertension. Epidemiological indicators of the prevalence and effectiveness of hypertension treatment depend not only on concomitant conditions and diseases, but also on the genetic characteristics of populations. The renin-angiotensin system plays an important role in the regulation of blood pressure and electrolyte homeostasis. In studies of genes of candidates for predisposition to essential hypertension, genes whose products provide individual biochemical links of the renin-angiotensin system were actively studied. Angiotensin II implements its biological effects through two types of receptors: type 1 (AT1R) and type 2. The AGTR1 gene encodes a type 1 angiotensin II receptor protein. The most studied SNP is rs5186, known as A1166C, which is located in the 3'-untranslated region of the type 1 angiotensin II receptor gene AGTR1. At position 1166, adenine (A) is replaced by cytosine (C), which alters the regulation of gene expression. In some populations, the link between being a carrier of the C allele rs5186 and an increased risk of developing essential hypertension has been demonstrated [7, 13].

The Republic of Sakha (Yakutia) is a region where extreme climatic factors have a depleting effect on the functional reserves of the human body. Stresses of adaptive mechanisms often manifest itself in the form of an increase in blood pressure. Changes in diet and physical activity have led to widespread overweight and obesity among indigenous populations of the North, which also contribute to higher blood pressure levels [1]. Under these conditions, the search for genetic markers of predisposition to the development of hypertension is of both scientific and practical interest.

The aim of the study was to research the distribution of alleles and genotypes of the A1166C polymorphic markers of the *AGTR1* gene (rs5186) and their link with essential hypertension in the group of representatives of the indigenous ethnic groups of Yakutia.

Materials and methods. A single-stage epidemiological study of the population of Nizhnekolymsky and Tomponsky districts of the Republic of Sakha (Yakutia) was carried out. The "case" and "control" groups were formed. A total of 351 participants (228 women and 123 men) were examined, including 56 Yakuts, 34 Chukchi, 77 Yukagirs and 184 Evens. The average age was 45.9 ± 12.5 years. The study was approved by the local ethical committee of the Yakut Science Centre of Complex Medical Problems. All participants were informed and have signed a voluntary informed consent for participation in the study and for blood sampling. Determination of nationality was carried out on the basis of self-identification of the participants.

The criteria for inclusion in the group of "cases" (AH +) include: belonging to an indigenous ethnic group of Yakutia (Yakuts, Evens, Chukchi, Yukagirs), being 18 years and older, and presence of hypertension at any stage. The group of "controls" (AH -) included persons without AH, representatives of the indigenous ethnic groups of Yakutia at the age of 18 and older. Exclusion criteria: belonging to a non-indigenous nationality, symptomatic arterial hypertension according to medical history and outpatient records.

The research program included the following sections: a questionnaire for objective assessment of state; blood pressure measurement, survey by a cardiologist, blood sampling from the cubital vein in the morning on an empty stomach with 12-hour abstinence from food, measurement of the waist circumference (WC) in cm was carried out below the chest above the navel, in the middle of the distance between the lower lateral edge of the ribs and the apex of the ridge the ilium (NIH, 1998); the circumference of the thighs at the level of the buttocks.

The abdominal obesity (AO) is exposed to the value of the waist measurement (WM) ≥ 80 cm on women, ≥94 cm on (VNOK, 2009).

Blood pressure (BP) was measured twice with an OMRON M2 Basic automatic tonometer (Japan) in a sitting position with calculation of average blood pressure with a margin of permissible measurement error of ± 3 mm Hg (ESH/ESC, 2013). Hypertension is present at the 140/90 mmHg or taking antihyperten-

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sive drugs during the study (2017 ACC/AHA Guideline). Out of 172 people who met these criteria, 4 people were excluded, whose condition was assessed as "secondary arterial hypertension". Thus, the “cases” group was represented by 168 participants, the “control” group by 179 normotensive individuals.

Laboratory methods of the research included analysis of total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL Cholesterol), low-density lipoprotein cholesterol (LDL Cholesterol), very low-density lipoprotein cholesterol (VHDL Cholesterol), levels glucose. The genetic study included the identification of the polymorphic marker A1166C of the *AGTR1* gene (rs5186).

When judging the incidence of disorders of the blood lipid profile in a population, we used the Russian recommendations of the VII revision of Society of cardiology of Russian Federation, 2020, into account the European recommendations, 2019. Hypercholesterolemia (HCS) is the level of TC ≥ 5,0 mmol/l (190 mg/dl) taking into account the risk of cardiovascular death on the SCORE scale, the high LDL Cholesterol level >3,0 mmol/l (115 mg/dl) with low, > 2.6 mmol/l with moderate, >1,8 mmol/l with high, > 1,4 mmol/l with very high and extreme risk, the low HDL Cholesterol level <1,0 mmol/l on men; <1,2 mmol/l on women, the hypertriglyceridemia (HTG) is the TG level is >1,7 mmol/l. The atherogenic index (IA) was determined by the formula: IA (cu) = (TC - HDL Cholesterol) / HDL Cholesterol (Klimov A.N., Nikulcheva N.G., 1999). A hyperglycemia (HG) on an empty stomach (a glucose in a blood plasma on an empty stomach > 5,6 mmol/l). Respondents with these disorders also included participants receiving specific medication for these conditions.

Genomic DNA was isolated from peripheral blood leukocytes by the method of phenol-chloroform extraction. Genotyping was carried out by means of sets (LLC NPF Litekh, Moscow) according to the manufacturing company instruction on “Real-time CFX96 amplifier” ("BioRad", the USA). For quality control, 10% of randomly selected samples were subjected to repeated genotyping.

The verification of the correspondence of the distribution of genotypes to the Hardy-Weinberg equilibrium law was carried out using an online calculator at https://wpcalc.com/en/ equilibrium-hardy-weinberg. Statistical analysis of the data was carried out using the SPSS STATISTICS 22 package. The frequencies of alleles and genotypes are presented with 95% confidence intervals (95% CI). When comparing groups depending on the number of groups and data type, the Mann-Whitney, Kruskal-Wallis, Pearson $\chi 2$ tests were used. The odds ratio (OR) was calculated with a 95% CI. The statistical significance of the differences (p) was taken equal to 5%.

Results and discussion. The distribution of genotype frequencies of the polymorphic marker A1166C of the *AGTR1* gene (rs5186) in the groups of Yakuts, Evens, and Yukagirs corresponded to the Hardy-Weinberg equilibrium. In the Chukchi group, which was represented by 34 participants, the distribution differed from the equilibrium (Table 1). The frequency of detection of the C allele varied from 0.13 in the Evens to 0.35 in the Chukchi. According to the ALFA (Allele Frequency Aggregator) project, the frequency of C allele carriage averages 0.28 (n = 238604), varying depending on population: from 0.009 among Africans (n = 354) to 0.30 among Hispanics with predominantly European and Native American origin (n = 6874). Among the populations of Southeast Asia, the prevalence of the C allele is 0.08–0.09 [8]. Thus, according to the presented study, the frequency of the C allele in the indigenous ethnic groups of Yakutia is, on average, higher than in the population of Southeast Asia and Africa.

Comparative analysis of the distribution of alleles and genotypes of the A1166C polymorphic marker of the *AGTR1* gene (rs5186) in the groups of cases and controls did not show statistically significant differences between the groups (Table 2). Thus, the study did not reveal a link between the studied polymorphic marker and the frequency of essential hypertension in groups of representatives of the population of the North. In academic literature, information on the link between the polymorphic marker A1166C of the *AGTR1* gene (rs5186) and essential hypertension is contradictory [2, 6, 7, 10, 12, 13]. In China, when comparing three genetically different populations with significant differences in the prevalence of essential hypertension, it was suggested that allele A may be a predisposing factor for essential hypertension in Tibetan men, while no association

Table 1

Distribution of alleles and genotypes of the polymorphic marker A1166C of the *AGTR1* gene (rs5186) in the indigenous ethnic groups of Yakutia

Allele/Genotype	Indicator	Yakuts n=56	Evens n=184	Chukchi n=34	Yukagirs n=77	All groups n=351
A	Total	90	321	44	116	571
	Frequency (95% CI)	80.4 (71.5-87.2)	87.2 (83.3-90.4)	64.7 (51.9-75.9)	80.6 (72.9-86.6)	81.3 (78.3-84.1)
C	Total	22	47	24	38	131
	Frequency (95% CI)	19.6 (12.8-28.5)	12.8 (9.6-16.7)	35.3 (24.1-48.1)	26.4 (19.5-34.5)	18.7 (15.9-21.7)
AA	Total	36	140	10	43	229
	Frequency (95% CI)	64.3 (50.0-76.7)	76.1 (69.2-81.9)	29.4 (14.5-48.5)	55.8 (44.0-67.1)	65.2 (60.0-70.1)
AC	Total	18	41	24	30	113
	Frequency (95% CI)	32.1 (20.2-46.4)	22.3 (16.6-29.1)	70.6 (51.5-85.5)	38.9 (28.1-50.9)	32.2 (27.4-37.3)
CC	Total	2	3	0	4	9
	Frequency (95% CI)	3.6 (0-14.3)	1.6 (0-5.3)	0	5.2 (0.7-14.0)	2.6 (1.1-5.0)
$\chi 2$ to the Hardy-Weinberg		0.019	8.08	10.1	0.179	1.284
p		0.892	0.99	0.001	0.673	0.257

Note. Table 1-3 p - the achieved level of significance; 95% CI - 95% confidence interval.

was found in the other two populations [2]. In a case-control study conducted in Poland (250 people with stable essential hypertension and 150 people with normal blood pressure), allele C and CC genotype were statistically significantly more frequent in patients with hypertension [12]. In a similar study conducted in India, individuals with CC genotypes were 2.4 times more likely to develop essential hypertension (p = 0.0001) than individuals with AC and AA genotypes [6]. At the same time, in the study by Suita, conducted in Japan, involving 1492 patients with hypertension and 2426 normotensive individuals, no association was found between the A1166C variants of the *AGTR1* gene and hypertension [10]. Researchers who studied similar groups in Tunisia came to the same results [3].

References contain information on the link between the polymorphic marker A1166C of the *AGTR1* gene and non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, liver fibrosis, dyslipidemia, insulin resistance and metabolic syndrome [4, 5, 9, 11]. In light of this data, in further analysis, the groups of carriers of alleles A and C, with different rs5186 genotypes, were compared in terms of the level of metabolic parameters and the frequency of its disorders (Table 3). Taking into account the fact that these parameters depend on the age and sex of the surveyed, a comparison of the groups by these indicators was carried out. The analysis showed that there were no differences in the age of the subjects, both between carriers of different alleles (p = 0.987) and different genotypes (p = 0.576). There were also no differences in the distribution of individual alleles (p = 0.786) and genotypes (p = 0.960) in men and women. Thus, the groups were comparable in terms of age and gender structure. The analysis did not reveal differences in blood pressure levels between carriers of different genotypes. It should be noted that systolic (SBP) and diastolic (DBP) levels were compared for all study participants, including those taking antihypertensive drugs, which could change the results of the assessment. Carriers of allele A were characterized by a statistically significantly larger waist circumference, lower serum HDL cholesterol levels, and high values of the atherogenic index (Table 3). These differences persisted when the group was divided depending on the genotype, including when the carriers of the AC and CC genotypes were combined.

When studying the link between the alleles and genotypes of polymorphic marker A1166C of the *AGTR1* gene (rs5186) with the frequency of metabolic disorders, statistically significant differences were obtained only in relation to hypo-alpha-cholesterolemia. Thus, the frequency of decreased levels of HDL cholesterol was 38.1% in carriers of the A allele versus 27.7% in carriers of the C allele (p = 0.026). When comparing carriers of different genotypes in terms of the frequency of hypo-alpha-cholesterolemia, the indicators were: AA genotype - 40.5%, AC - 28.6%, CC - 22.2%, respectively (p = 0.005).

Taking into account the data obtained, an analysis of the strength and direction of the link between the content of HDL cholesterol and certain metabolic parameters was carried out. The variable that can distort the reflection of these links may be the age of the surveyed. The concentration of HDL cholesterol did not correlate with the age of the subjects (r = -0.07, p = 0.076). No correlation was found between the content of HDL cholesterol and the levels of SBP (r = -0.06, p = 0.113), DBP (r = -0.09, p = 0.013), glucose (r = -0.07, p = 0.069), LDL cholesterol (r = -0.03, p = 0.412). A negative correlation was found with WC (r = -0.26, p <0.001) and TG content (r = -0.58, p <0.001). It is possible that the link discovered between carrying the *AGTR1* allele A and the level of HDL cholesterol is due to the association between waist circumference and HDL cholesterol. To test this assumption, we compared the levels of HDL cholesterol for different genotypes in groups divided by the presence of abdominal obesity. Nonparametric analysis of variance revealed no statistically significant differences in the group of persons with normal waist circumference (p = 0.180). Thus, it should be assumed that the revealed differences in the levels of HDL cholesterol in carriers of different genotypes and alleles of the A1166C polymorphic marker of the *AGTR1* gene are due to differences in WC.

Conclusion. Thus, the results of the study in the group of representatives of the indigenous ethnic groups of Yakutia did not reveal an association of

Table 2

Distribution of alleles and genotypes of the polymorphic marker A1166C of the *AGTR1* gene (rs5186) in hypertensive and normotensive individuals

Ethnos	Group	Allele/Genotype, n (%)			OR (95% CI), p
		A	**C**		
Yakuts	AH-	31 (77.5)	9 (22.5)		0.76 (0.29-1.97) p=0.750
	AH+	59 (81.9)	13 (18.1)		
Evens	AH-	176 (87.1)	26 (12.9)		0.98 (0.53-1.8) p=1.0
	AH+	145 (87.3)	21 (12.7)		
Chukchi	AH-	29 (65.9)	15 (34.1)		1.16 (0.41-3.27) p=0.988
	AH+	15 (62.5)	9 (37.5)		
Yukagirs	AH-	59 (73.8)	21 (2.2)		0.84 (0.40-1.75) p=0.776
	AH+	57 (77.0)	17 (23.0)		
All groups	AH-	295 (80.6)	71 (19.4)		0.90 (0.62-1.32) p=0.600
	AH+	276 (82.1)	60 (17.9)		
		AA	**AC**	**CC**	
Yakuts	AH-	12 (60)	7 (35.0)	1 (5.0)	AA and AC 0.79 (0.24-2.54). p=0.920 AA and CC 0.50 (0.03-8.71) p=1.0 AA and AC+CC 0.75 (0.24-2.33) p=0.835
	AH+	24 (66.7)	11 (30.6)	1 (2.8)	
Evens	AH-	77 (76.2)	22 (21.8)	2 (2.0)	AA and AC 1.06 (0.53-2.12) p=1.0 AA and CC 0.61 (0.05-6.89) p=1.0 AA and AC+CC 1.02 (0.52-2.0) p=1.0
	AH+	63 (75.9)	19 (22.9)	1 (1.2)	
Chukchi	AH-	7 (31.8)	15 (68.2)		AA and AC 1.40 (0.29-6.83) p=0.982
	AH+	3 (25.0)	9 (72.0)		
Yukagirs	AH-	22 (55.0)	15 (37.5)	3 (7.5)	AA and AC 1.05 (0.41-2.66) p=0.922 AA and CC 0.35 (0.03-3.63) p=0.696 AA and AC+CC 0.93 (0.38-2.29) p=1.0
	AH+	21 (56.8)	15 (40.5)	1 (2.7)	
All groups	AH-	118 (64.5)	59 (32.2)	6 (3.3)	AA and AC 0.97 (0.62-1.55) p=0.997 AA and CC 0.53 (0.13-2.18) p=0.581 AA and AC+CC 0.93 (0.60-1.45) p=0.841
	AH+	111 (66.1)	54 (32.1)	3 (1.8)	

Note. OR - odds ratio; AH + – the presence of essential hypertension, AH- – persons with normal blood pressure.

Table 3

Comparison of age and metabolic parameters in carriers of different alleles and genotypes of the polymorphic marker A1166C of the *AGTR1* gene (rs5186)

Indicator	Me (Q_1-Q_3)			p
Allele				
	A		**C**	
Age, years	48.0 (36.0-55.0)		47.0 (35.0-55.0)	0.987
WC, cm	88.0 (78.0-98.0)		83.0 (35.0-98.0)	**0.044**
SBP mm Hg	130.0 (120.0-150.0)		130.0 (35.0-150.0)	0.337
DBP, mm Hg	80.0 (80.0-90.0)		80.0 (35.0-90.0)	0.347
Glucose (mmol/l)	4.4 (3.9-5.0)		4.2 (35.9-5.0)	0.099
TG (mmol/l)	1.0 (0.7-1.4)		0.9 (35.7-1.4)	0.129
TC (mmol/l)	4.9 (4.4-5.5)		4.9 (35.4-5.5)	0.385
HDL Cholesterol (mmol/l)	1.2 (1.0-1.5)		1.4 (35.0-1.5)	**0.011**
LDL Cholesterol (mmol/l)	3.2 (2.7-3.7)		3.0 (35.7-3.7)	0.122
VHDL Cholesterol (mmol/l)	0.4 (0.3-0.6)		0.4 (35.3-0.6)	0.069
IA (cu)	2.9 (2.2-3.8)		2.7 (35.2-3.8)	**0.014**
Genotype				
	AA	**AC**	**CC**	
Age, years	47.0 (35.0-55.0)	48.0 (37.8-55.0)	33.0 (29.5-58.5)	0.576
WC, cm	88.5 (78.3-98.0)	86.0 (37.0-98.0)	81.0 (75.3-82.0)	**0.032**
SBP mm Hg	130.0 (120.0-150.0)	130.0 (37.0-150.0)	120.0 (117.5-145.0)	0.565
DBP, mm Hg	80.0 (80.0-90.0)	80.0 (37.0-90.0)	80.0 (77.5-90.0)	0.569
Glucose (mmol/l)	4.5 (4.0-5.0)	4.3 (37.8-5.0)	3.8 (3.3-4.4)	0.121
TG (mmol/l)	1.0 (0.7-1.4)	0.9 (37.7-1.4)	0.9 (0.7-1.1)	0.295
TC (mmol/l)	4.9 (4.4-5.5)	4.9 (37.3-5.5)	4.9 (4.1-5.5)	0.669
HDL Cholesterol (mmol/l)	1.2 (1.0-1.5)	1.3 (37.1-1.5)	1.4 (1.2-1.6)	**0.027**
LDL Cholesterol (mmol/l)	3.2 (2.7-3.7)	3.0 (37.6-3.7)	3.1 (2.6-3.6)	0.261
VHDL Cholesterol (mmol/l)	0.4 (0.3-0.7)	0.4 (37.3-0.7)	0.4 (0.3-0.5)	0.170
IA (cu)	3.0 (2.2-4.0)	2.7 (37.0-4.0)	2.6 (2.0-3.5)	**0.034**
	AA		**AC+CC**	
Age, years	47.0 (35.0-55.0)		47.5 (36.8-55.0)	0.750
WC, cm	88.5 (78.3-98.0)		84.5 (77.0-98.0)	0.127
SBP mm Hg	130.0 (120.0-150.0)		130.0 (120.0-150.0)	0.405
DBP, mm Hg	80.0 (80.0-90.0)		80.0 (80.0-90.0)	0.292
Glucose (mmol/l)	4.5 (4.0-5.0)		4.2 (3.8-5.0)	0.194
TG (mmol/l)	1.0 (0.7-1.4)		0.9 (0.7-1.4)	0.134
TC (mmol/l)	4.9 (4.4-5.5)		4.9 (4.2-5.5)	0.394
HDL Cholesterol (mmol/l)	1.2 (1.0-1.5)		1.3 (1.1-1.5)	**0.007**
LDL Cholesterol (mmol/l)	3.2 (2.7-3.7)		3.0 (2.6-3.7)	0.102
VHDL Cholesterol (mmol/l)	0.4 (0.3-0.7)		0.4 (0.3-0.7)	0.065
IA (cu)	3.0 (2.2-4.0)		2.7 (2.0-4.0)	**0.010**

Note. Me (Q1-Q3) - median and interquartile range.

the A1166C polymorphic marker of the *AGTR1* gene (rs5186) with essential hypertension. The limitations of the study were: the small number of groups, the inability of conducting a full, comprehensive examination of the participants to exclude the secondary nature of hypertension. A positive aspect of the research was the usage of controls from the same population, in the same time period. In further studies, an additional verification of the studied link is possible by using the hospital population as" cases", excluding the secondary nature of hypertension.

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INFLUENCE OF NORTHERN EXPERIENCE ON THE FREQUENCY OF METABOLIC SYNDROME AND ITS COMPONENTS AMONG MIGRANTS OF THE FAR NORTH AFTER MOVING TO NEW CLIMATIC AND GEOGRAPHIC LIVING CONDITIONS

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The article presents the findings devoted to the research on the influence of northern experience on the frequency of metabolic syndrome in migrants of the Far North after moving to new climatic and geographic living conditions. The highest frequency of metabolic syndrome among migrants was observed in the group with periods of residence in the Far North from 20 to 29 years. In all three groups of migrants, the three-component combination of metabolic syndrome was more common in comparison with other combinations. About a third of patients in all study groups had a combination of abdominal obesity, arterial hypertension and high levels of low-density lipoprotein cholesterol.

Keywords: migrants, the Far North, arterial hypertension, metabolic syndrome.

Introduction. Special attention paid recently to the problem of MetS is associated with its high prevalence among the population of economically developed countries and its unconditional role in the progression of cardiovascular pathology [4, 5, 8, 19]. The prevalence of MetS varies significantly in different populations from 10% to 84%, averaging 20-25% [4, 5, 8, 9, 10, 19]. According to various studies, the prevalence of MetS among the adult population of Russia varies from 7 to 50% [6, 7, 9, 10, 11, 16]. In large cities of Russia, the prevalence of a combination of 3 or more criteria for this condition reaches 40,3% - 50,5% [10, 11, 12, 16].

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It is known that the nature of human metabolism has pronounced regional differences that affect the predominance of certain pathogenetic mechanisms of MetS development and determine the features of its component structure [1, 4, 10]. This problem is particularly relevant in the harsh conditions of Siberia and the Far North [2, 3, 6, 7, 11, 13, 15, 18]. Under the influence of climatoecological and socio-economic factors characteristic of high latitudes, changes in metabolism occur in the human body, which are interrelated with the restructuring of mechanisms that maintain homeostasis [1, 2, 14]. Important reasons for the accelerated formation of cardiometabolic risk factors, which are components of MetS, in the alien population of the Far North are contradictions between the existing lifestyle and the nutrition stereotype, on the one hand, and pronounced metabolic changes caused by extreme environmental conditions, on the other [2]. This theory is confirmed by the results of studies indicating a high prevalence of MetS among the alien population of the northern regions [2, 3, 12, 16]. The regional features of the structure of MetS components identified in the course of the conducted studies may be due to significant differences in environmental effects on the human body [1, 2].

In the Far North, extreme climatic-meteorological and heliogeophysical factors come to the fore, making the body more demanding. This, in turn, leads to a specific restructuring of metabolism, pronounced stress and more rapid depletion of functional reserves of adaptive systems with an increase in «oxidative stress» due to the processes of hormonal and metabolic adaptation to the factors of the North [1, 14].

The aim. To study the influence of the northern experience on the frequency of metabolic syndrome in migrants of the Far North after moving to new climatic and geographical living conditions.

Materials and methods of the research. We examined 267 patients of the immigrant population (Caucasians) of both sexes with AH II - III stages (according to the recommendations of ESH / ESC, 2018) [17], who previously lived in the Far North (Norilsk, latitude: 69 ° 21.21 ′ N).) and moved to a permanent place of residence in Central Siberia (Krasnoyarsk, latitude: 56 ° 1.1034 ′ N), median age – 64,0 [59,0; 73,0] years. Migrants from the Far North divided into groups depending on the length of time they lived in the Far North (northern experience): first group – duration of residence from 10 to 19 years, second group – with duration of residence from 20 to 29 years, and third group - with a duration of residence of more than 30 years. The comparison group consisted of 267 patients with hypertension of the same age range-65,0 [59,0; 74,0] years (p=0,454), permanently residing in Krasnoyarsk. The study conducted in accordance with the ethical principles of conducting research with human participation of the Helsinki Declaration of the World Medical Association (Declaration of Helsinki), revised in 2013. All patients gave written informed consent.

To assess the frequency of MetS, the following definitions used ATP III (2005), IDF (2005), JIS (2009) and the recommendations of experts of the All-Russian Scientific Society of Cardiology (Russian criteria) in 2009. To analyze the frequency of combinations of three, four and

five components of MetS in the studied groups, the Russian criteria (2009) selected. According to Russian experts, the MetS criteria adopted in 2007 and corrected in 2009 are quite justified and correct, taking into account their pathogenesis, as well as the peculiarities of the healthcare system [4].

Statistical processing of the results of the study carried out using the Statistica 6.1 software package №. EXXR202F-256520FAN10 (StatSoft, USA). The obtained data are presented in the form of a median (Me) and interquartile interval [Q_1;Q_3]. Two independent groups compared using the Mann-Whitney U-test. The analysis of the frequency difference in two independent groups carried out using the χ^2 criterion with the Yates correction. The differences were considered statistically significant at p<0,05.

Results and discussion. In the course of the study, it was found that the highest frequency of MetS among migrants was observed according to the Russian criteria –76,0%, compared with similar indicators according to the criteria of the IDF – 58,1% (p=0,001), according to ATP III – 59,6% (p=0,001) and JIS – 65,5% (p=0,008) (Table 1).

Similar patterns were noted in the group of permanent residents in Krasnoyarsk: according to the Russian criteria – 65,2% compared to the IDF-51,3% (p=0,001), ATP III – 49,8% (p=0,001) and JIS-58,1% (p=0,091), respectively. At the same time, among migrants, the frequency of MetS according to the Russian criteria (p=0,005) and JIS (p=0,024) was higher in comparison with residents of Krasnoyarsk.

The obtained data on the frequency of MetS are consistent with the results of research by Romanova A. N. et al. (2011). It shown that in the non-indigenous population of Yakutia, men and women with coronary atherosclerosis, the frequency of MetS ranged from 26,6% to 68,8% according to the ATP III criteria and according to the Russian criteria from 61,1% to 96,9% [12].

It found that the most frequent variant of MetS in both groups was three-component (Fig. 1). There were statistically significant differences in frequency between three and five (p=0,001) and four and five (p=0,009) component variants of MetS among migrants.

In a comparative analysis among permanent residents in Krasnoyarsk, significant differences were found only between three and five (p=0,005) component variants of MetS.

The results of the study showed (Table 2) that among migrants and residents of Krasnoyarsk, the MS phenotype consisting of abdominal obesity (AO), AH and lipid disorders, which is characterized by a high level of low-density lipoprotein cholesterol (elevated LDL–C), is more common. The frequency of this combination in these groups was 26% among migrants and 27% among residents of Krasnoyarsk, respectively.

Next in frequency after the MetS variant with the above components among migrants was the variant consisting of four components: AO, AH, elevated LDL-C and lipid disorders characterized by a reduced level of high – density lipoprotein cholesterol (reduced HDL-C) – 16%. Among the residents of Krasnoyarsk, the second most common combination was also consisting of four components-AO, AH, elevated LDL-C and elevated triglycerides – 16%.

The third most frequent combination in both groups was a combination consisting of five components-AO, AH, reduced HDL-C, elevated LDL-C s and elevated triglycerides – 11%. Thus, among the examined groups, the most common combinations were those including AO, AH and lipid metabolism disorders.

Statistically significant differences revealed between migrants and residents of Krasnoyarsk in the frequency of MetS variants with the following combinations of components AO, AH, impaired glucose tolerance (IGT), elevated triglycerides; AO, AH, reduced HDL-C, elevated LDL-C; AO, AH, IGT, elevated triglycerides, elevated LDL-C and AO, AH, IGT, reduced HDL-C, elevated LDL-C. The frequency of other combinations of MetS components did not exceed 10% in all the studied groups.

The results obtained on the frequency of various MetS phenotypes in migrants

Table 1

The frequency of metabolic syndrome, taking into account various criteria in the examined migrants and residents of Krasnoyarsk

Diagnostic criteria metabolic syndrome	Migrants	Krasnoyarsk	*p*
1. Russian criteria (2009)	203 (76.0)	174 (65.2)	**p=0.005**
2. IDF (2005)	155 (58.1)	137 (51.3)	p=0.118
3. ATP III (2005)	159 (59.6)	133 (49.8)	**p=0.024**
4. JIS (2009)	175 (65.5)	155 (58.1)	p=0.075
p	**p_{1-2}=0.001**; **p_{1-3}=0.001**; **p_{1-4}=0.008**; p_{2-3}=0.725; p_{2-4}=0.075; p_{3-4}=0.153	**p_{1-2}=0.001**; **p_{1-3}=0.001**; p_{1-4}=0.091; p_{2-3}=0.729; p_{2-4}=0.118; p_{3-4}=0.056	

Table 2

Frequency of various combinations of metabolic syndrome components according to the Russian criteria (2009) in the examined migrants and residents of Krasnoyarsk

Combinations of metabolic syndrome components	Migrants	Krasnoyarsk	*p*
AO, AH, IGT	4 (2)	8 (5%)	p=0.147
AO, AH, elevated TG	12 (6)	10 (6)	p=0.946
AO, AH, reduced HDL-C	12 (6)	6 (3)	p=0.264
AO, AH, elevated LDL-C	53 (26)	47 (27)	p=0.506
AO, AH, IGT, elevated TG	1 (0)	6 (3)	p=**0.034**
AO, AH, IGT, reduced HDL-C	6 (3)	4 (2)	p=0.692
AO, AH, IGT, elevated LDL-C	8 (4)	6 (3)	p=0.801
AO, AH, elevated TG, reduced HDL-C	4 (2)	5 (3)	p=0.567
AO, AH, elevated TG, elevated LDL-C	21 (10)	28 (16)	p=0.098
AO, AH, reduced HDL-C, elevated LDL-C	33 (16)	8 (5)	p=**0.001**
AO, AH, IGT, elevated TG, reduced HDL-C	2 (1)	6 (3)	p=0.098
AO, AH, IGT, elevated TG, elevated LDL-C	4 (2)	16 (9)	p=**0.002**
AO, AH, IGT, reduced HDL-C, elevated LDL-C	10 (5)	0 (0)	p=**0.003**
AO, AH, elevated TG, reduced HDL-C, elevated LDL-C	22 (11)	19 (11)	p=0.980
AO, AH, IGT, elevated TG, reduced HDL-C, elevated LDL-C	11 (5)	5 (3)	p=0.222

Note: AO – abdominal obesity; AH – arterial hypertension; IGT – impaired glucose tolerance; elevated TG - elevated triglyceride; reduced HDL-C – lipid disorders characterized by low levels of high-density lipoprotein cholesterol; elevated LDL-C – lipid disorders characterized by high levels of low-density lipoprotein cholesterol.

are consistent with the data of previous studies [3, 11, 12, 16]. According to the results of the above-mentioned study by Romanova A. N. et al. (2011) the most common MetS phenotype in all surveyed men and women using different criteria for determining MetS is a combination of AO with AH and dyslipidemia (non-indigenous – from 31,8 to 75 % in men and from 28 to 81,8 % in women) [12]. According to another study, MetS in the form of a combination of AO, AH and dyslipidemia in patients with verified coronary atherosclerosis among the non-indigenous population permanently residing in Yakutia was manifested in 70,8% of cases [16].

Established that highest frequency of MetS among migrants and residents of Krasnoyarsk noted according to the criteria of Russian experts. The most frequent variant of MetS in both groups was a three-component one. More often in both groups there was a combination of such components of MetS as AO, AH and lipid metabolism disorders.

In addition, at the level of trends among people who lived in the Far North from 10 to 19 years, the frequency of MetS according to the Russian criteria was higher than when using the definitions of MetS ATP III, IDF and JIS. Among migrants with long periods of residence in the North, the frequency of MetS according to the Russian criteria was higher. There were statistically significant differences in the 20-29-year-old age group between the frequency according to the Russian criteria with IDF, ATP III and JIS, and in the third training group, between the Russian criteria with IDF and ATP III.

The study revealed that the most frequent variant of MetS in all internship groups was three-component (Fig. 2). However, statistically significant differences in frequency were found only in the third internship group between three and five (p=0,001) and four and five (p=0,001) component variants of MetS. There were also statistically significant differences in the frequency of the five component combination between the second and third study groups (p=0,016).

The results of the study showed that among the migrants in all the internship groups, the most common combination of such components of MetS as AO, AH, elevated LDL-C, the frequency of which in the first group as a whole was 29%, in the second group – 29% and in the third group – 28%, respectively. Next in frequency after the MetS variant with the above components among migrants was the MetS variant consisting of four components: AO, AH, elevated LDL-C and reduced HDL-C. In the first group, the frequency of this combination was 24%, in the second group – 12% and in the third group – 20%. It should be noted that in the second group, 12% of the examined patients had a five-component combination (AO, AH, IGT, reduced HDL-C, elevated LDL-C) and 12% had a six-component combination (AO, AH, IGT, elevated triglycerides, reduced HDL-C, elevated LDL-C). The frequency of other combinations of MetS components did not exceed 10% in all the studied groups. The revealed differences had no statistical significance.

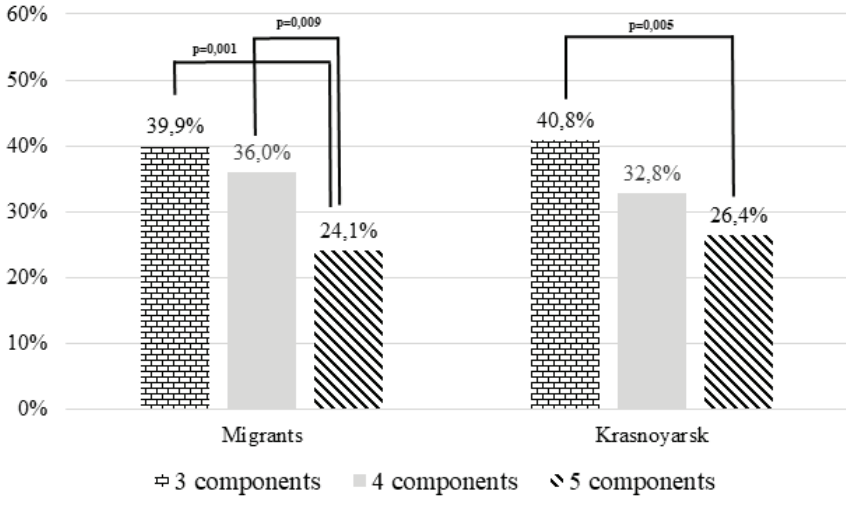

Fig. 1. Frequency of combinations of three, four and five components of metabolic syndrome according to the Russian criteria (2009) in the surveyed migrants and residents of Krasnoyarsk.

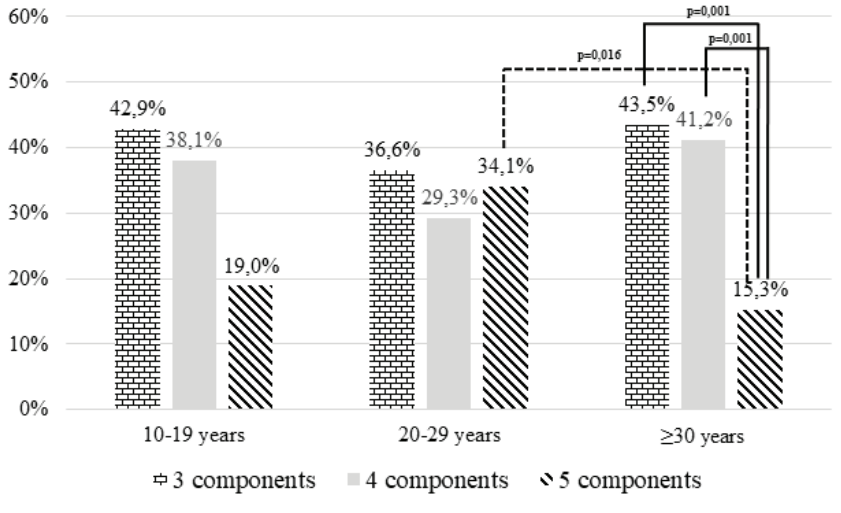

Fig. 2. The frequency of combinations of three, four and five components of metabolic syndrome according to the Russian criteria (2009) in the surveyed migrants, depending on the northern experience.

Summarizing the above, it should be noted that the highest frequency of MetS among migrants was observed in the group with periods of residence in the Far North from 20 to 29 years (from 72,4% to 95,3%), while, according to the Russian criteria, these indicators were the highest – 95,3%. In all three groups of migrants, the three-component combination of MetS was more common in comparison with other combinations. About a third of patients in all groups had a combination of MetS components of the type of AO, AH, elevated LDL-C.

According to Diaghileva V. B. (2013), MetS observed in 38,67% of permanent residents in the northern city and in 61.33% of shift workers, while the relationship between the increase in the frequency of MetS with the duration of residence in the northern city and work experience was established [2]. According to Nikolaev Yu. A. et al. (2012), with the experience of living in the Far North for 20-29 years, a phase of depletion of the body's adaptive reserves occurs,

which manifested in the accumulation of risk factors and a greater prevalence of MetS [1].

In conclusion, it should be noted that the results of studies devoted to the study of MetS in representatives of the indigenous and alien population living in extreme conditions of the Far North and Siberia are currently relevant [1, 2, 5, 9, 13, 17]. However, insufficient knowledge of this issue among migrants of the Far North living in new climatic and geographical conditions hinders the development and implementation of effective therapeutic and preventive measures to reduce the risk of cardiovascular complications in this category of the population. From the point of view of the formation of preventive strategies and medical examination of migrants of the Far North who have a high cardiometabolic risk, it is extremely important to know how the cardiovascular risk profile of these people will change after moving, at various stages of adaptogenesis.

Conclusion. According to the results of the study, it found that the highest frequency of MetS among migrants and residents of Krasnoyarsk noted according to the Russian criteria. The most frequent variant of MetS in both groups was a three-component one. More often in both groups there was a combination of such components of MetS as AO, AH and lipid metabolism disorders characterized by high levels of low – density lipoprotein cholesterol (elevated LDL-C), the frequency of which in these groups as a whole was 26% among migrants and 27% among residents of Krasnoyarsk, respectively.

The highest frequency of MetS among migrants observed in the group with the terms of residence in the Far North from 20 to 29 years (from 72,4% to 95,3%), while, according to the Russian criteria, these indicators were the highest – 95,3%. In all three groups of migrants, the three-component combination of MetS was more common in comparison with other combinations. About a third of patients in all groups had a combination of MetS components of the type of AO, AH, elevated LDL-C.

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SCIENTIFIC REVIEWS AND LECTURES

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CELL AND MOLECULAR MECHANISMS OF NEUROSTEROID ACTION IN DIFFERENT PARTS OF THE CENTRAL NERVOUS SYSTEM (Part 2)

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Neurosteroids (or neuroactive steroids) are a class of endogenous compounds which are synthesized in nervous tissue or\and able to modulate the brain functional activity, the class also includes steroids from gonads or adrenals, which are capable of migrating through the blood brain barrier to achieve their biological targets. The second part of the review is focused on describing multiply features of synthesis, functioning of such neurosteroids as progesterone, allopregnanolone, dehydroepiandrosterone and dehydroepiandrosterone sulfate as well as their targets, synthesized analogs. It also highlights the peculiarities of their production in different regions of rat central nervous system in ontogenical aspect. Many outstanding processes which depend on progesterone level were described: differentiation of oligodendrocytes precursors, changes in Schwann cells' activity, which lead to myelination intensification, stimulation of dendrite, motoneurons' growth - the basis of nervous tissue recovery; synaptogenesis in Purkinje neurons. Special emphasis was made on the modulation of GABAA receptors by allopregnanolone, anti-apoptotic and neuroprotective effects of dehydroepiandrosterone and dehydroepiandrosterone sulfate. Diverse and controversial data on changes in neurosteroid effects in dependency on the target's localization in the brain were systematized. Human and rat neurosteroid profiles were compared and contrasted in order to estimate the possibility of rat usage as a biological model in studies, which are focused on the correlation between changes in neurosteroid status and such diseases as epilepsy, schizophrenia, autism, anxiety, depression, posttraumatic stress disorder.

Keywords: neurosteroids, neurotransmitters, neuronal plasticity, progesterone, PROG, allopregnanolone, ALLO, dehydroepiandrosterone, DHEA, dehydroepiandrosterone sulfate, DHEAS, regions of central nervous system, ontogenesis.

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Abbreviations: ALLO – allopregnanolone; DHEA, DHEAS – dehydroepiandrosterone, dehydroepiandrosterone sulfate; PROG – progesterone; P450scc - cholesterol side-chain cleavage enzyme; P450c17 - steroid 17α-monooxygenase; ST – sulfotransferase; PKA – protein kinase A; AMPA – α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; BDNF – brain derived neurotrophic factor; GABAA – receptor of γ-aminobutyric acid type A; 3βHSD – 3β-hydroxysteroid dehydrogenase; NGF – nerve growth factor; NMDA – N-methyl-D-aspartate; PREG, PREGS – pregnenolone, pregnenolone sulfate; 5-HT-receptors – membrane 5-hydroxytryptamine or serotonin receptors; mIPSCs – miniature inhibitory postsynaptic currents; TSPO - translocator protein; LTP - long-term potentiation; MPA - medroxyprogesterone acetate; PXR - nuclear pregnane X receptor; MAP-2 - microtubule-associated protein 2.

Progesterone

Progesterone is derived from pregnenolone by the 3βHSD enzyme [20]. Maximum pregnenolone and progesterone levels in hippocampal tissue may be detected right after the birth and then their progressive decrease comes. Maximum enzyme's activity may be detected in ependymal cells of brain's ventriculars and in cerebellar Purkinje cells (0,717+\-0,37 c.u. and 0,400+\- 0,06 c.u. respectively). This level of activity is comparable to its level in cells of peripheral endocrine glands (zona fasciculata of adrenal glands, Leydig cells in testis [1]. Progesterone and 5α-dehydroprogesterone conduct their effects trough the progesterone nuclear receptor, whereas 3α-hydroxi-4-pregnen-20-on and allopregnanolone don't seem to have such a molecular mechanism. Moreover, 5α-dehydroprogesterone and allopregnanolone may interact with $GABA_A$ receptors, effect that progesterone lacks [7,15], in other words, the intracellular presence of 5α-reductase and 3α-hydroxysteroid-dehydrogenase may result in genesis of extra neuromodulators. Still, some of sources state that in unsaturated concentrations progesterone and androsterone interact with glycine and $GABA_A$ receptors, which leads to activation of ion transport [29].

Role in the myelination processes

Progesterone is synthesized by Schwann cells and considered to play

an important role in myelination in the peripheral nervous system, including the myelination stimulation after cold damage mediated by locally synthesized progesterone. This effect is mediated mainly by the interaction with nuclear receptor and more rarely by the neuroactive metabolites' formation. Showed that progesterone provokes the intensification of the main myelin's protein's expression, increase of myelin's expression's speed, reduction of time needed for the initiation of synthetic processes. [8]. Progesterone increases the expression level of myelin-specific proteins' mRNA, including mRNA of protein zero (P0), peripheral myelin protein 22 (PMP-22) [23], transcriptional factor Krox-20, which also regulates the myelination in the peripheral nervous system [8]. When Schwann cells and neurons are cultivated together, progesterone dose-dependently increases myelin formation speed, involving the increase in expression level of P450scc and 3βHSD. Consequently, progesterone modulates the process of myelin's formation directly in Schwann cells and indirectly by the nuclear receptors' activation and transcription's stimulation in neurons, which was proved by the elimination of neuronal progesterone's effect when the receptors were blocked by RU-486, progesterone's antagonist. The intensification of myelination takes place in the CNS as well [11].

Role in oligodendrocytes' differentiation

The level of enzymes' expression, needed for progesterone's and its metabolites expression, is different for oligodendrocytes and their progenitors, therefore it may be suggested that neurosteroids take part in progenitors' proliferation and their terminal differentiation [10]. Pre-progenitors, which have the highest concentrations of 3βHSD и 3αHSD, synthesize progesterone from pregnenolone actively. Mature oligodendrocytes are characterized by high level of 5α-reductase as well, but can not convert pregnenolone into progesterone due to the termination of 3βHSD expression [22].

Progesterone's influence on the spinal cord motoneurons' functional activity

Progesterone is involved in recovery of the spinal cord motoneurons' functioning [25]. The increase in pregnenolone and progesterone concentrations without significant changes in P450scc and 3βHSD expression levels was detected in the tissue of damaged spinal cord, but not in its serum, which implies the existence local neurosteroidogenesis [17]. Progesterone stimulates the mRNA's expression of such substances as acetylcholinesterase, Na^+,K^+-ATPase, GAP-43, synthesis of the main myelin's protein, proliferation of oligodendrocytes' progenitors [24] in damaged motoneurons. Nevertheless, another point of view states that classic progesterone's nuclear receptor's can't mediate recovery effects due to the fact that transcriptionally inactive ligand of the progesterone's receptor, enantiomer of progesterone, provokes the same recovery effect on the animals' damaged spinal cord [29]. Allopregnanolone, progesterone's metabolite, may also contribute positively to the recovery process as both 5α-reductase and 3αHSD are present in the spinal cord cells. Some of progesterone's effect may be mediated by the growth factors' stimulation, for instance, BDNF - brain-derived neurotrophic factor [6]. Spinal cord's damage leads to the decrease in BDNF's mRNA expression as well as in quantity of the BDNF protein itself. 3 days-long progesterone treatment doesn't change the BDNF's mRNA expression level in spinal cord motoneurons of animals in control group, whereas mRNA's and BFNF's levels increase significantly in motoneurons in dorsal horns of the spinal cord [24]. Nonetheless, progesterone stimulates the immune binding of BDNF in motoneurons of healthy animals, suggestively because of the increase in BDNF synthesis in non-neuronal cells.

Cerebellum as progesterone's target

Progesterone induces cerebellum's development [27]. Purkinje cells express P450scc, 3βHSD, 5α-reductase and 3αHSD, within their membrane, PREG, PREGS, PROG and ALLO are synthesized [13]. Purkinje neurons produce PROG and ALLO in the neonatal period, when the ecpression level and enzyme activity of 3βHSDare heightened. It is widely known that cortex formation in rodents is performed by the migration of external granular cells, neuronal and glial growth, synaptogenesis in the neonatal period, Purkinje cells also differentiate just after the birth, therefore, cerebellum's development coincides with the neurosteroidogenesis intensification [22]. Progesterone's influence on the Purkinje cells differentiation, which reveals itself in stimulation of dendritic growth, formation of dendritic spines, regulation of synaptic distribution density, is identified in cultivated cerebellar slices of rat pups and in mature rat's brain *in vivo* [25], [Table 1]. These effects may be mediated by classical nuclear receptor PGRMC1, undifferentiated receptors or progesterone's metabolites.

Allopregnanolone

Allopregnanolone's interaction with $GABA_A$- receptors leads to both tonic and phase generation of inhibitory currents, resulting in neuroprotective and anti-convulsant effects [14]. Nonetheless, PREGS acts as a $GABA_A$-receptors antagonist, and if the balance between PREGS and ALLO is upset, seizers may occur [19, 27].

Behavioral and electrophysiological effects of ALLO and all structurally similar compounds are specific for $GABA_A$ receptors: they have a low influence on or simply don't interact with glycine, AMPA, NMDA and 5-HT_3 receptors [18]. ALLO increases the frequency of mIPSCs – miniature inhibitory postsynaptic currents dose-dependently in preorbital zone of hypothalamus, probably, because of the stimulation of a spontaneous GABA release. This effect is strictly determined by extracellular concentration of Cl^- ions, in other words, it's determined by primary activation of presynaptic $GABA_A$ receptors followed by increased membrane's permeability for chorine ions [30]. Animal models showed that enhancing of 3α,5α-THP (ALLO) and 3α,5α-THDOC (allotetrahydrodeoxycorticosterone) expression during stress may be a homeostasis mechanism normalizing the activity of GABA-ergic system as well as hypothalamus-pituitary-adrenal system. Nevertheless, neurosteroids concentrations and actions are pathologically changed in the conditions of chronic stress, for instance, ALLO may be neurotoxic. It impairs long-term potentiation mechanism, which consequently leads to the disruption of short-term and long-term memory. It morphologically results in the reduction of hippocamp's mass. [5]. Moreover, ALLO in chronically high concentrations may increase the quantity of soluble Aβ-amyloid, which is a predictor of grave Alzheimer disease, in a synaptic gap.

If the exposure to high concentrations of ALLO is short-time and is interrupted by considerable breaks, ALLO intensifies the differentiation of the unipotent progenitor cells in the dentate furrow of the hippocampus, therefore, it enhances the recovery of nervous tissue. GABA plays the role of excitory mediator in those cells, as if they were embryonic, because of the increased chlorine's intracellular concentrations [16].

ALLO doesn't have influence on the spontaneous glutamate release in neurons of prefrontal cortex [Table 2], on the opposite, it inhibits this process, if it's induced by depolarizing agents and electrical stimulus [3]. Inactivation of Ca^{2+} ion channels of L, N, R, and P-types by

the formation of chelate complex with extracellular Ca^{2+}, inhibition of protein enzymes of vesicular transportation, blockage of signaling cascades, including calmodulin, adenylate cyclase and PKA underlies this process. The inhibitory effect of ALLO on the glutamate's release reduced the speed of neuronal death under conditions of oxygen-glucose deprivation and H_2O_2 in the experiment [14], which may determine such ALLO's neuroprotective effects as analgesic, anxiolytic, and antidepressant.

It is already known that ALLO increases spontaneous norepinephrine secretion in the cerebral cortex, this process is also menstrual phase-dependent. Interestingly that activating influence is maximum during estrus and I diestrus, decreases gradually during II diestrus, proestrus and 7 days after ovariectomy. ALLO potentiates K^+-stimulated norepinephrine secretion through α2-noradrenergic receptors throughout the estrus. As norepinephrine takes part in modulation of cortical neurons' excitability, its influence on ALLO may either enhance excitability or determine sex-dependent and menstrual phase-dependent features of modulation [26, 30].

Another mediator secreting under the ALLO's influence is dopamine. The animal model, striatum slices, isolated from rat's brain in estrus phase, demonstrated the enhancement of NMDA-induced dopamine secretion. Ovariectomy eliminates this effects, whereas subcutaneous injection of the combination of endogenous estrogen and progesterone regains it. ALLO triggers significant suppression of spontaneous dopamine secretion *in vivo* during the estrus, but still enhances it in animals after ovariectomy on hormone-replacement therapy with estrogen and progesterone [2]. Probably that stimulation of NMDA-induced dopamine secretion is a regulator of sensorimotor functions with sex and cycle features.

ALLO's microdialysis in nucleus accumbens of freely moving rats demonstrates controversial influence of the neurosteroid on the dopamine secretion, both activation and suppression of its spontaneous secretion, increase in secretion with morphine stimulation and decrease in stress-induced dopamine release were detected. Nucleus accumbens plays an important role in formation of mood and motivation, that is why, ALLO may be considered as a modulator for those processes as well as for drug addiction, depression and abstinent syndrome [30].

It was stated that ALLO inhibits spon-

Table 1

List of progesterone's (PROG) main effects in various regions of the central nervous system (without developmental characteristics)

Location	Basic effect	Required conditions	Receptors	Meaning
Hippocampus	NMDA-stimulated norepinephrine release (o)	-	NMDA-receptors	Increase of the intensity of mental processes and physical activity
Prefrontal cortex	1. Spontaneous release of glutamate (o) 2. 5-HT stimulated release of glutamate (o) 3. Dopamine-stimulated Glutamate Release (-)	-	Sigma-1 (-) Sigma-1/D1 synergy (-)	Impact on cognitive function, participation in the pathogenesis of neuropsychiatric diseases
Hypothalamus	Release of serotonin (-)	Ventromedial nuclei	5-HT receptors	Elimination of serotoninergic tonic inhibition, (+) lordosis behavior (arching of the back in mammals during estrus)
	Release of serotonin (o/+)	Preoptic zone		Blockade of the LG surge (peak lutropin concentration and stimulation of ovulation)
Cerebellum	Dendrite growth (+), dendritic spine formation (+), synaptogenesis (+)	Purkinje neurons	Nuclear progesterone receptor PGRMC1	Formation of the cerebellar cortex in the neonatal period, differentiation of Purkinje neurons
Spinal cord	1. Expression of myelin basic protein (+), protein 0 (P0) (+) 2. Peripheral myelin protein 22 (PMP-22) (+) 3. Transcription factor Krox-20 (+)	Schwann cells	Effect is mediated by PROG metabolites	Intensification of myelination processes and regeneration after damage
	Expression of nuclear progesterone receptors (+)	Dorsal root ganglia neurons	Nuclear receptor PROG	
	Expression of acetylcholine transferase, Na, K-ATPase, GAP-43, myelin basic protein, BDNF	Motoneurons	Metabolites, PGRMC1, nuclear receptor PROG (?)	Restoration of functions of damaged motoneurons

taneous dopamine secretion dose-dependently in neurons of prefrontal cortex, which take part in modulation of emotional state, whereas elimination of ALLO's effects by finasteride significantly increase stress-induced (foot shock) and drug-induced (anxiogenic druf FG7142) neurotransmitters' release [Table 2]. It was also stated that fluoxetine treatment in patients with depression and panic disorders stabilize neurosteroids' level in the CNS [2].

Dehydroepiandrosterone (DHEA) and Dehydroepiandrosterone sulfate (DHEAS)

The concentrations of DHEA and DHEAS change dynamically during the ontogenesis of the human body [12]. They decrease rapidly just after the birth due to the involution of the fetal zone of the adrenal cortex and then remain at a stable minimum level until individual is 7-8 years old. Adrenarche starts at this age due to the maturation of the adrenal cortex and therefore increased synthesis of DHEA and DHEAS in it, a process develops parallelly to puberty. The concentration of these neurosteroids continues to increase until 20-25 years of human's life, then the slow decline begins, subsequently, at the age of 60 years, the concentrations of DHEA and DHEAS barely reach 20-30% of their concentration at 20 years' age. These changes are directly related to the functions of these neurosteroids, which are vital in childhood,

Table 2

List of allopregnanolone's (ALLO) main effects in various regions of the central nervous system (without developmental characteristics)

Location	Basic effect	Required conditions	Receptors	Meaning
Hippocampus	K + -stimulated release of γ-aminobutyric acid (-)	-	Ca2+-canals L-type	Antipsychotic effect
	1. Spontaneous release of acetylcholine (-) 2. Stress-induced release of acetylcholine	Intraventricular injection	-	Modulation of memory, stress reactions
Prefrontal cortex	1.Spontaneous release of glutamate (o) K + -stimulated release of glutamate (-) 2.Spontaneous release of acetylcholine (-) 2.Spontaneous release of dopamine (-)	-	-	Modulation of cognitive processes, changes in emotional status during the menstrual cycle, pregnancy, menopause
Frontal cortex	1. Spontaneous release of norepinephrine: estrus, diestrus I (+); diestrus II and proestrus (o) 2.K + -stimulated release of norepinephrine: estrus (+)	-	α2 - noradrenergic receptors (+)	Regulation of excitability of cortical neurons, determination of sex differences in modulation of cortical functions
Striatum	1.Stimulated release of glutamate (o) 2.Spontaneous release of acetylcholine (-)	-	-	-
	1. Spontaneous release of dopamine: estrus (-) 2. NMDA-stimulated release of dopamine: est-rus (+); diestrus (o)	*In vivo* Striatum slices	-	Modulation of sensorimotor processes
Hypothalamus	1. Spontaneous release of γ-aminobutyric acid (+) 2.K + -stimulated release of γ-amino-butyric acid (o)	Preoptic zone	Primary activation of presynaptic GABAA receptors, Na + -K + -Cl-cotransporter, extracellular Ca2 +	GABA-ergic regulation of secretion of gonadotropin-releasing factors produced by neurons in the medial preoptic zone
	Spontaneous release of dopamine (-)	Ventromedial nuclei	-	Inhibition of the release of luteinizing hormone, reduction of reproductive activity

Table 3

CList of dehydroepiandrosterone's (DHEA) main effects in various regions of the central nervous system (without developmental characteristics)

Location	Basic effect	Required conditions	Receptors	Meaning
Hippocampus	NO production in response to NMDA receptor stimulation (-)		Sigma-1 (+)	Protection against glutamate, AMPA and kainate excitotoxicity, regulation of processes of syn-naptogenesis
Prefrontal cortex	Elongation of axons and dendrites (+) (+)	-	NMDA-рецепторы (+)	-
Forebrain	1. Spontaneous release of glutamate (+) 2. K + -stimulated release of glutamate (o) 3. Spontaneous release of glutamate (+) 4. K + -stimulated release of glutamate (+) 5. Spontaneous release of glutamate (-)	Synaptosomes Rats 12 months old Rats over 12 months old		Increase in physiological tonic glutamatergic impulses that regulate plastic processes

Table 4

List of dehydroepiandrosterone sulfate (DHEAS) main effects in various regions of the central nervous system (without developmental characteristics)

Location	Basic effect	Required conditions	Receptors	Meaning
Hippocampus	1. Strengthens the processes of dendrite formation 2. Spontaneous release of glutamate (+) 3. Spontaneous release of acetylcholine (+) 4. Release of norepinephrine: spontaneous (o); 5. K + -stimulated (o), in the presence of D2-antagonists (+); 6. NMDA-stimulated (+)	In nanomolar concentrations Microdialis *in vivo* Hippocampus slices	Sigma-1(+) Ca2 + -channels L, N-types Sigma-1(+)	Improving learning ability, memory
Prefrontal cortex	Spontaneous release of glutamate (+)		D1-receptors (+) Sigma-1 (+)	Providing cognitive processes

adolescence and adulthood: DHEA and DHEAS stimulate the development of the cerebral cortex [4], mediate neuronal plasticity, which underlies the adaptation to changing environmental conditions, through the influence on the amygdala reduce the level of fear and anxiety, which naturally increases due to the abundance of new impressions and experiences, are involved in providing hippocampal mechanisms of memory and learning by influencing synaptogenesis processes [Tables 3,4], have a protective function: decrease the ability of the GCS-receptor complex to affect the stress factors' transcription level in the cell; reduce GCS-mediated excess glutamate's release from neurons (reduce neurotoxicity, associated with excess glutamate); protect hippocampal neurons from AMPA and kainate excitotoxicity by inhibiting of NO-production stimulated by a NMDA-receptor mediated increase in the concentration of intracellular Ca2+, determine the development of sexual behavioral stereotypes before puberty, inhibit the mictoglia activation during the brain damage, reduce the activity of apoptosis and synthesis of proinflammatory cytokines [9], they are Sigma-receptor agonists that regulate Ca2+, Cl -, K+, NMDA-mediated ion currents, neurotransmitters' release, lipid neuronal transport, brain neurotrophic factor's signal transmission, myelination, neurogenesis and synaptogenesis [3], DHEA stimulates basal glutamate secretion and doesn't affect the K+ - stimulated release of the mediator in synaptosomes of the forebrain at the early stages of intrauterine ontogenesis both *in vivo* and *in vitro*. Since glutamatergic transmission of the forebrain provides

the plastic processes, which are necessary for learning and memorizing, it was assumed that DHEA increases the basal level of tonic impulsion of the forebrain cortex and confirmed by an improvement of rats' performances in the avoidance test.

It was shown that DHEA promotes elongation of τ-immunopositive axons in nanomolar concentration and insignificantly stimulates the growth of MAP-2 immunospecific dendrites, whereas DHEAS increases the formation of dendrites without affecting the length of the axon. DHEA dose-dependently increases calcium admission into cells in embryonic cortical neurons, apparently through interaction with NMDA receptors [Table 3], since this effect is blocked by MK801 and D-AP5 NMDA-receptors' antagonists, which may be one of the mechanisms underlying the regulation of axon growth. However, the fact whether such effects are limited to the period of intranatal development or persist in adult animals still remains unknown [21]. DHEAS can act as a DHEA depot [23].

DHEA microdialysis stimulates spontaneous acetylcholine secretion in the hippocampal neurons of anesthetized rats, a similar effect is observed when DHEAS plasma concentration increases, provoked by an inhibition of sulfatase activity. Cholinergic transmission provides the functional changes in hippocampal neurons that underlie memory, that is why enhancing of the spontaneous acetylcholine release may be one of the important mechanisms for improving memory under the action of DHEAS [30].

Conclusion. The spectrum of neuroactive steroids is specific to various parts of the CNS, their neurons and glial cells, and changes during the ontogenesis. Generally, steroidogenesis' intensification occurs in the pre- and neonatal periods of individual development, when these substances regulate the formation of the cerebellar cortex, elongation of processes, myelination, synaptogenesis, etc. Decrease or even the complete cessation of steroidogenesis enzymes' expression is observed then in adults. However, neurosteroids continue to influence learning and memorizing processes, emotional status, motivation as well as cognitive and motor functions of an individual. Increased steroidogenesis is observed in a number of physiological and pathophysiological conditions, such as cold and ischemic damage, pregnancy, stress, schizophrenia, Parkinson disease.

Reference

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5-NUCLEOTIDASE ACTIVITY IN THE PLACENTA IN CYTOMEGALOVIRUS INFECTION AS A MARKER OF DEVELOPMENT OF PREECLAMPSIA

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5'-nucleotidase is an enzyme that catalyzes the phosphorylytic cleavage of 5'-nucleotides, including adenosine monophosphate, which is converted to adenosine. The world literature contains a large amount of works concerning the role of the enzyme in the cardiovascular, immune, nervous, digestive, respiratory and other systems. Nevertheless, despite the long study period, the features of the 5'-nucleotidase activity in various infectious processes have practically not been studied. The goal of our research was to study the activity of the 5'-nucleotidase enzyme in the placenta in reactivation of cytomegalovirus infection in the third trimester of pregnancy. We studied placenta samples obtained in childbirth from women with laboratory confirmed reactivation of chronic cytomegalovirus infection and a diagnosis of moderate preeclampsia in the third trimester of pregnancy, as well as from clinically healthy women with latent cytomegalovirus infection (control group). The method with the formation of lead phosphate by Wachstein and Meisel in a slight modification was used for the histochemical localization of 5'-nucleotidase activity. A significant increase in the intensity of the histochemical reaction to 5'-nucleotidase in the syncytiotrophoblast of the placental villi was revealed, indicating an increase in the enzyme activity in the reactivation of cytomegalovirus infection during pregnancy. The cytophotometric index in the syncytiotrophoblast of the placentas of the first group increased to 37.4 ± 2.23 relative units compared with the control group (29.2 ± 2.55 relative units, p <0.05). A significant increase in the intensity of the enzyme in the placenta was noted in cases where the reactivation of the cytomegalovirus process was accompanied by preeclampsia. The cytophotometric index increased by almost 40% (p <0.001) in comparison with the data of the control group and amounted to 42.2 ± 2.99 relative units. Thus, an increase in 5'-nucleotidase activity created a high level of adenosine, which, in our opinion, could contribute to the development of characteristic signs of preeclampsia in cytomegalovirus infection during pregnancy. As a reason for the development of this phenomenon, we assume that hypoxia, inflammation and a decrease in energy supply formed as a result of an exacerbation of the infectious process.

Keywords: cytomegalovirus infection, pregnancy, placenta, 5'-nucleotidase, preeclampsia.

Introduction. The 5'-nucleotidase enzyme was first cloned from the rat and human placenta [21], an active study of its biochemical properties and localization, isolation and purification began since the 1970s and 1980s [14, 20]. Despite this, studies on the role of the enzyme in the reproductive system and the pathogenesis of various pathological conditions are still ongoing. The specific features of 5'-nucleotidase activity in various infectious processes are practically not studied.

5'-nucleotidase is an enzyme that catalyzes the phosphorylytic cleavage of 5'-nucleotides, including: adenosine monophosphate (AMP), cytosine monophosphate, uridine monophosphate, inosine monophosphate, guanosine monophosphate, as well as nicotinamide mononucleotide and NAD, thereby regulating their availability. The most effective substrate for 5'-nucleotidase is AMP. In this case, AMP is broken down to adenosine. The earliest studies of the function of nucleotides and adenosine in the morphosis were discussed in terms of their role as a source of energy and an integral part of other compounds. It is now generally accepted that purines and pyrimidines have potent effects mediated by the activation of specific membrane receptors. Adenosine acts as a P1 purinergic receptor agonist. The second type of purinergic receptor, called P2, is selective for ATP / ADP [24]. However, an increase in adenosine production can lead to the development of pathological conditions during pregnancy. Since adenosine regulates the formation of blood vessels, its high concentration can inhibit the growth of the placenta in the early pregnancy [6]. It is also known that an increased level of adenosine causes vasoconstriction of the placenta vessels [7].

An increase in the concentration of adenosine is observed in women with preeclampsia [13,17,18,19]. A number of researchers have determined that an increase in adenosine in the placenta contributed to the development of characteristic signs of preeclampsia, including hypertension, proteinuria and intrauterine growth restriction [6,12,17,19]. The reason for the increase in the content of adenosine during pregnancy, according to experts, is an increase in the activity of the enzyme 5'-nucleotidase in the placenta [12]. This placental enzyme is considered as a candidate marker for the formation of adenosine in this organ during preeclampsia [6]. **The goal of research** was to study the activity of the 5'-nucleotidase enzyme in the placenta in reactivation of cytomegalovirus infection in the third trimester of pregnancy.

Materials and methods. Samples of placentas from 102 women obtained from childbirth at 37-40 weeks of gestation were studied. The criteria for the inclusion of patients in the first group (37 cases) were: the presence of laboratory confirmed reactivation of chronic cytomegalovirus (CMV) infection in the third trimester of pregnancy, age from 18 to 37 years, consent to the study; in the second group (35 cases) - laboratory confirmed reactivation of chronic CMV infection and diagnosis of moderate preeclampsia (ICD O14.0) in the third trimester of pregnancy, age from 18 to 37 years, consent to the study; in the control group (30 cases) – absence of reactivation of chronic CMV infection during pregnancy, age from 18 to 37 years, consent to the study.

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The exclusion criteria from both groups were: exacerbation of any extragenital and infectious diseases, apart from CMV infection, primary CMV infection, age under 18 and over 37 years, smoking, alcohol and drug use, lack of voluntary informed consent.

Laboratory methods were used: PCR on a DT-96 apparatus using a set of NPO DNA-technology (Russia) for the diagnosis of CMV DNA in material from buccal epithelium scrapings, blood serum and urine; ELISA on a spectrophotometer "Stat-Fax 2100" (USA) using a set of ZAO "Vector-Best" (Russia) in blood serum for diagnostics of the form (acute, chronic, primary) of CMV process.

Assessment of the severity of preeclampsia was carried out on the basis of clinical guidelines (treatment protocol) “Hypertensive disorders during pregnancy, childbirth and the postpartum period. Preeclampsia. Eclampsia", approved by the Ministry of Health of the Russian Federation on 07.06.2016. No. 15-4 / 10 / 2-3483.

For the histochemical localization of 5'-nucleotidase activity, we used the method with the formation of lead phosphate by Wachstein and Meisel [3] in a slight modification. The enzymatic reaction was carried out on cryostat tissue sections for 30 minutes at 37° C in 50 mM tris-maleate buffer (pH 7.4) supplemented with 5 mM MnCl2, 2 mM Pb $(NO3)_2$, and 2.5 mM levamisole as an alkaline phosphatase activity inhibitor and in the presence of 1 mM AMP as a substrate. The control was incubated in a medium without a substrate. The reaction was detected in the presence of 1% Na_2S. Then the samples were placed in a glycerol gel, analyzed, and photographed under a Meiji Techno light microscope (Japan). The slides were studied using the Scion Image software (USA) according to the method described in our previous works [2].

The results of the study were statistically processed using the "Statistica 10.0" computer program after the Lilliefors and Kolmogorov-Smirnov normality tests using the Student's t-test.

Results and discussion. Placental slides showed the activity of 5'-nucleotidase in the plasma membrane of syncytiotrophoblast (Fig. 1). No enzyme activity was detected in the villi stroma. In control sections incubated in a medium without a substrate, the enzyme activity was not determined (Fig. 2). A number of specialists suggest that 5'-nucleotidase is involved in the regulation of blood microcirculation in the placenta [23].

Adenosine formed as a result of the reaction can selectively modulate growth, proliferation, migration, invasion and differentiation of cells during embryonal development, and regulate fetal metabolism [6,7]. Its important role in the angiogenesis and vasculogenesis of the fetus and placenta is suggested. In vitro studies have shown that this nucleoside under physiological conditions stimulates a significant production of pro-angiogenic factors, such as vascular endothelial growth factor and membrane-bound fms-like tyrosine kinase-1, and simultaneously inhibits anti-angiogenic factors - soluble fms-like tyrosine kinase-1 [11]. The functional properties of adenosine include the regulation of vascular tone and nutrient transport [16].

In reactivation of CMV infection in the third trimester of pregnancy, an increase in the activity of the histochemical reaction to 5'-nucleotidase was found (Fig. 3). The cytophotometric index in the placental syncytiotrophoblast in the first group increased to 37.4 ± 2.23 relative units compared with the control group (29.2 ± 2.55 relative units, $p < 0.05$). Thus, the activity of the reaction increased by 25%. Our data are consistent with in vitro studies describing increased expression and enzymatic activity of 5'-nucleotidase in CMV infected endothelial cells compared to uninfected ones [10]. The most pronounced changes were noted in the material of the second group of the study. The cytophotometric index increased by almost 40% ($p < 0.001$) in comparison with the data of the control group and amounted to 42.2 ± 2.99 relative units.

The mechanisms leading to an increase in 5'-nucleotidase activity in the placenta are poorly understood. It is known that the enzyme is activated under conditions of hypoxia, in the presence of a number of pro-inflammatory factors (TNFα, IL-1β, interferons, prostaglandin E), as well as a weakening of energy supply [8]. Earlier, in active CMV infection during pregnancy, an increase in the content of HIF-1α [5], TNF-α, IL-1β [1,22] and a decrease in the intensity of energy metabolism [4] were revealed. We believe that the reactivation of the CMV process, which promotes the formation of conditions of hypoxia and inflammation, led to an increase in the activity of 5'-nucleotidase in the placental villi syncytiotrophoblast, and to the formation of a large amount of adenosine. Its high level is supposed to compensate for the negative effect of inflammatory components, depletion of energy supply, and counteracts the progression of further complications [7]. However, a long-term increase in the expression of 5'-nucleotidase can

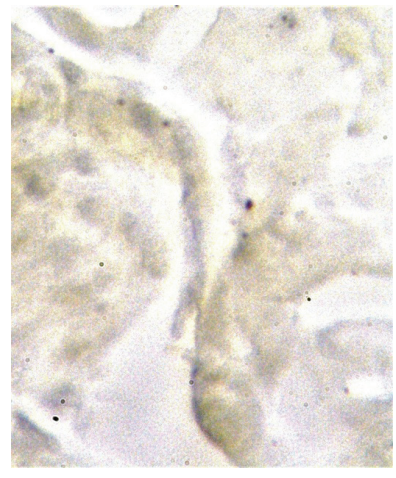

Fig. 1. Placenta from the control group. Dark brown deposits in micrographs correspond to 5'-nucleotidase activity in syncytiotrophoblast. Magnification 15×40.

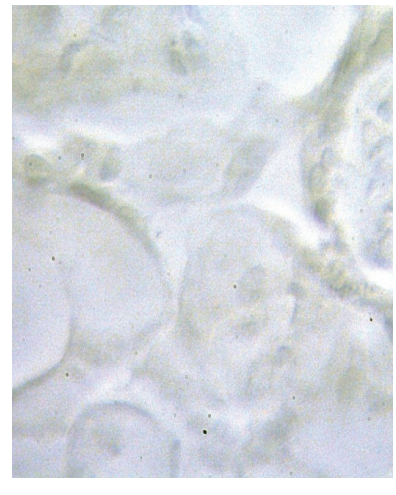

Fig. 2. Lack of 5'-nucleotidase activity in syncytiotrophoblast in control sections of the placenta. Magnification15×40.

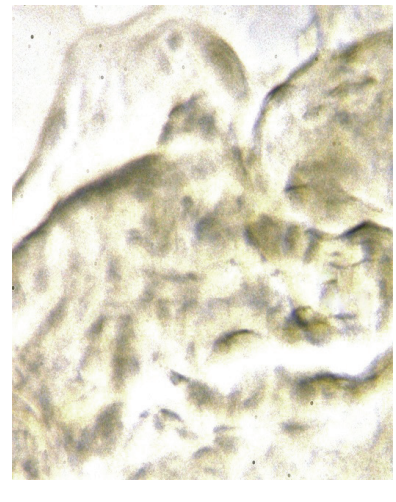

Fig. 3. Placenta from the group with reactivation of CMV infection. The 5'-nucleotidase activity is higher compared to the control group. Magnification 15×40.

lead to depletion of the pool of extracellular nucleotides, local formation of adenosine, activation of the corresponding purinergic receptors and induction of preeclampsia symptoms, which is one of the most serious complications of pregnancy, including associated with CMV infection [9,15].

Conclusion. A significant increase in the intensity of the histochemical reaction to 5'-nucleotidase in the placental villi syncytiotrophoblast was revealed, indicating an increase in the enzyme activity in reactivation of CMV infection during pregnancy. A significant increase in the intensity of the enzyme in the placenta was noted in cases when the reactivation of the CMV process was accompanied by preeclampsia. Thus, an increase in 5'-nucleotidase activity, in our opinion, could create a high level of adenosine, which contributed to the development of characteristic symptoms of preeclampsia in CMV infection during pregnancy. As a reason for the development of this phenomenon, we assume that hypoxia, inflammation and a decrease in energy supply formed as a result of an exacerbation of the infectious process.

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УДК 612.11+612.12

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THE FUNCTIONAL ROLE OF TRANSFERRIN RECEPTOR – TfR1

The article presents modern data on the functional role of the transferrin receptor - TfR1. The information on the significance of this receptor in the functioning of various cells of the body is generalized, and the diverse role of this receptor depending on the type of cells and the stage of their activation is shown.

Keywords: receptor, transferrin, TfR1, CD71, iron, erythrocytes, lymphocytes, ferroptosis, oncology.

Transferrin receptor (TfR1) - CD71 is expressed on virtually all cells in the body. It is a multiligand receptor that is able to bind and transfer into the cell not only transferrin, but also various proteins, viruses, chemotherapy drugs, bacterial toxins, plant toxins, DNA, oligonucleotides, short inhibitory RNA (siRNA) and enzymes [4, 11, 32, 40, 47]. TfR1 is most strongly expressed on placental syncytiotrophoblasts, myocytes, basal keratinocytes, hepatocytes, endocrine pancreas, spermatocytes and immature erythroid cells, reticulocytes, hepatocytes, endothelial cells of the blood-brain barrier [30, 41]. Transferrin is an essential component of cell growth and metabolic processes that require iron, including DNA synthesis, oxygen sensitivity, and the G1 to S phase transition in the cell cycle, electron transport, mitogenic signaling pathways and, in turn, cell proliferation and survival [10, 12, 23]. Consequently, fast-growing cells require more iron for growth and actively proliferating cells have a much higher level of CD71 expression than cells at rest [33]. In research [36] It has been shown that CD71 and ki-67 (the nuclear protein of actively proliferating cells) have the same expression pattern after stimulation of CD4 + and CD8 + cells, which makes it possible to assess the level of activation of cell proliferative activity by the level of CD71 +, without intracellular staining of the ki-67 protein. Simultaneous increase in CD71 + and ki-67 levels has also been proven in malignant neoplasms [19].

Transferrin belongs to endogenous signaling proteins called alarmines. During the invasion of pathogens, alarms result from the breakdown of cells and the subsequent release of enzymes that break down proteins such as transferrin into fragments [38, 46]. At the cellular level, the expression of the transferrin receptor protein is interrelated with the expression of the ferritin protein through the interaction of proteins sensitive to iron, with the regulatory element of iron on the 5'-untranslated region of ferritin mRNA and regulatory elements of iron on the 3 '-translated region of the mRNA of the transferrin receptor. Expression of TfR1 increases, and expression of ferritin decreases at a low concentration of cytosolic iron, an increase in the level of cytosolic iron has the opposite effect [22]. Regulation of TfR1 expression is provided not only by the level of intracellular iron, but also largely by the oxygen status of the cell, the presence of reactive oxygen species, and hypoxia [26, 51]. Hypoxia leads to stabilization of hypoxia-inducible factor-1α (HIF-1α), the main regulator of transcription of genes responding to hypoxia, including the transferrin receptor gene [44]. HIF-1α then moves to the nucleus, where it binds to a hypoxia-inducible element in the promoter region of the transferrin receptor gene. Hypoxia increases the formation of reactive oxygen species, increases the cytosolic labile iron pool and TfR1 expression, while proliferation is activated, but not ferroptosis [39].

CD71 + on tumor cells. The level of TfR1 expression by malignant cells is many times higher than that of normal cells of the body. It has been shown that TfR1 expression can correlate with tumor stage or cancer progression, and an increase in the TfR1 level on transformed cells is a poor prognosis in various types of cancer [33, 43, 49]. Recently, there has been a lot of research on the use of the transferrin receptor to «capture» tumor cells. The use of TfR1 for the isolation of malignant cells has an advantage over other methods, mainly based on the detection of epithelial cell adhesion molecules, because as an affinity target, it can separate almost any type of cancer cells, regardless of the origin of the disease, including non-epithelial [1, 33]. The transferrin receptor is an attractive target for targeted therapy [24, 42]. On the one hand, due to constant recirculation, TfR1 is used to deliver drugs directly to malignant cells; on the other hand, antibodies are actively used that block its natural function, which leads directly to the death of cancer cells [2, 34]. The drugs used for chemotherapy are toxic to the body due to their rapid diffusion and accumulation in the body, which leads to high intoxication. Through the use of TfR, drug delivery is significantly improved, which makes it possible to increase their intracellular concentration. This results in more efficient tumor targeting and enhances overall therapeutic efficacy. In addition, some drugs are conjugated with transferrin, which also allows the delivery of active substances directly to the cancer cell, preventing their degradation in the extracellular space. This significantly increases the safety of their use and patient survival. However, the problem of using drugs aimed at blocking the function of the transferrin receptor, or blocking the functioning of cells, indirectly through TfR1, is that virtually any cell in the body expresses the TfR1 receptor to a greater or lesser extent. Thus, when using drugs by means of TfR1, for the treatment of patients with malignant neoplasms, it is necessary to assess the harm / benefit ratio.

CD71 + erythroid cells (EC). High expression of transferrin receptors is characteristic of early precursors of erythrocytes in the intermediate phase of normoblasts, after which the expression decreases in the phase of reticulocytes [18, 35, 37, 45]. Maturation to erythrocytes results in loss of transferrin receptor expression in combination with suppression of the hemoglobin synthesis mechanism. Most mature erythrocytes do not express CD71. A high level in the circulation of CD71 + EC in newborns and in various pathologies of hematopoiesis, oncological processes. The detection of high concentrations of CD71 + EC in newborns, which have an immunosuppressive

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effect, allows a new look at the problem of «failure» of immune protection in newborns. EC CD71 + suppress innate, anti-infectious and tumor immunity [6]. Removal of these cells from the circulation restores resistance to neonatal infections, but, for example, the introduction of EC CD71 + in adult patients leads to the suppression of anti-infectious protection [17]. CD71 + erythroid cells - like other immunomodulatory cells such as Tregs - can use a variety of mechanisms to mediate immune regulation. They can suppress or modulate immune cell function through soluble factors such as TGF-β, arginase-2, cytokines and reactive oxygen species through cell-cell interactions (e.g. PD-1: PDL-1 / PDL-2, VISTA) [14].

A decrease in the level of CD71 + erythroid cells leads to an increase in the activation of immunocompetent cells in the intestine and the synthesis of pro-inflammatory cytokines (IL-6, TNFα) [15]. CD71 + cord blood erythroid cells suppress the proliferation of CD4 + and CD8 + cells [9]. The immunosuppressive role of CD71 + erythroid cells in relation to anti-infectious / innate immunity is balanced by the need to suppress active inflammation in the intestine as a result of active colonization by microflora in the first months of a child's life. Thus, a decrease in the activity of immunity in newborns can be considered as a physiological norm aimed at the formation of successful adaptation to new conditions. A similar immunoregulatory effect of CD71 + -ECs is exerted during pregnancy and is likely to play a decisive role in gestation and the formation of fetal and maternal tolerance. Maternal CD71 + erythroid cells suppress an aggressive allogeneic response against the fetus, such as a decrease in TNF-α and IFN-γ production through arginase-2 and PD-1 / PDL-1 activities. Their depletion leads to the failure of gestation due to the immunological rejection of the fetus. Similarly, fetal liver CD71 + erythroid cells exhibit immunosuppressive activity [8]. Expression of the transferrin-1 receptor and ferroportin was found on the placental syncytiotrophoblast and was polarized such that TfR1 was on the apical maternal membrane and ferroportin was on the basal fetal membrane, consistent with unidirectional iron transport from mother to fetus. Ferritin is highly expressed in the stroma, suggesting that fetal tissue can store and store iron. A decrease in CD71 + erythroid cells leads to the formation of inflammatory reactions due to a decrease in the production of IL-4 and IL-10, against the background of an increase in the concentration of TNF-α and IL-6 in the tissues of the placenta [8]. A decrease in the level and dysfunction of CD71 + erythroid cells was noted in patients with inflammatory bowel disease during pregnancy. Dysfunction of CD71 + erythroid cells contributes to the formation of a pro-inflammatory environment in the gastrointestinal tract, dysbiosis, a decrease in the level of Tregs is characteristic, against the background of a high concentration of IL-6 and TNF-α [31].

The number of erythroid cell precursors CD71 + increases significantly in patients with COVID-19, especially in moderate to severe disease [16, 25]. The high level of these cells is partly due to the strong immunosuppressive effect that forms in patients with COVID-19. CD71 + ECs are vulnerable to infection with SARS-CoV-2, due to the high expression of angiotensin-converting enzyme 2 (ACE2), which is used by the virus to attach and invade the cell, so the virus can spread through the bloodstream, causing local inflammation in the tissues. Immature erythrocytes CD71 + play an important role in the pathogenesis of HIV, due to the presence on their membrane of a large number of CD35 adhesion receptors and DARC chemokine receptor, which are the main target molecules for HIV. Immature CD71 + erythrocytes promote the persistence and transmission of HIV to uninfected CD4 + T cells [5]. In hepatocellular carcinoma tissues, intratumoral CD45 + CD71 + erythroid cells are able to suppress the activity of T cells due to the generation of reactive oxygen species, IL-10 and TGF-β by paracrine and intercellular contact, which plays an important immunosuppressive role in the tumor microenvironment and serves as a marker for predicting hepatocellular recurrence carcinomas [29].

The role of CD71 + on somatic cells. Expression of TfR1 on somatic cells plays a decisive role in the early stages of development. Thus, deletion of the TfR1 gene causes severe muscle atrophy, growth retardation, metabolic disorders and premature death. Deletion of TfR1 in adults is not so critical and does not affect survival, but it causes skeletal muscle atrophy and motor functional disorders similar to muscle atrophy observed after denervation [50]. A decrease in TfR1, but an increase in the level of the Slc39a14 protein on the cell membrane promotes labile iron accumulation in skeletal muscles, which leads to the activation of ferroptosis (FP) in old skeletal muscles [48]. Ferroptosis, the process of cell death caused by cellular metabolism and iron-dependent lipid peroxidation [20, 52]. Ferroptosis does not depend on the activation of caspases, the release of cytochrome C, an increase in intracellular calcium and other mediators of programmed cell death [21]. Ferroptosis is associated with diseases such as ischemic organ damage, cancer, and neurological diseases. The transferrin receptor can be a specific marker of ferroptosis. Ferroptosis is fatal for virtually all types of tumor cells, and the regulation of its activity is considered as one of the promising options for treating tumors [13, 53]. The transferrin receptor is activated in beta cells of the pancreas in the first weeks of the postnatal period, surface expression of CD71 is regulated in a glucose-dependent manner. Beta cells express higher levels of several other genes involved in iron metabolism, and iron deprivation significantly impairs beta cell function. With a deficiency of glucose in the beta cells of the pancreas, the expression of CD71 + increases. CD71 - is a postnatal beta-cell-specific marker and plays a central role in iron metabolism in the functioning of beta cells [3]. However, high expression of CD71 in the mesangium is responsible for the progression of IgA nephropathy. CD71 functions as a mesangial IgA receptor, binding of CD71 to circulating immune complexes (CICs) containing IgA1 leads to the deposition of CICs on the glomerular mesangium [7, 27]. SIgA bound to the apical CD71 + receptor on the surface of enterocytes avoids lysosomal degradation; this process ensures the transcytosis of bound proteins [28].

Conclusion. The function of the transferrin receptor is diverse, due to its ability to bind substances of various natures and transport them across the cell membrane. The use of TfR in the targeted treatment of cancer patients, actively studied in recent years, is a rather promising direction and is justified from the point of view of high expression of TfR by tumor cells. This method significantly reduces the toxic effect of drugs, but the question remains about how such drugs affect healthy cells in the body, which also express TfR. The emergence of new data on the functional immunoregulatory role of CD71 + erythroid cells allows a new look at the issue of the functioning of the immune system in children. Thus, new perspectives are opening up in studying the issue of «failure» of immunity in children and solving the issue of the need for immunostimulating therapy at an early age.

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THERAPEUTIC APPROACHES TO RESTORING THE ANTIATHEROGENIC FUNCTION OF HIGH DENSITY LIPOPROTEINS

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A low level of high density lipoproteins (HDLs) in blood plasma is considered to be an important risk factor for the development of atherosclerosis. The antiatherogenic function of HDLs is associated with their participation in the reverse transport of excess cholesterol from peripheral tissues to the liver. In addition, they have antioxidant, anti-inflammatory, antithrombotic and anti-infectious properties, which also contribute to their atheroprotective effect. Recent studies have shown that the structure and composition of HDLs can change under various pathological conditions, thus leading to the appearance of dysfunctional HDLs, which cannot perform protective functions in the body. This review is devoted to the analysis of modern approaches aimed both at increasing the level of HDLs in blood plasma and at maintaining and restoring their native functional properties.

Keywords: high density lipoproteins, approaches to increase antiatherogenic function.

Introduction. Large epidemiologic studies have demonstrated an inverse relationship between the concentration of serum cholesterol (Chol) in high density lipoproteins (HDLs) and the risk of developing cardiovascular diseases (CVDs). Each increase in the concentration of HDL-Chol by 0.026 mmol/l decreases the risk of developing coronary heart disease (CHD) by 2–3% [56]. In this connection, various approaches were devised to increase the HDL-Chol level. However, recent studies, particularly those where the transfer of Chol esters (eChol) was estimated using an inhibitor of cholesteryl ester transfer protein (CETP), revealed that an increase only in the level of HDL and HDL-Chol does not prevent cardiovascular diseases [48]. At present, the research interest is focused on estimating the functional state of HDL and HDL-Chol rather than their level.

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The main antiatherogenic function of HDLs is related to the reverse Chol transport (RCT); in addition, they possess antioxidant, anti-inflammatory, antithrombotic and anti-infectious functions, which also determine their atheroprotective action [6,8]. HDLs enhance the production of nitric oxide (NO), stimulate proliferation and migration of endothelial cells, and suppress inflammation and apoptosis processes [39]. HDL-associated enzymes paraoxonase 1 (PON-1), lecithincholesterolacyltransferase (LCAT), platelet-activating factor acetylhydrolase (PAF-AH), and myeloperoxidase (MPO) are responsible for the antioxidant functions [45]. Antidiabetic [22,38] and cardioprotective functions of HDLs are discussed [6].

The aim of this review is to analyze therapeutic approaches to restoring the native structural-functional properties of HDLs. Among such approaches are physical exercise and low-calorie diet, CETP inhibitors, and raising the level of apolipoprotein A-I (apoA-I) with the use of recombinant HDLs (rHDLs) or apoA-I mimetic peptides.

Physical exercise. Regular physical exercises and diet not only increase the level of HDL-Chol but also improve the functional properties of lipoprotein particles, particularly HDLs [29]. Different researchers have demonstrated that the HDL level significantly increases after a training load. Sportsmen showed a higher maximum oxygen consumption (VO_2max), concentrations of HDL-Chol and apoA-I in blood plasma were also higher; along with this, the efficacy of RCT increased due to a growth in the amount of large mature α1-HDL particles that deliver Chol esters to the liver [29,20]. In women with obesity, physical exercise for 9 weeks (5 sessions a week) reduced their body weight and decreased the concentration of triglycerides (TG) and apoB; therewith, the content of total Chol, LDL-Chol and HDL-Chol did not change. However, RCT has improved; this was estimated from the HDL ability to remove $^{(14)}$C-Chol from the human monocyte/macrophage line THP-1 [42]. In marathoners, the concentrations of LDL-Chol, apoB and TG did not differ from the control group of patients with a sedentary lifestyle. However, marathoners had higher levels of HDL-Chol and apoA-I; therewith, an increase in the transfer rate of labeled lipids (Chol, eChol, TG, and phos-

pholipids (PLP)) from donor blood serum to HDLs of sportsmen was observed [53].

HDLs were shown to protect the vessel endothelium in patients with chronic cardiac insufficiency (CCI) in response to physical exercise. At the beginning of the study (before physical exercises), the patients showed a substantial decrease in the NO production by human aortic endothelial cells (HAEC) upon incubation in the presence of HDLs isolated from CCI patients. HDLs taken from patients slightly stimulated the phosphorylation of Ser(1177), Thr(495), PKC-ßII-Ser(660) and p70S6K-Ser(411) in endothelial NO synthase (eNOS). The production of nitric oxide (NO) virtually reduced to zero. Daily training during 15 weeks considerably improved the ability of HDLs to activate eNOS and increased the production of NO in HAEC [2]. In comparison with the control group, blood plasma of patients with metabolic syndrome (MS) showed a high level of TG, a low concentration of HDL-Chol, and a low activity of paraoxonase-1 (PON-1) in HDLs. After three months of moderate intensity training without any specific diet, a decrease in the TG level without changes in HDL-Chol and LDL-Chol was observed. However, physical exercises increased the transfer of free Chol to HDLs and enhanced the activity of PON-1 in them [12].

A positive correlation was found between aerobic exercises and enzymes involved in the regulation of RCT (LCAT, CETP, and lipoprotein lipase (LPL)) [29]. An important role in RCT is played by ABCA1 and ABCG1 transporters and SR-B1 receptors. Experimental models performed with Wistar rats revealed an increase in the concentration of HDL-Chol, apoA-I, pre-β-HDL particles and LCAT in blood serum after six weeks of aerobic training with physical exercise. An increased expression of mRNA of ABCA1 transporter was observed in the animals' liver and intestines. After 12 weeks of aerobic training, expression of the ABCA1 gene increased also in the heart [17]. An increased expression of SR-B1 and ABCA1 genes was observed in spayed rats after eight weeks of aerobic training [33]. Physically active persons have higher concentrations of apoA-1 and ABCA1 mRNA in leucocytes as compared to people with a sedentary lifestyle. Moreover, a positive correlation was found between ABCA1 in leucocytes with pre-β-HDL and activity of LCAT, as well as between apoA-I and RCT [21]. In mice with the type 2 diabetes model, physical training also increased expression of the ABCA1 gene [55].

It was shown that physical exercise increases the gene expression and the content of PPAR-alpha (Peroxisome Proliferator – Activated Receptor α) in the liver, which plays an essential role in the metabolism of HDL [55]. The PPAR nuclear transcription factors enhance the synthesis of main HDL proteins, thus facilitating RCT. In addition, PPAR-α boost the HDL capture by the liver [7]. PPAR-α are also involved in the regulation of inflammation processes, expression of cell adhesion molecules, and production of chemotaxis factors; in addition, they suppress the proliferation of smooth muscle cells and the activity of fibroblasts. Their activation may promote the regression of atherosclerotic plaques [7]. In people with a sedentary lifestyle, the prolonged low intensity aerobic exercises activate PPARγ and lead to a positive modulation of receptors CD36, transporters ABCA1 and ABCG1, which are directly involved in RCT [10].

Thus, physical exercises accelerate RCT, which is related to an increase in the concentration of apoA-I, activity of enzymes (LCAT, LPL), and expression of ATP-binding cassette transporters ABCA1 and ABCG1. Strengthening of physical activity improves the ability of HDL to activate eNOS and NO production, thus affecting the state of endothelium-dependent vasodilation. In addition, the indicated conditions increase the activity of PON-1 and the antioxidant activity of HDL subfractions.

Low-calorie diet. Nutrition plays a key role in the metabolism of lipoproteins, particularly HDL. Each kilogram of the body weight loss increases HDL-Chol by 0.01 mmol/l (0.4 mg/dl). Aerobic physical exercises, such as 25-30 km of sharp walk per week (or an equivalent activity), also promote weight reduction and increase the level of HDL-Chol by 0.08-0.15 mmol/l (3.1-6 mg/dl) [1]. In obese women, intense physical exercises decreased the body weight from 2.3 to 15.5 kg, on the average. A significant correlation was found between body weight losses and RCT [42].

A one-year randomized controlled study PREDIMED monitored the functions of HDLs after conventional antioxidant-rich Mediterranean diet. The study revealed an increase in RCT relative to the initial level, a decrease in activity of CETP, an increase in activity of LCAT and PON-1, and the ability of HDL to induce a release of NO in endothelial cells. Thus, the Mediterranean diet was shown to improve atheroprotective functions of HDLs [20]. In men with excess body weight and obesity, 12 weeks of a low-calorie diet decreased not only the body weight, but also the level of microRNA (miR-223) in HDLs. MiR-223 is represented by small endogenous noncoding RNA that are associated with metabolic disorders in the case of obesity [46].

A pronounced decrease in the body weight after bariatric surgery also restores the HDL functions. Bariatric surgery – the Roux-en-Y gastric bypass (RYGB) – is a surgical operation leading to a considerable decrease in the gastric volume and amount of absorbed nutrients. A retrospective observation of adult patients with obesity and type 2 diabetes showed a decrease in the frequency of macro- and microvascular complications of diabetes in the group of patients after RYGB [3]. It was demonstrated in experimental models that RYGB facilitates an early improvement of the HDL function, including their anti-apoptotic, antioxidant and anti-inflammatory activity as well as the ability to transfer cholesterol. In addition, RYGB rapidly restores endothelial dysfunction and enhances the ability of HDL to produce NO. The restoration of the HDL function was stable for a long time after bariatric surgery [36].

Thus, RYGB or a considerable decrease in the excess body weight achieved with the use of a low-calorie antioxidant-rich diet is aimed to increase the HDL level, enhance RCT and lower the activity of CETP. In the process, the activity of LCAT and PON-1 enzymes and the ability of HDLs to induce NO production by endothelial cells are also improved.

The effect of niacin (nicotinic acid – NA, vitamin B3). The main target organs for NA are liver and adipose tissue. In the liver, NA inhibits diacylglycerol acyltransferase 2, thus hindering the secretion of very low density lipoproteins (VLDL) and lowering the LDL level in blood plasma. Therewith, the level of HDL-Chol and apoA-I in blood plasma considerably increases by stimulating the synthesis of apolipoproteins in the liver [1].

An early assessment of the niacin monotherapy for reducing the CVD risk happened to be promising. In a randomized placebo-controlled study (Coronary Drug Project), the 15-year observation demonstrated the following advantages of NA monotherapy: it decreased the occurrence of CVD and reduced the risk of lethal outcomes [11]. According to the meta-analysis published in 2010, a long-term administration of NA decreased the frequency of cardiovascular complications by 25% [9]; in a combination with statins, it promoted the regression of atherosclerosis estimated from changes in the carotid artery intima-media thickness [6]. However, later studies did not confirm

the positive clinical effect of monotherapy with this preparation; moreover, the number of serious unwanted effects (hot flashes to the head, neck and upper part of the body) increased. Side effects of NA are attributed to the formation of a large amount of prostaglandin D2 (PGD2); as a result, the niacin therapy did not become popular [52]. The sustained release forms of niacin in a combination with laropiprant (a selective antagonist of the PGD2 receptor) have been developed to suppress the side effect. However, a study on the efficacy of the Tredaptiv (NA + laropiprant) preparation did not reveal additional advantages when the preparation was added to therapy with statins. Besides, in the group of patients cured with niacin and laropiprant, the development of insulin resistance was observed and the risk of diabetes increased by 32%. Therapy by the Tredaptiv preparation increased the risk of other side effects, including myopathy, gastrointestinal hemorrhage, stroke and infections [52].

Examination of healthy volunteers revealed that therapy with the (NA + laropiprant) preparation at a dose of 2 g/day during 16 weeks produced a typical effect on the lipid characteristics (HDL-Chol increased by 16%, LDL-Chol decreased by 20%, and TG decreased by 15%); however, apoA-I was replaced by SAA (Serum Amyloid A) in the composition of HDL [19]. The HDL particles enriched with SAA are dysfunctional, they lose atheroprotective properties of native HDLs and can act as proinflammatory agents [40]. A J774 macrophage culture and a primary culture of endothelial cells of bovine aorta were used to demonstrate that niacin does not affect the outflow of Chol from the cells. Niacin did not activate the phosphorylation of eNOS (Ser1179) and Akt (Ser473); therefore, it does not affect the ability of HDL to improve the function of endothelium. These facts may explain the absence of cardioprotective effect of the NA therapy [19].

By now, none of the medicinal preparations containing NA has been approved [52].

The role of CETP inhibitors. A glycoprotein that carries Chol esters (CETP) is synthesized in the liver and plays an important role in the transfer of eChol and TG between lipoproteins. Chol esters are transferred from HDL to potentially atherogenic VLDL and LDL particles, which are then removed with the use of LDL liver receptors (LDLR). The inhibition of CETP decreases the transfer rate of eChol from HDL to TG-rich lipoproteins, thus increasing the content of eChol in HDL. The inhibition of CETP or the CETP gene knockout in rabbits decreases the intensity of atherosclerosis of aorta and coronary arteries even at a diet with high Chol content [48]. A genetic polymorphism determining the low level or activity of CETP is accompanied by a higher concentration of HDL-Chol and a low concentration of LDL-Chol in blood, which reduces the risk of developing CHD. This formed a basis for the development of medicinal preparations belonging to the class of CETP inhibitors, the application of which was expected to reduce the risk of CVD complications [48,6,24].

Four CETP preparations have passed through phase III trials, but the results for two of them – dalcetrapib and torcetrapib – were negative. Although an increase in HDL-Chol and apoA-I (the main HDL protein) was observed, the ILLUMINATE study of CVD patients cured with torcetrapib was stopped prematurely. An electrolyte imbalance, an increased concentration of aldosterone in blood plasma, hypertension, and frequent heart attacks were observed in patients in comparison with the control group obtaining atorvastatin [5]. In the dal-OUCOMES study, therapy with the CETP inhibitor, dalcetrapib, did not reduce the risk of complications and mortality from CVD despite the absence of side effects in comparison with torcetrapib. This study was also stopped prematurely due to the evident ineffectiveness of the preparation [43].

The ACCELERATE study estimated the efficacy of a powerful CETP inhibitor – evacetrapib. Administered in a high dose (500 mg/day) as a monotherapy, it increased the HDL-Chol level and decreased LDL-Chol. However, the observation was also stopped approximately after two years because it was inefficient in reducing cardiovascular events [27].

The REVEAL study dealt with the action of anacetrapib. The use of anacetrapib increased the level of HDL-Chol more than twofold, and that of apoA-I by 36%. Efficacy and safety of the preparation were investigated during four years. No side effects of anacetrapib were observed in the study. Anacetrapib showed a moderate decrease in the main cardiovascular complications already in the first year of therapy and a considerable decrease during four years as compared to placebo [51]. The mechanism of the beneficial effect of anacetrapib is attributed to improvement of the qualitative composition of HDL. In persons cured with anacetrapib, HDLs increased RCT from the macrophages loaded with Chol by a factor of 1.5 – 2 [48]. Other inhibitors of CETP – evacetrapib and TA-8995 – increased the RCT rate by 34 and 50%, respectively [6]. It was noted that HDLs retain their anti-inflammatory and antioxidant properties during the administration of CETP inhibitors [6,51].

An important positive effect produced by CETP inhibitors is a decrease in the level of glycated hemoglobin, glucose and insulin resistance index (HOMA-IR). Thus, CETP inhibitors can prevent the prodiabetogenic effect of statins and niacin [30]. In this connection, investigation of the effect of various CETP inhibitors on the functional properties of HDLs is considered to be promising.

The effect of recombinant HDLs. The effect of the phospholipid apoA-I complexes with a low content of lipids, the so-called recombinant HDLs (rHDLs), was earlier studied using experimental models with animals. Intravenous infusion of rHDLs and an increase in the apoA-I level in transgenic animals were shown to enhance RCT from macrophages, which is accompanied by a pronounced regression of atherosclerosis in mice and rabbits [6,37,13].

Chen and co-authors [13] studied the effect of the CSL-111 preparation, which is a complex of native apoA-I with soybean lecithin. A single infusion of CSL-111 at a dose of 80 mg/kg decreased the volume and changed ultrasonic characteristics of a plaque [13]. Similar results were obtained in the case of four weekly infusions of CSL-111 at a dose of 40 mg/kg in patients with coronary atherosclerosis [44]. In patients after atherothrombotic stroke or acute myocardial infarction, four weekly infusions of 40 mg/kg CSL-111 increased the mobilization of endothelial progenitor cells, which led to restoration of endothelium and neovascularization [16]. In patients with atherosclerotic lesion of peripheral arteries of the lower extremities, even a single infusion of the preparation considerably decreased in 5–7 days the Chol content and expression of cell adhesion molecules (VCAM-1) in plaques [14]. Clinical trials of the improved CSL-111 species, the so-called CSL-112, are being performed now. Intravenous infusion of CSL-112 was shown to increase fourfold the ABCA1-mediated outflow of Chol [18].

rHDLs (CER-001), which are the modified negatively charged lipoprotein particles containing recombinant human apoA-I and natural phospholipids, have been proposed. In mice on a diet with high Chol content, CER-001 were shown to promote RCT [50]. In patients with familial hypo-alpha-lipoproteinemia, the CER-001 therapy not only increased the Chol content in HDL but also accel-

erated RCT. In addition, a considerable decrease in the activity of inflammation in aortic and carotid plaques as well as a decrease in the wall thickness of the carotid artery were found [26]. A similar effect was obtained for patients with homozygous familial hypercholesterolemia [23]. In patients with acute coronary syndrome (ACS), six weakly infusions of CER-001 at a dose of 3 mg/kg facilitated a decrease in the total volume of atheroma as compared to placebo [49,25,31].

Of particular interest are rHDLs containing apoA-I *Milano* (ETC-216), which differs from the wild-type apoA-I by arginine substituted for amino acid cysteine in position 173. The distinctive feature of this rHDL species is a longer removal from blood. The apoA-I *Milano* mutation was first detected in a cohort of Italians with low occurrence of atherosclerosis despite a very low level of HDL-Chol [47]. Intravenous infusion of ETC-216 substantially decreased the content of lipids in atheromatosis plaque and led to a rapid and pronounced regression of atherosclerosis in rabbits and mice [49]. Five weekly intravenous infusions of ETC-216 at a dose of 15 mg/kg to patients with ACS also produced a significant decrease in the volume and average maximum thickness of atheroma of the coronary vessels [35]. It should be noted that in some studies the infusion of rHDLs with apoA-I *Milano* did not exert a distinct positive effect. Thus, in the studies carried out in 22 hospitals of Canada and Europe, intravenous infusion of the standardized rHDL containing apoA-I *Milano* (MDCO-216) to the patients with ACS who received statins did not lead to a regression of atherosclerotic plaques [31].

A new rHDL species, which was called tetranectin-apoA-I (TN-apoA-I), is being tested now. This species was created by merging three apoA-I molecules with human protein tetranectin and phospholipids in different ratios. The TN-apoA-I complex is not filtered from blood by kidneys and has a longer half-life, which potentially increases its efficacy. It was shown that TN-apoA-I stimulates RCT, activates LCAT and exerts anti-inflammatory effect [37]. In rabbits, a single intravenous infusion of TN-apoA-I stabilized atherosclerotic lesions due to a considerable decrease in the migration of monocytes and the content of macrophages in carotid artery plaques. The quantitative estimation of RCT in rabbit plasma samples, which was carried out using the mouse macrophage line J774, revealed that TN-apoA-I significantly increases the Chol outflow via the ABCA1-mediated route. In addition, the infusion of rHDL considerably decreased the endothelial expression of cell adhesion molecules (VCAM-1, ICAM-1 and MCP-1), exerting the anti-inflammatory effect and protecting the endothelial cells [37]. rHDLs are supposed to induce an antioxidant protein 24-dehydrocholesterol reductase (DHCR24) in endothelial cells, thus enhancing their anti-inflammatory properties [41].

Therefore, studies demonstrate that rHDLs are the efficient Chol acceptors of macrophages, increase RCT and exert anti-inflammatory action on the artery wall.

The use of apoA-I mimetics. The apoA-I mimetic peptides are the artificially synthesized peptides possessing the biological properties of native apoA-I. The most studied and efficient preparation among mimetic peptides is D-4F, which comprises 18 amino acids, including four phenylalanine (F) residues. This peptide is not destroyed by digestive enzymes and can penetrate into plasma at oral administration [54, 15]. D-4F shows antiatherogenic effects similar to native HDLs: it accelerates the formation of pre-β-HDL, increases RCT, enhances the expression of eNOS and the production of NO, and suppresses the development of oxidative stress. The mechanism of D-4F antioxidant action is associated with the activation of heme oxygenase 1 (HO-1) enzyme, which neutralizes reactive oxygen species induced by the oxidized LDLs, which prevents the development of apoptosis in endothelial cells and promotes the restoration of their functions. It was shown that D-4F facilitates the migration and reparation of HAEC by inhibiting the expression of cell adhesion molecules and monocyte chemotactic protein 1 (MCP-1) [28].

RVX-208, which is also a small synthetic molecule, has been developed at the Resverlogix Corporation laboratory (Calgary, AB, Canada) for the treatment of ACS, atherosclerosis and Alzheimer's disease. A peroral preparation RVX-208 selectively activates the nuclear transcription factor, which increases the hepatic and intestinal production of apoA-I. In HepG2 cells, the RVX-208 preparation induced the synthesis of mRNA of apoA-I and protein, thus increasing the level of pre-β-HDL and α-HDL [34]. In African green monkeys receiving RVX-208 (at a dose of 7.5, 15 and 30 mg/kg twice a day or 60 mg/kg once a day during two months), the level of apoA-I and HDL in blood serum increased by 57 and 92%, respectively. Therewith, the incubation of macrophages in the presence of serum taken from these animals enhanced RCT [4]. The Ib/IIa phase clinical study demonstrated safety and good tolerance of RVX-208. In healthy volunteers, this preparation considerably increased the amount of apoA-I and pre-β-HDL and intensified the outflow of Chol from macrophages [34]. Unfortunately, patients with CHD, in contrast to monkeys, did not show a considerable increase in the apoA-I level even when a high dose of RVX-208 was administered. In addition, high doses of the preparation increased threefold the level of serum transaminases. A promising result of this study was the revealed increase in RCT (approximately by 20%), which was caused most likely by rapid maturation of HDLs [32].

Thus, an increase in the apoA-I level at intravenous infusion of rHDL or apoA-I mimetic peptides produces a beneficial effect on RCT, the function of endothelium and the regression of atherosclerotic plaque. The improvement of the function of endothelium under the action of apoA-I may proceed via the activation of intracellular signaling pathways associated with the regulation of inflammation and apoptosis.

Conclusion. Experimental and clinical studies have demonstrated that a significant role in pathogenesis of atherosclerosis and, hence, cardiovascular diseases is played by the modified or dysfunctional HDLs. Such HDLs lose their antiatherogenic, antioxidant and anti-inflammatory properties. Several approaches to restoring the lost functions of HDL are known. Some of them are simple but quite effective: intensification of physical activity and a diet promoting a decrease in excess body weight. In recent years, it was proposed to correct lipoprotein metabolism with the use of CETP inhibitors, apoA-I mimetic peptides, rHDLs and HDLs containing apoA-I *Milano*. Such therapeutic approaches make it possible not only to increase the level of HDLs and apoA-I in blood plasma, but also to enhance their antiatherogenic action by accelerating RCT, increasing the activity of LCAT and PON-1 enzymes, and restoring the functions of vascular endothelium. However, additional clinical trials are required to estimate safety and efficacy of these approaches.

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POINT OF VIEW

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«RIGHT NOT TO KNOW» AS AN ETHICAL PRINCIPLE FOR DNA TESTING OF LATE-ONSET DISEASES

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The article examines the ethical principle - the "right not to know", associated with DNA testing of diseases with late onset of development, based on the materials of foreign publications. For geneticists and doctors of the Republic of Sakha (Yakutia), this problem will require discussion and decision-making, since type I spinocerebellar ataxia - a hereditary late manifestation disease, DNA testing of which has been used in practical medicine of the republic since the 2000s - is widespread in the population. Huntington's chorea is the most researched hereditary disease on bioethical issues. According to experts, it is necessary to update the recommended testing guidelines for Huntington's chorea in the context of the principle of “right not to know” with a joint committee of geneticists, neurologists, and legal and ethical experts.

Keywords: bioethics, right not to know, DNA testing, prenatal diagnostics, Huntington's chorea, spinocerebellar ataxia type 1.

Introduction. The rapid development of molecular genetic research, high-throughput methods of genome sequencing and the widespread use of DNA diagnostics of various diseases is, undoubtedly, a mark of progress of science and practical medicine, but on the other hand, this further aggravates ethical problems of interference with the human genome, such as the autonomy of the individual, confidentiality of genetic information, moral and psychological consequences for the individual with a complex choice of decisions related to DNA testing [2,9, 36].

Throughout life, an individual can resort to various types of genetic testing, depending on the goals that he sets for himself: with the need to conduct DNA testing to find out the cause of his disease or detect a hidden genetic health issue, testing to establish kinship, testing to determine the compatibility of body tissues, to predict the tolerability of different drug options. Most often, people turn to medical genetic testing for diagnosing hereditary pathologies. Currently, with the help of DNA testing, a huge number of various diseases and predispositions are diagnosed [32,39]

In Russia, a small number of publications of a philosophical nature are devoted to the problem of the “right not to know”, in particular, there is an opinion that the predictive direction of medicine, based simultaneously on universal biological laws and personalized genetic preclinical diagnosis of potential pathologies of a particular person, will inevitably put the individual in a difficult moral and existential situation [6]. A rethinking of the currently established ethical and normative attitudes is taking place, taking into account the new possibilities of genomic medicine, and we are not talking about a return to paternalism, but asserting the need for a broader concept of autonomy, taking into account the existing restrictions on informing and understanding the family specifics of genetic information [1].

The purpose of this article is to discuss the ethical "right not to know" principle associated with DNA testing for late-onset diseases. For geneticists and physicians, this problem will definitely require discussion and decision-making, since In the Republic of Sakha (Yakutia), a hereditary disease with a late onset of development is widespread - type I spinocerebellar ataxia (SCA1), DNA testing of which has been used in practical medicine of the republic since 2000. Our research experience in the field of ethics of genetic counseling and DNA testing has revealed a complex layer of social, legal and psychological problems that require close attention of specialists [4,37].

The «Right not to know» Principle and DNA Testing of Hereditary Diseases. There are fundamental ethical principles associated with DNA testing of hereditary diseases: non-directiveness of genetic counseling, respect for individual autonomy, preservation of confidentiality of genetic information of any kind, the principle of fairness and awareness [2,

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27]. For a long time, the doctor has been the patient's confidant, who entrusted him with his health. The professionalism of medical workers lies in their competence, the ability to protect the interests of not only their patients, but also the public interests, so as not to lose the trust of society in medicine as a whole [34].

In the last decade, with the onset of the "genomic era", there has been some transformation of established ethical rules. The most discussed and challenging for doctors and geneticists is the ethical principle of "the right not to know" [10,11]. Legal researcher Andorno (2004) writes: "The 'right not to know' statement may seem strange. Over the past decades, it has been strongly emphasized that the patient has the right to be informed about the risks and benefits of treatment or intervention and, on this basis, to give consent to them or not. Having reaffirmed the "patient's right to know" as a fundamental ethical and legal principle, we are now faced with a clearly opposite requirement. This happens, in particular, in the field of genetics: as the predictive power of genetic tests increases, more and more people learn that they are at risk of serious disease without any real chance to reduce this risk or receive effective treatment "[7,22].

Recent research in cognitive psychology has shown that people often prefer not knowing complete information. For example, a recent study showed that 85-90% would not want to know in advance what negative events will hit them in the future (eg, cause of death, divorce) [23]. However, the preference for not knowing about potentially threatening upcoming life events seems to be less pronounced in the context of genetic testing; genetic testing is generally positively assessed by the public [18]. It has been shown that the majority want to know about their results and that there is little difference between information about risk (e.g. information about the status of the carrier) and information about a possible diagnosis (e.g. about the onset of dementia) [15, 40]. Interestingly, some patients in the 50% risk group for Huntington's disease (HD) wanted to know their genetic status with any, even a positive result, according to them, they could plan further work and outline priorities in life [30,31,42]

The ethical principle of the "right not to know" is recognized in international and national legislation. According to the acts and conventions: - "everyone has the right to know any information received about his health, the wishes of individuals not to be informed about this must be respected"; "The patient has the right not to be informed at his / her direct request, unless it is required to protect the life of another person"; "The right of every person to decide whether or not to be aware of the results of genetic examination and the consequences arising from this should be respected" [30,31,41].

Nowadays, the entire human genome can be quickly sequenced and analyzed at a constantly decreasing financial cost. Such high-performance methods are very likely to lead to random findings and conclusions [21]. In a study by Hofmann, 2016, this is defined by the expression "incidental findings of uncertain significance" (IFUS) - random inferences of uncertain significance. As an example, consider a case published in The New York Times in 2014. *"Jennifer was 39 years old and she was perfectly healthy, but her grandmother died young from breast cancer, so she decided to get tested for mutations in two genes known to increase the risk of the disease. When a genetic consultant suggested additional tests for 20 other genes associated with various types of cancer, Jennifer said yes. "The more information the better", - she thought. The results, she said, were "surreal." She did not have mutations in her breast cancer genes, but one of the genes was associated with a high risk of stomach cancer. In people with a family history of the disease, this mutation is considered so risky that patients who are not even sick are often advised to have their stomachs removed. But no one knows what this discovery might mean in someone like Jennifer, whose family did not have this disease "[24].* As you can see, genomic technologies are producing unexpected finds, such as cancer susceptibility genes, that have clinical implications for those tested and their families. Whether people or their families who were seeking for a test are ready to know these results remains questionable [16, 22, 26]. In a Canadian sample of patients from burdened families with HD, a three-year program of predictive and prenatal DNA testing for HD was conducted - 88% of patients from this sample refused the predictive DNA test [17]. In another study, when questioning participants in a hypothetical scenario on the problem of presymptomatic diagnosis of incurable diseases, 50% of respondents would not want to receive negative information about their health, including the diagnosis of HD. The reasons were as expected - incurability of the disease, fear of disability, possible depression and stress [43]. Melnyk (2012) showed that: lack of resources for coping with the disease, expected regret and learning about uncontrolled predictors were associated with avoidance of information about the risk of breast cancer [16,33]. In other reports, the specific decision on the need to find out genomic information by an individual in hypothetical scenarios representing cases of disabling diseases with late onset was clearly predetermined by the characteristics of the disease scenario, namely "ability to control the disease" and "DNA test accuracy" [12,22].

Failure to disclose positive results can be problematic for both genetics physicians and those conducting genetic testing. It is a difficult ethical situation when they work within medical ethical boundaries based on the principles of autonomy, charity and fairness [27, 38].

The ethical principle of the "right not to know" is also linked to the problem of DNA testing of minors. In studies of bioethical problems of medical and genetic counseling of patients at risk of SCA1, several precedents of presymptomatic DNA testing of minors in families with SCA1 have been described. One of them was associated with the mother's understandable desire to protect her daughter from psychological stress during her future admission to a higher educational institution. The mother believed that if her daughter is a carrier of the SCA1 mutation and becomes ill in adulthood, then it makes no sense for her to make efforts to get higher education. With this attitude, the girl's mother asked to reveal the results of DNA testing to her, but the request was refused, because this precedent was regarded as involuntary discrimination within the girl's own family. The next case was connected with the transfer of a boy from boxing to a less traumatic sport at the convincing request of his parents, who were very worried about the result of the DNA test and the health of their son. Despite the principle of non-disclosure, doctors had to satisfy the parents' request to disclose the child's genetic status. The cases described reveal complex ethical problems, the solution of which depends on the level of education of the parents, on the material support of the family and on many different nuances that doctors may not know about. It is not excluded that a family has a special attitude towards a child, the possibility of discrimination in obtaining education or in the field of insurance. Premature disclosure of his genetic status to a child can lead to a loss of confidence, self-identification in society, distortion of values and goals in life [8, 20, 29].

«Right not to know» in prenatal diagnosis of diseases with late onset of development. Since 2002, in clinical

medicine of the Republic of Sakha (Yakutia), prenatal diagnosis (PD) of type I spinocerebellar ataxia has been carried out in compliance with the fundamental bioethical principles: full informing the family about the PD procedure, the priority right of the pregnant woman to decide on the fate of the fetus, performing the PD procedure in the early stages up to 12 weeks pregnancy, patient autonomy and confidentiality [29]. However, we do not exclude the emergence of more complex ethical situations in PD SCA1 associated with presymptomatic DNA testing of carriers of the mutation in the *SCA1* gene.

For a more complete disclosure of the issue, let us turn to the research of ethical problems of PD of another neurodegenerative disease - Huntington's disease (HD), which also belongs to the group of monogenic diseases with dynamic mutations and late onset of development, like SCA1. The case described by Erez et al., (2010) raises an important ethical question: is there a right to genetic ignorance (the right not to know) when it puts others (partner) at risk of unnecessary medical procedures.

A 34-year-old woman went to an antenatal clinic for genetic counseling. She was 12 weeks pregnant and recently found out that her husband's father had HD. After receiving genetic counseling, she and her husband underwent prenatal fetal testing. The results of the examination of the husband showed that he had no risk of developing HD and, as expected, the fetus also had no risk of developing HD. This is a case of familial HD, when a proband at risk chose not to know his disease status, but wanted to know the status of his unborn child. Three years later, the couple returned to antenatal consultation to receive pre-implantation genetic diagnostics (including in vitro fertilization) for future pregnancies, while the proband's spouse had to undergo an unnecessary procedure and, accordingly, an unjustified risk to her health.

In this case, doctors are faced with a dilemma of how to use the accumulated experience in favor of the patient, without subjecting him to unnecessary procedures and at the same time try to disclose information useful for the family in the most delicate way in order to make it possible to make the most correct decision on the procedure for prenatal testing and further prospects[21,35].

HD is not only the most studied hereditary disease on bioethical issues, but also the most advanced in terms of interaction of researchers and doctors with the Association of HD burdened families and patients with [35].

Historically, back in 1985 in France and in 1989 in Canada, an international group for the study of Huntington's Disease at the World Federation of Neurology discussed bioethical and legal issues related to scientific research in HD. As a result, a set of ethical principles and rules for presymptomatic DNA testing of HD was adopted. These rules were recommended to be followed not only by doctors - geneticists and specialists performing DNA testing, but also by patients at risk for HD, since the interests of both the doctor and the person being tested were respected. Later, significant progress was made in the development and implementation of direct DNA diagnostics in medical practice, this analysis has become a routine procedure. In 1994, an expanded and refined protocol of DNA testing and medical genetic counseling for HD became the main document for specialists in many countries, where it was possible to organize molecular genetic laboratories [14, 25, 28].

Now, in the era of genomics and the application of high-throughput genome sequencing, experts believe it is time to return to the issue of updating the recommended guidelines for HD testing by a joint committee of geneticists, neurologists, and legal and ethical experts. It is proposed to focus the attention of specialists on the following points: the key specialists in the consultation process should be: a geneticist, psychologist, and neurologist. In order to reduce the risk of unnecessary testing on the parent and fetus, it is imperative to confirm the diagnosis of HD in the family. Most genetic tests are most informative if a clinically affected family member is tested first before using the test to predict genetic status for a clinically unaffected family member. When considering prenatal testing, the procedure and cell type affect the interpretation of the results. In HD, there is a category of intermediate alleles that can potentially spread to a range of diseases within a single generation. Genetic counseling of the highest standards should be available in every country and provided by a specialized genetic counseling unit. Joint discussion of the issue between the consultant and the consulted should be aimed at obtaining free and informed consent of the test taker to conduct DNA testing. Obtaining a positive DNA test result should not be an obstacle to childbearing if the tested person has made a decision to prolong the pregnancy [35].

Conclusion. The “right not to know” is widely discussed in foreign literature, there are both supporters and opponents of this ethical principle. The arguments “for” the compliance with the principle are, first of all, the fears of violating basic ethical principles, namely, the rights and autonomy of the individual. There will be an inevitable increase in paternalism in medical practice, as well as the loss of the principle of confidentiality of genetic information. It will be impossible to assess the moral and psychological suffering of the patient, especially when he undergoes pre-symptomatic DNA testing of an incurable disease. On the other hand, experts in this field - opponents of the principle of "the right not to know", believe that the patient should be fully informed about any disease, hiding data that is important to relatives also violates their rights and risks unnecessary procedures, such as prenatal or pre-implantation diagnostics.

Talks and discussions will continue, the ethical rules of DNA testing, whole genome DNA sequencing and related "random findings" of the human genome will be researched. Bioethical research is especially relevant in the Republic of Sakha (Yakutia), where routine DNA testing and prenatal DNA diagnostics of late-onset hereditary diseases are performed.

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CONNECTIVE TISSUE DYSPLASIA AS A CAUSE OF GASTROESOPHAGEAL REFLUX DISEASE: A CASE REPORT

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The article presents a clinical case of detecting gastric metaplasia of the esophageal mucosa in combination with anatomic and physiological changes in the organs of the gastroduodenal zone in a young woman with no bad habits, hereditary burden and other risk factors for reflux disease.

Pronounced changes in the osteoarticular apparatus in combination with changes in the skin, muscles and disorders of the internal organs suggest that this patient may have connective tissue dysplasia.

The patient underwent endoscopic ablation of the pathologically altered esophageal mucosa, followed by the appointment of an esophageal protector in order to maintain a stable remission.

Keywords: Gastroesophageal reflux disease, Barrett's esophagus, gastric metaplasia, reflux esophagitis, connective tissue dysplasia.

Introduction. Gastroesophageal reflux disease (GERD) has become one of the important problems of recent years in the modern clinic of internal diseases. A decisive role in the pathogenesis of the disease is played by a violation of the motor-evacuation function of the upper organs of the gastrointestinal tract, namely, insufficiency of the lower esophageal sphincter and dysfunction of the phrenicoesophageal ligament [9]. Frequent refluxes into the esophagus of gastric, and in some cases of duodenal contents contribute to damage to the mucous membrane of the distal esophagus with the development of catarrhal or erosive-ulcerative esophagitis, and in some patients with cylindrical cell metaplasia and the appearance of clinical symptoms that worsen the quality of life [9]. According to some authors, one of the possible causes of GERD can be considered connective tissue dysplasia (CTD), in which pathological structural changes can occur, leading to dysfunctions of internal organs and body systems. [7,8,11].

According to clinical guidelines, CTD is distinguished into differentiated and undifferentiated. The primary defect in collagen synthesis, which is characterized by a characteristic type of inheritance and a vivid clinical picture, is manifested by the syndromes of Marfan, Ehlers-Danlos, osteogenesis imperfecta, "flaccid skin", etc., which are referred to as differentiated CTD, while, with undifferentiated CTD, there may be organ manifestations without clear symptoms and morphological changes in the affected organs [2,4,11].

In the literature, reports are more common about the influence of undifferentiated variants of dysplasia on the course and manifestations of the pathology of the digestive tract, mainly in children and adolescents [8,11]. At the same time, as the researchers note, the problems of diagnosing undifferentiated connective tissue dysplasia lie in the variety of phenotypic features and the absence of uniform diagnostic criteria [8,11].

Purpose of the study. We analyzed the clinical case of detecting gastric metaplasia of the esophageal mucosa in combination with anatomophysiological changes in the organs of the gastroduodenal zone in a young woman (31 years old) with no bad habits, hereditary burden and other risk factors for reflux disease.

Material and research methods. We present the interesting clinical observation of a young patient (woman, 31 years old), she was urgently hospitalized in the emergency department of the Republican Hospital No. 2 of the Center for Emergency Medical Aid in 2019 with a characteristic clinic of exacerbation of chronic pyelonephritis. Against the background of antibiotic therapy, the patient developed aching pains in the epigastrium and in the right hypochondrium, aggravated after eating, a constant feeling of nausea, sometimes vomiting of stomach contents, and therefore was consulted by a gastroenterologist.

In addition to the above complaints, a detailed survey revealed that the patient constantly noted heartburn, regurgitation and belching with air, aggravated after eating, with a change in body position during the day, including at night. Recently, she has been worried about the burning sensation of the tongue, poor tolerance of hunger (the occurrence of headaches, weakness).

Medical history: The patient has been ill since childhood. She notes that there was always belching with air, sometimes regurgitation. Also, from an early school age, periodically after an error in the diet (eating fatty foods), she had vomiting of bile. She first sought medical help in 2010, when, at the 26th week of pregnancy, after taking fatty foods, severe heartburn, repeated vomiting of bile, and fever arose. The patient was taken urgently to the department of pathology of pregnant women. Against the background of infusion treatment and adherence to the diet, the state of health improved. Constant heartburn, epigastric pain, aggravated after eating, increased frequency of belching with air appeared in 2013. She lost three kilograms in a year. She went to the local polyclinic. Endoscopic examination revealed reflux esophagitis, superficial gastritis (protocol not provided), treatment was prescribed with omeprazole 20 mg per day for three weeks. At the end of the course of treatment, epigastric pains subsided, heartburn and belching became less frequent and unexpressed. In September 2017, epigastric pain and persistent heartburn reappeared. Along with the above symptoms, for the first time, the patient began to notice a constant feeling of nausea, periodically ending with vomiting, soreness and discomfort in the throat, and hoarseness. Esophagogastroscopy revealed focal hyperemia of the esophageal mucosa of the lower third of the type of "tongues of flame" and targeted biopsy was performed for morphological verification of structural changes

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in the esophageal mucosa. The result of histopathological examination: stratified squamous epithelium with areas of transition to the epithelium of the gastric type with moderate lymphoplasmacytic infiltration of the stroma. After a three-week course with proton pump inhibitors in a standard dosage, the patient noted an improvement in well-being. A year later, heartburn at night, a constant burning sensation of the tongue, began to disturb again. She was treated by a dentist for inflammation of the papillae of the tongue and deterioration of the teeth. She took antacids on her own and did not seek specialized medical help. She associates a real deterioration in well-being with antibiotic therapy for exacerbation of pyelonephritis.

Life history: The patient grew and developed according to her age. At the same time, she differed from her peers in flexibility, tall stature and slenderness. Work is associated with psycho-emotional overload. No bad habits. Eating irregular, unbalanced, sufficient calories.

On the maternal side, heredity is not burdened by connective tissue disorders, the presence of associated anomalies and malformations, cardiovascular accidents and oncological diseases, on the paternal side this is unknown. The family has two children. According to the patient, the brother is tall, slender, and suffers from spinal scoliosis.

Chronic diseases: OU myopia medium, mitral valve prolapse, hypotonic vascular dystonia, hypokinetic biliary dyskinesia, chronic pyelonephritis.

Gynecological history: Pregnancy - 1, childbirth - 1, on time, fast, spontaneous.

Data of examination, research and their discussion: General condition is satisfactory. Asthenic body type. Height 170 cm, weight 56 kg. BMI 19.4 kg / m². Musculoskeletal system: asthenic chest, spinal scoliosis, hallux valgus. Hypermobility of the interphalangeal joints of the hands. Muscle hypotrophy and hypotonia. The skin is pale in color, thin translucent, the turgor is reduced. The tongue is moist, with unevenly pronounced papillae, the root is coated with a white bloom. The abdomen is regular in shape, with superficial palpation, soft, moderately painful in the epigastrium. The tapping symptom is weakly positive on both sides. Physiological functions are normal. The rest of the organs and systems are unchanged.

According to laboratory data, moderate hypoproteinemia (total protein 64 g / l, with a norm of 66-83 g / l), indicators of fat and carbohydrate metabolism without deviations. In general blood tests, ESR is 20 mm / h. The content of micro- and macronutrients, the study of atrial sodium uretic peptide has not been carried out.

In dynamics after 2 years on esophagogastroscopy, pronounced hyperemia in the form of "tongues of flame" occupies circularly the entire mucous membrane of the lower esophagus with proximal spread of various lengths and morphologically confirmed gastric metaplasia, without epithelial dysplasia.

Computed tomography of the abdominal cavity and retroperitoneal space using bolus contrast enhancement did not reveal any pathological changes.

X-ray examination of the upper gastrointestinal tract with contrast revealed no closure of the cardia; with the Trendelenburg test, a reverse throw of contrast from the stomach into the esophagus was noted. Elongation of the stomach, its lower pole is located above the entrance to the small pelvis, at the -S1 level. Conclusion: X-ray signs of reflux esophagitis. Insufficiency of the cardia. Grade 2 gastroptosis (Fig.).

Diagnosis of the functional state of the esophagus and the esophageal-gastric junction using intraesophageal daily pH-metry, pH-impedance and high-resolution manometry has not been carried out, due to the lack of research methods in the hospital.

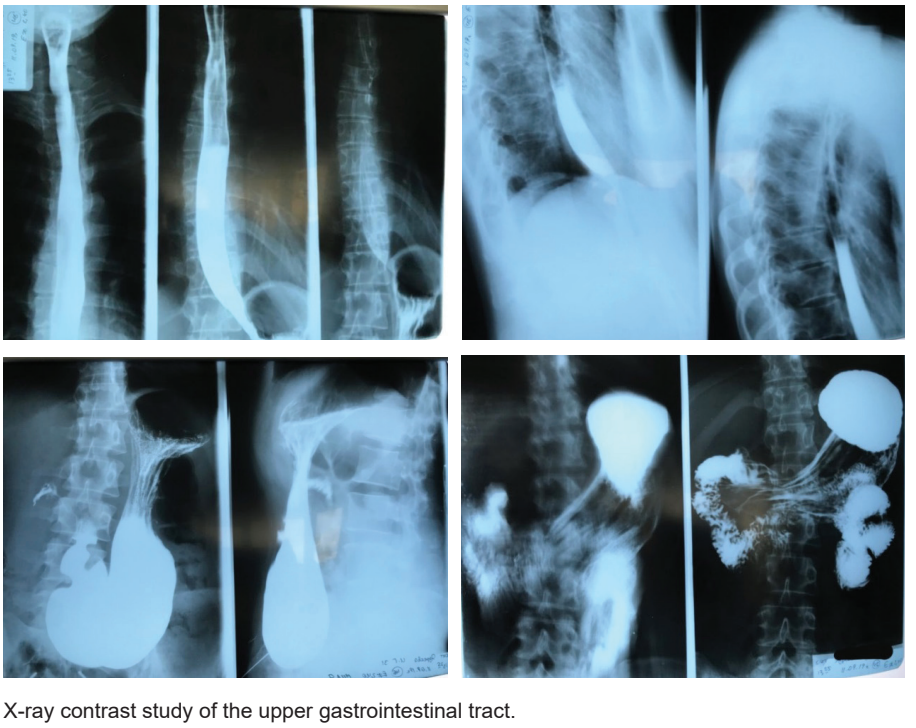
On the basis of complaints, history, physical examination, laboratory and instrumental studies, the diagnosis was made: Gastroesophageal reflux disease. According to the Los Angeles classification of reflux esophagitis, grade D. Barrett's esophagus. Gastroptosis 2 tbsp. Background: Undifferentiated connective tissue dysplasia.

The patient was given dietary and lifestyle recommendations, medicinal therapy was started with use of a proton pump inhibitor, prokinetic and antacid, the purpose was to effectively treat clinical symptoms and maintain a stable remission of the diseases. Of the proton pump inhibitors, dexlansoprazole was selected with a prolonged antisecretory effect with a single dose regardless of food intake [9], it provided effective control of the nighttime symptoms of heartburn in this patient. At the same time, irregular medication intake, non-compliance with dietary and lifestyle recommendations affected the quality of therapy.

According to the literature, with gastric emptying due to deceleration of motility, disorders of sympathicotonia, duodeno-gastric and gastroesophageal refluxes may occur, leading to destructive changes in the mucous membrane of the gastroesophageal zone [7,8,11].

Given the young age, pronounced gastroptosis, non-adherence to drug therapy, the patient was consulted by a gastro-surgeon and endoscopic ablation of the pathologically altered esophageal mucosa was proposed, after which the clinical manifestations of reflux disease were relieved within a year, and in order to maintain stable remission, the esophagoprotector alfazox was prescribed in a standard dosage.

The American Gastroenterological Association (AGA) and the American Society of Gastroenterological Endoscopy



X-ray contrast study of the upper gastrointestinal tract.

(ASGE) propose radiofrequency ablation for Barrett's esophagus in patients at high risk of developing adenocarcinoma and with hereditary oncological burden.

However, the American College of Gastroenterology (ACG) and the European Society for Gastrointestinal Endoscopy (ESGE) do not recommend endoscopic ablation in patients without dysplasia due to possible complications and high cost [1,3,10].

The article presents a clinical case of detecting gastric metaplasia of the esophageal mucosa in combination with anatomophysiological changes in the organs of the gastroduodenal zone in a young woman (31 years old). She did not have bad habits, hereditary burden and other risk factors for reflux disease.

The revealed pronounced changes in the osteoarticular apparatus, such as an asthenic type of constitution, deformation of the chest, spine, feet, hypermobility of the joints, in combination with changes in the skin, muscles and disorders of the internal organs - the heart, organs of vision and the digestive system, suggest that this patient may have connective tissue dysplasia. According to the patient, the manifestation of symptoms was noted already in childhood and, especially, in adolescence, it did not greatly affect the quality of life, after 25 years the severity of clinical symptoms of pathologies of the digestive system increases, which are confirmed by endoscopic and histological diagnostic methods, which is consistent with literature data [2.4-8.11].

To clarify the background disease of the patient, we need to conduct an in-depth comprehensive clinical, genealogical, laboratory instrumental and molecular genetic studies with the determination of biochemical diagnostic markers of connective tissue dysplasia, in particular, hydroxyproline [2,4].

When confirming undifferentiated CTD, taking into account the progressive course of the disease, which affects the patient's quality of life, dispensary observation is recommended with laboratory and instrumental research methods, depending on the leading clinical syndrome [2,4].

Conclusion: In the presented observation, clinical, endoscopic and morphological signs dominate, they are specific for Barrett's esophagus without epithelial dysplasia in combination with anatomophysiological changes in gastroduodenal organs. Changes in the osteoarticular apparatus, skin, muscles in combination with disorders of the internal organs suggest that this patient may have connective tissue dysplasia as the basic factor of reflux disease. In a standard dosage, the esophagoprotector Alfazox was prescribed to maintain stable remission and prevent epithelial dysplasia.

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MULTISYSTEM INFLAMMATORY SYNDROME IN CHILDREN AND ADOLESCENTS IN YAKUTIA

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Generally, COVID-19 is milder in children than in adults. But already in May 2020, information about a multisystem inflammatory disease that affects children and adolescents began to arrive from around the world. The pathogenesis and pathological picture of COVID-19 are characterized by the development of thrombus inflammation, generalized microangiopathy in the form of destructive-proliferative viral vasculitis and coagulopathy with secondary damage to the skin, internal organs, central nervous system, hemophagocytosis. This paper presents the observation of ten patients aged from 6 months to 14 years treated at the Children's Clinical Infectious Diseases Hospital in Yakutsk from September 2020 to March 2021 with a diagnosis of U07.2 - Coronavirus infection caused by COVID- 19, no virus identified, M30.3 Kawasaki-like syndrome. The presence of prolonged fever, damage to two and whiter systems of the body, laboratory markers of inflammation without obvious foci of an acute infectious process was the criteria for making a diagnosis. Also, a prerequisite for establishing a diagnosis was the presence of contact with patients with COVID-19 or antibodies to SARS-CoV-2. There were no patients with complicated cases that required intensive care among the observed patients, but there were severe cardiac lesions in some children. All our patients were of Yakut nationality. In contrast to the available literature data, we did not observe a significant violation of the blood coagulation system; in some patients, thrombocytosis was prominent. We observed a subarachnoid haemorrhage in one patient.

Keywords: multisystem inflammatory syndrome, children, fever.

Introduction. Symptoms of the multisystem inflammatory syndrome (MIS) are similar to those of Kawasaki disease. Kawasaki disease characterized by self-limiting vasculitis. As a rule, the disease affects only children, and a prolonged increase in body temperature is its first sign [1,2]. The new syndrome is called pediatric multisystem inflammatory syndrome or MIS. It is now clear that this condition is a delayed consequence of new coronavirus infection. According to the WHO recommendations, preliminary criteria for recognizing a case of MIS require the presence of at least one of the following two symptoms: rash; hypotension or shock; cardiac arrhythmia; signs of coagulopathy; acute gastrointestinal symptoms; elevated markers of inflammation without obvious microbial causes of inflammation; infection with coronavirus or direct contact with patients with COVID-19 [3]. They indicate that the time interval from contact with a Covid-19 patient can range from 6 to 51 days, and in most cases, children with MIS had antibodies to SARS-Cov-2 [2]. The most frequently affected gastrointestinal tract (92%), cardiovascular (80%) and respiratory system (70%), haematological changes had 76% of patients [1,2]. Also, several authors note a high frequency of MIS cases among the Mongoloid and Afro-Caribbean population, mainly boys who are ill [4]. Intravenous immunoglobulins and systemic corticosteroids are effective treatments [2,4]. The incidence of confirmed SARS-CoV-2 infection in children and adolescents was 322 per 100,000 people in this age group, and MIS-C - 2 per 100,000 people [5]. In 2020, 6098 children were diagnosed with Covid-19 in the Republic of Sakha (Yakutia), 857 of them needed inpatient treatment.

The purpose of this study is an analysis of clinical and laboratory data of multisystem inflammatory syndrome in children, determination of the features of the course of this disease in patients of Yakut nationality.

Research methods and materials. We observed 10 patients with the multisystem inflammatory syndrome. The criteria for establishing the diagnosis were: severe condition, fever for at least 48 hours, damage to 2 or more body systems, laboratory signs of an inflammatory process, signs of a new coronavirus infection (determination of antibodies to SARS-Cov-2 IgG class by enzyme-linked immunosorbent assay, detection of the virus by polymerase chain reaction), contact with patients with Covid-19.

Results and discussion. Between September 17, 2020, to March 19, 2020, we observed 10 patients with multisystem inflammatory syndrome, of which nine were boys and one girl. The ages of the children were very different: 6 months, 1 year, 5 years - 2 children, 6 years old, 7 years old, 8 years old, 10 years old, 13 years old and 14 years old. All patients were of Yakut nationality (Sakha). When analyzing the epidemiological history, the fact that six patients had family contact with patients with Covid-19. The terms of contact were: 14 days (in 2 cases), 1 month (3 cases) and 1.5 months (1 case). The PCR examination took place among contacted persons, results were negative in all cases. It worth noticing that in all cases, the diagnosis of Covid-19 in family members was laboratory confirmed. Three children had clinical signs of ARVI, and two of them also had anosmia, which was short-lived and stopped within 2 weeks. The course of ARVI in everyone on the usual basic therapy had no complications, and recovery came on the 5-7th day. Three patients showed no clinical signs of the disease at the time of contact. One patient, 11 days before this hospitalization, underwent inpatient treatment with a diagnosis of "Coronavirus in-

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fection caused by the COVID-19 virus." Three patients had no history of contact with COVID-19 patients. At the time of admission to the children's infectious diseases hospital, all patients by ELISA determined antibodies to SARS-Cov-2 of the IgG class in titers above 20. It was not possible to isolate the virus genome by PCR in any patient.

Children were admitted to an infectious diseases hospital with referral diagnoses of acute respiratory viral infections in 7 patients and two with a diagnosis of acute respiratory infections. One boy was admitted with a referral diagnosis "Coronavirus infection caused by the COVID-19 virus, no virus identified." He was initially hospitalized in the Central District Hospital with a diagnosis of Kawasaki Syndrome, the examination revealed antibodies to SARS-Cov-2 of the IgG class, and he was transferred to the DIKB in Yakutsk. In all cases, the onset of the disease (multisystem inflammatory syndrome) was acute - with a rise in body temperature from 38.4 ° C to 40.2 ° C, there was hyperemia of the throat, signs of intoxication. Three had a dry cough in two cases, and one child had a wet cough with a discharge of mucopurulent sputum. At the time of hospitalization, three patients had lymphadenitis in varying degrees of severity and with different localization. Seven children had skin rashes of various localization. The diarrheal syndrome was noted in two children. Hospitalization was carried out on the 4th day in four patients, in three on the 2nd day, one patient was admitted to the hospital on the 3rd, 7th and 9th days of illness.

On admission, the condition of all children was assessed as serious. At least two systems were involved in the pathological process.

Fever (febrile or hyperthermic) occurred in all children. The duration of the fever ranged from 5 to 12 days and depended on the duration of hospitalization and the appointment of glucocorticosteroid therapy. One patient had a second wave of fever after two days of normal body temperature.

Damage to the cardiovascular system, to one degree or another, was detected in all patients according to ECG data. One child was diagnosed with myocarditis, two children were diagnosed with coronaritis. In the remaining patients, the instrumental examination was diagnosed with violations of the cardiac conduction system: in three cases - atrioventricular block of 1 degree, in two children - incomplete blockade of the right bundle branch. Three children have diagnosed with grade 2 cardiomegaly.

Lymph node involvement was quite common (in 7 patients). In addition to the submandibular lymph nodes, the axillary, cubital and inguinal lymph nodes were affected. The lymph nodes were dense, not welded to the underlying tissues, painful on palpation ranging from 1 cm to 2.5 cm. Ultrasound of the cervical lymph nodes was performed in one child, due to a sharp pain in the lymph nodes, to exclude an abscess. Also, scleritis and photophobia were noted in 6 patients.

In 4 patients from the first days of illness (2-3 days) maculopapular rashes were noted. The rash had a confluent character, protruded above the level of the skin. In one patient, the rash spread, almost all over the body and bullae with serous contents were noted. In this patient, the rash was practically the only clinical sign. In one patient, the rash was petechial in nature. The duration of the rash ranged from 2 to 6 days and also depended on the timing of the administration of glucocorticosteroids. In one patient, without exanthema syndrome, on the 12th day of illness, peeling was noted on the tips of the fingers. In one child, in the acute period, there was pasty and soreness of the skin of the soles and palms, there was no peeling later. The defeat of the respiratory system was diagnosed in 6 patients. In four cases - acute bronchitis, in two - community-acquired bilateral lower lobe pneumonia, moderate severity. No patient had respiratory failure. The lesion of the gastrointestinal tract was noted in 5 patients and was represented by pain in the abdomen and four patients had loose stools. The duration of the episode of diarrhoea ranged from 4 to 6 days. Thus, the observed patients showed damage from 2 to 4 body systems.

In laboratory research, we revealed significant changes. In the general analysis of blood, all patients showed a neutrophilic shift in the leukocyte formula: stab neutrophils accounted for up to 59%, seminiferous neutrophils up to 79%. Moreover, leukocytosis occurred only in four patients. An increase in ESR to high values was characteristic, and there was a negative trend during the course of the disease. The maximum ESR values were in a six-month-old child who was diagnosed with myocarditis. On the 2nd day of illness, ESR was 57 mm. rt. Art., on the 12th day - 70 mm. rt. Art. Three children had severe thrombocytosis, which also increased over time. One patient had thrombocytopenia (88 × 109).

In a biochemical blood test, the cytolytic syndrome was determined in all patients: an increase in ALAT levels (up to a 7-fold increase) and ASAT (up to a 6-fold increase). It should be noted that in all cases during the therapy there was a rapid normalization of these parameters. Indicators of total bilirubin increased slightly in two children, less than 1.5 times.

All patients had increased CRP indices, and significantly: from 20-fold to 100-fold increase. Against the background of ongoing therapy and clinical improvement, the CRP value reached the norm in only two patients. Also, five patients showed an increase in procalcitonin: two up to 10 ng/ml, two - 2 ng / ml. and 0.8 ng / ml. All children had a significant increase in the level of D-dimer, the maximum value was 2280 ng/ml.

An increase in ceatinine values was noted in 5 patients, in three of them, the norm was slightly exceeded, in two - 2 times higher than the norm. The parameters of creatinine kinase were not changed in any patient. Unfortunately, the level of ferritin was investigated only in four patients, in two cases an increase in this indicator was noted by 1, and 1.9 times.

The study of the blood coagulation system did not reveal significant changes. Two patients had a slightly increased

activated partial thromboplastin time (APTT), and in one patient the level of fibrinogen reached 6.3 g / L. The prothrombin index was not changed in any child. The duration of bleeding in Duka and coagulation according to Sukharev remained normal in all patients.

All patients received pathogenetic therapy: three children - normal human immunoglobulin, six - dexamethasone and prednisolone - two children. Therapy began within 1 to 3 days after hospitalization and, respectively, from 2 to 11 days from the onset of the disease. Against the background of the therapy, all patients showed positive dynamics: the body temperature returned to normal within 2 to 4 days, on average by 2.9 days. Moreover, the duration of the fever before hospitalization ranged from 1 to 11 days. On average, patients were in the infectious diseases hospital for 8.4 days (from 1 to 14 days). Four patients were transferred to the Department of Cardioreheumatology of the Pediatric Center of the National Center of Medicine for further examination and treatment. Six children were discharged home in satisfactory condition under the supervision of an outpatient cardio rheumatologist.

Conclusion. Thus, all observed children had signs of MIS. Despite the incomplete symptom complex, the clinical

and laboratory picture made it possible to establish this diagnosis of "Multi-inflammatory syndrome". Some peculiarities were revealed in patients in the Republic of Sakha (Yakutia). All patients were representatives of the Mongoloid race, the majority were boys. The most frequently affected cardiovascular and respiratory systems. The blood coagulation system was not significantly impaired, while half of the patients had significant thrombocytosis. Also, a dynamic increase in changes in some laboratory parameters was revealed, even against the background of stabilization of the general condition of the patients. This raises the question of the need to study the follow-up of patients with MIS.

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A CLININCAL CASE OF LOUIS-BAR SYNDROME EARLY ONSET IN A CHILD

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Abstract: The article represents a clinical case of Louis-Bar syndrome. The pediatricians should be alert to genetic disorders when revealing signs of immunodeficiency. Immunoassay examination is of extreme importance in early diagnosis of primary immunodeficiency.

Keywords: immunodeficiency, immunoglobulins, cerebellar ataxia, telangiectasia, replacement therapy

Introduction. Louis-Bar syndrome (OMIM#208900) is an autosomal recessive disorder which is characterized by ataxia, oculocutaneous teleangioctasis, immunodeficiency, predisposition to oncological disorders, infertility and premature aging. Louis-Bar syndrome refers to syndromes characterized by chromosomal instability, which occurs in balanced chromosomal rearrangement within the immune system cells. The characteristic feature of the syndrome is in cerebral neurodegeneration resulting in early fatality [1,4]. Recent investigations revealed that aneuploidy for chromosome is increased from 3 to 5 times in cerebral cells of the patients, thus, up to 30-50% of cerebral cells turn to be aneuploid [4].

Progressing cerebral ataxia with early onset is a principal clinical manifestation, epileptic attacks are not rare. Teleangioctasia of the conjunctiva, auricles and cheeks appear at the age of 3-6 years. 80% of cases are predisposed to infections due to immunodeficiency [2,3,5].

Immune abnormality is resulted from selective IgA deficiency. It is characterized by the signs of the damaged cell immunity leading to circulating T-lymphocytes reduction [3,5].

Clinical features of Louis-Bar syndrome. The authors represent a clinical feature of Louis-Bar syndrome in a 6-year old girl. A girl is of Russian ethnicity, born from the 6th pregnancy and 3rd labor. Pregnancy was characterized by gestational toxicosis and gestosis. The delivery was on the 40th week. The birth weight was 3800g, height was 50 cm. The child was lactated till the 6th month. Psychomotor development: she could raise her head since 2 month, roll over since 5 months, she walks since 1 year and 4 months and talks from 1 year and 2 months.

Family history is not complicated. The parents refuse chronic disorders.

At the age of 6 months the general condition was assessed as satisfactory. No signs of abnormalities were revealed. The skin was clean and pale. No fever revealed. The pharynx was with no signs of abnormality. Peripheral lymphatic nodes were impalpable. Nasal breathing was free without discharge. Respiration was puerile, weak in the lower lobes of the lungs, without rales. Respiratory rate was up to 35 per minute. Heart rate was 120 per minute. The abdomen was soft and painless. The liver and the spleen were not enlarged. Stool and diuresis were not disturbed. The complete blood count showed: hemoglobin (HGB) – 120 g/dL (Reference range (RR): 120-160 g/dL); erythrocytes (RBC) – 4.4x10^{12}/L (RR: 4.1-5.2x10^{12}/L); platelets (PLT) - 250 10^{9}/L (RR: 150 – 450x10^{9}/L); leukocytes (WBC) -2.2x10^{9}/L (RR: 5.5 – 12.5x10^{9}/L); lymphocytes (LYMF) – 55% (RR: 50-65%); monocytes – 3% (RR: 4-10%);

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stab neutrophils - 5% (RR: 1-5%); segmented neutrophils - 35% (RR: 20-35%); eosinophils - 2% (RR: 0-5%); erythrocytes sedimentation rate (ESR) by Panchenkov's micromethod -10 mm/hour (RR: 1-15 mm/hour). Complete blood count revealed leukopenia.

The child suffered of acute viral respiratory infections 6 times during his first year of life, had 3 cases of tonsillitis thus confirming clinical signs of immunodeficiency.

The patient was referred to the specialized hematological department at the age of 1 year. There he was examined. The complete blood count showed: hemoglobin (HGB) - 122 g/dL; erythrocytes (RBC) – $4.35x10^{12}$/L; platelets (PLT) – $200x10^{9}$/L; leukocytes (WBC) – $3.5x10^{9}$/L (RR: 4.5 – $13x10^{9}$/L); lymphocytes (LYMF) - 65% (RR: 45-65%); monocytes –10% (RR:4-10%); stab neutrophils - 5% (RR: 1-5%); segmented neutrophils - 15% (RR: 20-35%); eosinophils – 5.0% (RR: 1-4%); erythrocytes sedimentation rate (ESR) by Panchenkov's micromethod – 6.0 mm/hour. Complete blood count revealed leukopenia and neutropenia. Biochemical analysis of blood was within the norm.

Bone marrow sample revealed 1% of stromal cells (RR: 0-2%). The number of blasts was 1% (RR: 0-2%). Index of neutrophil maturation was 0.7 (RR: 0.6-0.8). Index of erythroblast maturation – 0.8 (RR: 0.8-0.9). The correlation of white and red blood cells lines 3:1 (RR: 3-4:1). Diagnostic decision: According to the obtained results no pathology was revealed.

After the medical examination the patient was clinically diagnosed with neutropenia of unknown genesis. A regular pediatric check-up was recommended.

Weakness, fatigue, and waddling were revealed at the age of 1.5 years. The general condition was assessed as poor. The child was of correct habitus with malnutrition. The layer of subcutaneous fat was low. The skin was pale. The conjuctiva of the both eyes showed signs of teleangiectasia. The skin surface of the face and auricles were pigmented with freckles, the skin was dry. The visible mucous membranes were pale. The lymphatic nodes of the neck and sublingual part are not matted together and painless. The pulse was 95-100 per minute. Respiratory rate was 30 per minute. Clear pulmonary sound was percussed above the lungs, respiration was vesicular. Heart sounds were muffled, rhythmic. The abdomen was soft and painless to palpation. The liver and the spleen were not enlarged. Stool and diuresis were not disturbed. The neurological status revealed clinical signs of central nervous system damage. The child was slow to contact. Consciousness was clear. The cognitive functions were impaired with unstable attention and poor mimics. The face was symmetrical, the movement of the eyeballs was full. Mild signs of cerebral ataxia were noticed: walked on his own but waddling and feeting his legs apart. Babinski syndrome was absent for the both sides. The mascular tonus was decreased. Tendon reflexes of the arms and the legs were decreased.

The suggested diagnosis: neutropenia of unknown genesis. Cerebral ataxia. Hypotrophy of the 2nd degree. Perinatal encephalopathy. Cerebral atrophy. Readiness for convulsions.

The child was referred to the Russian children's clinical hospital (Moscow) for the further examination. The child was examined by the allergologist-immunologist, who paid attention to the signs of leukopenia, neutropenia and frequent cases of respiratory infections in the child's case history.

The results of ummunogram showed: Ig A - 0 g/L (RR: 0.21-2.82g/dL); Ig M – 0.38 mg/mL (RR: 0.47-2.40 mg/mL); Ig G – 23.7 mg/ммL (RR: 4.83-12.26 mg/mL); Ig E - 1 IU/mL (RR: 0-60Un/mL); CD3+ - 55.00% (RR: 62.0-69.0 %); CD4+ - 35.00% (RR: 28.1-65.0%); CD8+ - 25.00% (RR: 26.0-68.0%). Diagnostic decision: immunoglobulin A absence, sharp decrease of immunoglobulin M, CD3+ subpopulation. The obtained results confirm primary immunodeficiency.

Alpha-fetoprotein test showed 90 IU/mL (RR: 0-58IU/mL). Diagnostic decision: test results reveal an increased level of alpha-fetaprotein.

MRI of the brain revealed signs of the cerebral atrophy, enlargement of IV ventricle.

The electroencephalography (EEG) revealed pulse sharpening between the ranges θ and δ and epileptic spike-and-wave complexes, spikes; hypsarrhythmia.

The geneticist examined the child and described the phenotype as dysplastic with stooping back. Hypersalivation. The palpation of the backbone was painless. The backbone line was not curved. The length of the lower limbs D=S. The movement of the joints was full and painless. Waddling. The skin was pale with coffee-like spots. The abdomen was soft and palpable. The spleen and the liver were within the norm. External genitals were of female type. Stool and diuresis were not disturbed.

The genetic data are: ATM (c.5932G>T, c.450453delTTCT, c.15641565delGA, c.5170G>T (p.Glu1724Ter) + c.748C>T (p.Arg250Ter), g.108115650G>A (p.Trp266*), p.Ala1945_Phe1952delV, p.Glu376fs, p.Ile2629fs/.

The clinical diagnosis is primary immunodeficiency. Louis-Bar syndrome (confirmed by molecular genetic analysis).

The patient is recommended to follow regular check-ups at the local pediatrician and the allergologist-immunologist. Immunogram control. Human immunoglobulin (gabroglobin, Gamunex-C, Ig VENA) is administered as a lifelong therapy.

The patient is regularly examined at the Pediatric center of the Republican hospital #1, National medical center, for the last 4 years. The patient receives replacement therapy of immunoglobulin (Ig VENA) and is examined twice a year. The patient's state is considered as stable at the moment. There is still cerebral ataxia, she suffers from respiratory infections 4-5 times a year. The patient is considered as disabled and she is on home education.

This clinical case reconfirms that the pediatricians should be alert to genetic disorders when revealing signs of immunodeficiency. Early immunoassay examination of the child is of extreme importance in early diagnosis of the primary immunodeficiency. Replacement therapy may balance development of long-lasting subacute and chronic infectious disorders [5].

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Menkes disease. A clinical case report of a rare disorder of copper metabolism caused by a mutation in the *ATP7A* gene

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The article presents the features of diagnostics and dynamic monitoring of a patient with Menkes disease, a rare disorder of copper metabolism caused by a mutation in the *ATP7A* gene. The data of scientific literature on the epidemiology, etiology, pathogenesis of this disease are analyzed, and the basic principles of therapy and the outcomes of the disease are considered. The described patient was admitted for treatment with a diagnosis of consequences of severe perinatal lesions of the central nervous system with a typical clinical picture for this group of diseases: spastic tetrapresis, pseudobulbar syndrome, structural focal epilepsy. However, during the follow-up process, uncharacteristic symptoms were noted: short blonde coarse hair, bladder diverticulosis and progressive atrophy of the cerebral cortex in dynamics according to MRI. During the diagnostic search, tests for copper concentration and serum ceruloplasmin levels were performed twice, indicating Menkes disease, and complete exome sequencing was performed, which confirmed the presence of a point mutation in the *ATP7A* gene.

Keywords: Copper, ceruloplasmin, hypsarrhythmia, Menkes disease.

Introduction: Most hereditary metabolic disorders are extremely rare, but together they represent a fairly common group of diseases. As a rule, they are incurable and often lead to early disability and death, however, for many hereditary diseases, pathogenic therapy has been developed and with timely diagnosis and early start of treatment therapy, it is possible to achieve a favorable prognosis for life. One of these is hereditary disorders of copper metabolism.

The most famous neurodegenerative disease associated with impaired copper metabolism is Wilson-Konovalov disease, caused by a mutation in the ATP7B gene, which is responsible for the synthesis of copper-transporting ATPase and is characterized by the accumulation of copper in various organs and tissues, with predominant damage to the liver and central nervous system [2. 5, 13]. However, in addition to Wilson-Konovalov disease, there is a much less well-known hereditary disorder of copper metabolism, in which there is an uneven copper deficiency in various organs. This disease is called Menkes disease or curly hair disease and is an X-linked recessive neurodegenerative disease caused by mutations in the ATP7A gene located on chromosome Xq21.1 [1, 13]. Being an X-linked disease, clinical manifestations are possible only in males, in women who are carriers of ATP7A mutations, with the exception of rare cases associated with sex chromosome aneuploidy or X-autosome translocations, the disease is asymptomatic. However, some carriers of the mutant gene have minor hair and skin abnormalities [6, 8, 14].

The ATP7A gene is responsible for the synthesis of the transport protein of the same name, the function of which is to transport copper across cell membranes; this protein is expressed in all organs except the liver. In the small intestine, the ATP7A protein is necessary for the absorption of copper from food by active transport; in the cells of other organs, it acts as a carrier of copper from the cell membrane to the Golgi apparatus, where the synthesis of proteins and enzymes takes place, for the functioning of which copper [5] is required. One of these enzymes is lysyl oxidase, which binds tropocolagen into strong fibrils of mature collagen. When the mechanism of lysol oxidase synthesis is disturbed, defective collagen is formed, the properties of which determine many of the symptoms of Menkes disease. Normally, when there is an excess of copper inside the cell, the same ATP7A protein transports it to the cell membrane and removes the excess from the cell. Mutations in the ATP7A gene lead to the synthesis of a defective protein that is incapable of normal functioning. As a result, the absorption of copper from food in the intestine is sharply reduced, the synthesis of enzymes in the Golgi apparatus is disrupted, and the transport of excess copper from cells is blocked. Ultimately, copper accumulates in the small intestine and kidneys, and in the brain and other tissues, its content becomes catastrophically low, which together contributes to the formation of a kind of clinical picture of the disease [3, 4].

Menkes disease occurs in 1: 250,000-1: 350,000 newborn boys. Manifests, as a rule, in the neonatal period. Early symptoms include hypothermia, hyperbilirubinemia, growth retardation, and multiple stigmas of dysembryogenesis. Hair in the neonatal period, as a rule, looks normal [3, 11, 13].

Upon completion of the first 2-3 months of relatively normal development, a regression of previously acquired skills occurs, followed by a gross delay in psychomotor development. These children are characterized by the appearance of various types of epileptic seizures (focal, generalized, myoclonic) [11], general weakness with further formation of spastic tetraparesis [8]. Over time, a striking distinctive feature of these children becomes obvious - trichoplidystrophy, their hair becomes matted, coarse, gray or ivory [12]. A characteristic feature of the course of the disease is multiple diverticula of the urinary tract, often leading to rupture and secondary infection. Among other signs, it is important to note gen-

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eralized osteoporosis with frequent rib fractures [3, 4].

When conducting MRI studies, progressive atrophy of the cerebral cortex is detected. On angiograms, the vessels look twisted, elongated, with multiple stenoses, dilatations and aneurysms, which further leads to vascular complications - subdural hemorrhages, rupture of arteries and thrombosis, which, as a rule, become the cause of death

Pathogenetic treatment includes the use of copper histidinate 0.2-0.45 mg / day intravenously, which is able to restore normal levels of copper in plasma, cerebrospinal fluid and liver, however, the drug is ineffective for the treatment of neurological symptoms [3, 7, 13].

Life expectancy usually does not exceed 3 years.

Materials and methods:

- analysis of literary sources in the databases MEDLINE, Embase, US National Library of Medi-cine's PubMed database, ISI Web of Knowledge, Google Scholar, uMEDp and e-library, etc.

- analysis of the medical history of copper metabolism disorders caused by a mutation in the ATP7A gene.

Results and discussion. Patient M., 2 years old, was admitted to the palliative department of the regional clinical children's hospital No. 2 in Vladivostok on 17.03.20 with a diagnosis of West syndrome, in order to correct anticonvulsant therapy. He was hospitalized immediately after suffering acute community-acquired pneumonia, during which he was transferred to invasive mechanical ventilation (ALV). On admission, the following complaints were noted: frequent, up to several dozen per day, polymorphic convulsive seizures (adversive, myoclonic), severe retardation of psychomotor development, lack of independent urination, inhibition of swallowing with the possibility of feeding only through a nasogastric tube, as well as respiratory failure 2-3 degrees.

From the anamnesis of life, it is known that the child is from the first pregnancy, which proceeded physiologically. First birth, at thirty-eight weeks by emergency caesarean section. In childbirth, pronounced intrauterine fetal hypoxia was diagnosed, an Apgar score of 1/3 point, resuscitation measures were carried out. Birth weight 2500 g, height 48 cm. Breastfed up to 2.5 months, then transferred to an adapted formula. From birth he grew and developed with a delay in neuropsychic development. Was observed by a neurologist with a diagnosis of severe perinatal lesion of the central nervous system, received symptomatic treatment.

At the age of three months, convulsive syndrome first appeared, and the emergency medical team admitted the child to the neurological department of the regional clinical hospital No. 1 in Vladivostok. During the initial examination on the electroencephalogram (EEG) of 10/22/2018, acute - slow wave complexes were found in the left frontal regions, on the magnetic resonance imaging (MRI) of the brain from 10/23/2018, signs of linear periventricular zones of gliosis in the region of the seven-oval centers, foci of gliosis in the caudate nuclei, signs of replacement hydrocephalus, asymmetry of the lateral ventricles. A consultation with a neurosurgeon was carried out, the conclusion: PPTSNS, episyndrome, delayed psycho-motor development. The following treatment tactics were used: intravenous dexamethasone at the rate of 2 mg per kg of body weight per day, with a gradual decrease in dosage, a general course of 2 months and a solution of valproic acid (depakin) at a dosage of 30 mg per kg of body weight for a long period. At discharge, outpatient supervision of a neurologist, epileptologist and continuous administration of valproic acid are recommended. Against the background of the ongoing treatment, the condition in the future without positive dynamics. The results of dynamic observation and additional research methods:

- 6 months (01/14/19): computer EEG - specific epileptiform activity was revealed in the form of bilateral synchronous high-wave activity with a focus in the frontotemporal leads up to 200 μV, “shark teeth”.

- At the age of nine months, the correction of anticonvulsant therapy was carried out - a prolonged form of valproic acid (depakin chronosphere) at the rate of 40 mg / kg per day, topiramate tablets at the rate of 2 mg / kg per day for 2 doses and phenobarbital tablets at the rate of 8 mg / kg per day for 2 receptions.

- 11 months EEG (05/28/2019) - pronounced changes in cortical rhythm by the type of gypsum rhythm. The diagnosis was revised to West syndrome. In addition to the treatment, a solution of levetiracetam (keppra) was added at the rate of 30 mg / kg per day.

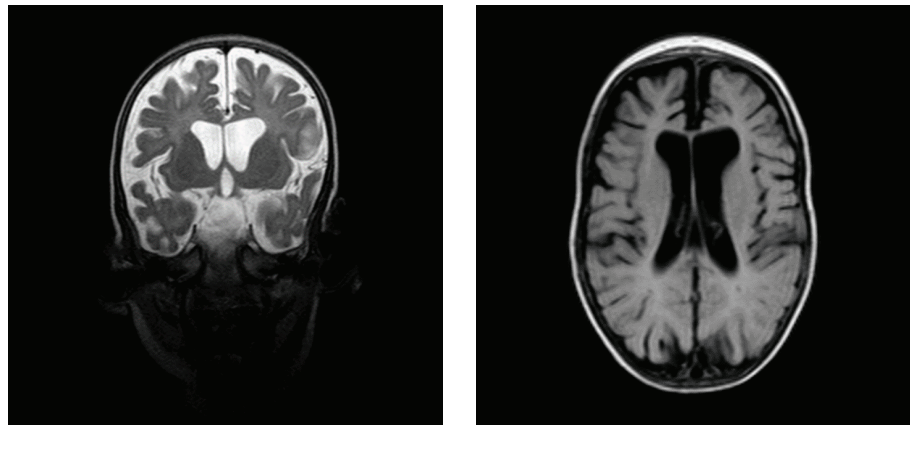
- 1 year 2 months EEG (08/21/19) - a pronounced slowdown of cortical rhythmics, registration of epileptiform activity in the form of diffuse discharges of deformed broadened "acute-slow wave" complexes with predominant localization in the central regions.

- 1 year 6 months EEG (01/16/2020) - an increase in the paroxysmal activity index, deformed, broadened “acute - slow wave” complexes of various localization, forming a pattern like hypsarrhythmia. MRI of the brain and cervical spine - a picture of diffuse cerebral atrophy with the formation of cerebrospinal fluid cysts of the temporal lobes (photo 1).

Photo 1. MRI of the brain with diffuse cerebral atrophy, formation of cerebrospinal fluid cysts of the temporal lobes.

On January 17, 2020, he was urgently hospitalized in the urology department of the regional clinical hospital No. 1 in Vladivostok with a referral diagnosis: acute urinary disturbance. While in the urology department, the child was diagnosed with congenital malformation of the genitourinary system: urinary bladder devirticulosis, secondary pyelonephritis, active stage, bilateral cryptorchidism.

On admission to the palliative care unit, the condition is consistently severe. During examination, the contact is not available, consciousness is not determined, the reaction to examination is negative. Breathing through the ventilator. The range of movements is significantly reduced, the movements carried out are chaotic, uncoordinated. Periodic myoclonic seizures. When as-



Patient M. MRI of the brain with diffuse cerebral atrophy, formation of cerebrospinal cysts of the temporal lobes

sessing neuropsychic development - a gross delay along all lines, unable to hold his head and turn over. The skin is pale, pronounced acrocyanosis. Hair that is light, fine and coarse to the touch, has a tendency to "caking". A pronounced decrease in the subcutaneous fat layer, to the level of protein-energy malnutrition of the II-III degree. Muscle tone is diffusely reduced. Tetraparesis of central genesis. Joint movements are limited by spasticity, and there are no joint contractures. Examination of the cranial nerves: visual concentration is undetectable, the pupils are round. The reaction of the pupils to light is direct, friendly, lively. Signs of oral automatism, pseudobulbar syndrome. On auscultation of the lungs, there are many moist rales. The bladder is enlarged on palpation, tense. Urination is only possible through a urethral catheter.

The severity of the condition and the progression of neurological symptoms, combined with a detailed analysis of the course of the disease, as well as the presence of characteristic phenotypic signs (features of hair), made it possible to suspect the presence of a hereditary metabolic disease with brain damage in the child. The presumptive diagnosis was a copper metabolism disorder caused by a mutation in the ATP7A gene - Menkes's disease.

On March 24, 2020, a blood sample was taken to study the levels of ceruloplasmin and copper (in the child's analyzes, ceruloplasmin was reduced to 65.65 at a rate of 200-600 mg / l), serum copper was reduced to 4.3 mkM / l (at a rate of 13 -24 mkM / l). In March 2021, full exome sequencing was performed on the Illumina NovaSeq 6000 at the Genetico Center for Genetics and Reproductive Medicine. The rs797045338 G> C mutation in intron 7 (out of 22) of the ATP7A gene in the hemizygous state was revealed, which made it possible to confirm the presumptive diagnosis of Menkes disease.

By the age of two, the palliative service achieved a phased cancellation of artificial ventilation, and now the child is breathing independently through a tracheostomy tube. Feeding is carried out through the nazagastric tube (grated food, Nutridrink nutritional therapy). A satisfactory anticonvulsant effect was achieved with phenobarbital monotherapy at a rate of 10 mg / kg per day. Periodically, courses of levocarnitine, B vitamins are carried out, as well as repeated courses of massage and physiotherapy. The child does not receive pathogenetic therapy that can prolong life span, since the drug for intravenous life-long administration of copper histidinate is not registered in the Russian Federation.

At the moment (05/23/2021) the child is three years old, the state is without clear dynamics. Neurological symptoms are relatively stable, there are rare myoclonic seizures up to 5-7 per day, lasting no more than 1 minute. Tolerance to phenobarbital is satisfactory, from undesirable effects - hypersalivation and the need for frequent sanitation of the respiratory tract. The prognosis for life is not favorable.

Conclusion. The key feature of this clinical case is the complexity of differential diagnosis of perinatal hypoxic-ischemic brain lesions with hereditary neurodegenerative diseases. A timely diagnosis will help parents consciously plan the birth of healthy children under the supervision of a geneticist. A description of the clinical case of Menkes's disease, as well as the peculiarities of diagnosis and the experience of managing a child with this disease can be useful for specialists, including those of a palliative profile.

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