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E.P. Ammosova, T.M. Klimova, R.N. Zakharova, A.I. Fedorov, M.E. Baltakhinova

POLYMORPHIC MARKER RS137100 OF THE *LEPR* GENE AND METABOLIC DISORDERS IN THE INDIGENOUS POPULATION OF YAKUTIA

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The aim of the study was the search for associations of polymorphic marker rs1137100 of *LEPR* gene with lipid specter, metabolic syndrome and its components among indigenous population of Yakutia. The study involved 227 representatives of the Yakut nationality, permanently residing in the village of Berdigestyakh, Gorny Ulus of Yakutia. We found a significant relationship between allelic variant A, genotypes AA/AG of *LEPR* gene with hypoalphacholesterolemia. No association with metabolic syndrome or its components was found.

Keywords: *LEPR* gene, polymorphic marker rs 1137100, obesity, metabolic syndrome, population, genetics, dyslipidemia, indigenous population, Yakutia, North.

Introduction. Leptin is a hormone produced in adipocytes and is involved in the regulation of satiety, energy balance, and fat metabolism. In recent studies it was shown that leptin stimulates glucose uptake and fatty acid oxidation, participates in the secretion of insulin through *LEPR* receptors, which are localized in the β-cells of the pancreatic gland, adipose tissue, and muscle [10]. Leptin receptor is a transmembrane protein belonging to the superfamily of cytokine receptors; it has several alternative isoforms.Leptin receptors form homodimers that can activate paths JAK2/STAT3, which regulate food intake and energy balance. The *LEPR* gene localized on chromosome 1 (1p31.3) has been studied in various ethnic groups [9]. We describe polymorphic variants of the *LEPR* gene associated with obesity, insulin resistance, diabetes, and increased risk of cardiovascular and oncologic disease [7]. However, the results of studies in different ethnic populations are contradictory.

In polymorphism of rs1137100 (K109R), lysine is replaced by arginine (AGG by AAG) at codon 109, exon 4 (K109R). Currently, there are many studies focusing on the role and relationship of K109R polymorphism with lipoproteins, metabolic syndrome [15, 17], obesity [4, 8, 10, 12], diabetes mellitus [16], increased risk of developing cardiovascular diseases [6], but the research results are ambiguous.

This study aims to study the association of *LEPR* gene polymorph marker (rs1137100, K109R) with lipid profile, metabolic syndrome and its components in a sample of representatives of the Yakut population.

Materials and Methods. The sample was formed in the course of a one-stage observational study among the unorganized population of the village Berdigestyakh, Gorny Ulus of the Sakha (Yakutia) Republic. The genetic study was carried out in 227 representatives of the Yakut nationality, permanently residing in this settlement. The age of the respondents was 18 and older, the av-

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Table 1

Distribution of Alleles and Genotypes of *LEPR* Gene Polymorphic Marker (Rs137100) In A Group of Yakut Nationality*

Group	n (%)			R
	Allele			
	A	G		
Men Women	28 (30.8) 71 (34.6)	63 (69.2) 134 (65.4)		0.515
Both sexes	99 (33.4)	197 (66.6)		
20-39 years old 40-59 years old 60 years and older	39 (32.5) 43 (35) 17 (32.1)	81 (67.5) 80 (65) 36 (67.9)		0.896
Genotype				
	AA	AG	GG	
Men Women	8 (11.2) 22 (14.1)	20 (28.2) 49 (31.4)	43 (60.6) 85 (54.5)	0.675
Both sexes	30 (13.2)	69 (30.4)	128 (56.4)	
20-39 years old 40-59 years old 60 years and older	9 (10) 13 (14) 8 (18.2)	30 (33.3) 30 (32.2) 9 (20.4)	51 (56.7) 50 (53.8) 27 (61.4)	0.453

Note: * Distribution of genotypes in all groups is consistent with the Hardy-Weinberg law of equilibrium; p is the achieved level of statistical significance of differences when comparing groups using the Pearson $\chi 2$ test.

erage age was 43±17.5. Gender-wise the respondents were primarily women with 156 (68.4%), with 72 men (31.6%). The research project was approved by the local Committee on Bioethics of the Yakutsk Scientific Center for Complex Medical Problems (extract from Minutes No. 39 dated June 26, 2014). All participants signed informed voluntary consent to participate in the study.

The criteria of the International Diabetes Federation (IDF, 2005) were used to verify the metabolic syndrome. All participants were examined according to a single program, which included: anthropometric examination according to the standard method, analysis of body composition using a Tanita Bioimpedance Analyzer (Japan) SSC 330, double measurement of blood pressure (BP), and fasting venous blood sampling. The content of glucose, total cholesterol (TC), triglycerides, high density lipoproteins (HDL cholesterol) was determined on an Express Analyzer CardioChek PA, USA. We have also taken into account the conducted medicinal treatment for hypertension, diabetes, and lipid disorders. The following criteria were used to diagnose obesity: body mass index ≥ 30 kg/m2; the size of the waist circumference more than 80 cm in women and 94 cm in men (according to the IDF criteria for European populations) [13]. Obesity was diagnosed in 46 (20.8%) individuals. The metabolic syndrome was detected in 36 respondents, which amounted to 16.4% of the total number of those surveyed. Among women, metabolic syndrome occurred significantly more often than among men – 20.7 and

Table 2

Frequency of Allelic Variant G of the *LEPR* Gene Polymorphic Marker (Rs137100) In Different Ethnic Groups*

Authors, year	Country	Population	N	Floor	Allele frequency G (95% CI)
Data from this study, 2017	Russia	Yakuts	228	Both sexes	0.66 (0.68; 0.72)
Matsuoka et al., 1997.	Japan	Asians	115	Both sexes	0.78 (0.70; 0.85)
Koh et al., 2002	Korea	Asians	220	Men	0.83 (0.78; 0.88)
Gotoda et al., 1997	England	Europeans	322	Both sexes	0.27 (0.22; 0.31)
Mamme's et al., 2001	France	Europeans	566	Both sexes	0.25 (0.21; 0.28)
Wauters et al., 2001	Belgium	Europeans	280	Women	0.28 (0.22; 0.33)
Yiannakouris et al., 2001	Greece	Europeans	118	Both sexes	0.12 (0.06; 0.18)

Note: N – the number of examined; CI – confidence interval.

Table 3

Anthropometric and Metabolic Characteristics of Respondents Depending on the Allelic Variant and Genotypes of The *LEPR* Gene Polymorphic Marker (rs137100)

Indicators	Genotype	Me (Q_1;Q_3)	p	Allele	Me (Q_1;Q_3)	p
SBP, mm Hg	AA и AG	112.7 (106.7; 126.7)	0.143	A	112.6 (106.6; 126.6)	0.508
	GG	120 (108.3; 134.0)		G	115.8 (107.3; 130.0)	
DBP, mm Hg	AA и AG	75.3 (70.0; 82.0)	0.195	A	75.3 (70.0; 82.0)	0.603
	GG	77.7 (70.0; 88.5)		G	76.1 (70.0; 85.0)	
Total cholesterol, mmol/l	AA и AG	4.2 (3.6; 5.3)	0.358	A	4.1 (3.5; 5.2)	0.452
	GG	4.4 (3.6; 5.2)		G	4.4 (3.5; 5.2)	
HDL cholesterol, mmol/l	AA/AG	1.7 (1.47; 2.1)	0.300	A	1.6 (1.4; 2.0)	0.370
	GG	1.7 (1.4; 2.2)		G	1.7 (1.4; 2.2)	
Triglycerides, mmol/l	AA / AG	0.9 (0.8; 1.1)	0.683	A	0.8 (0.7; 1.0)	0.810
	GG	0.9 (0.8; 1.118)		G	0.8 (0.7;1.0)	
LDL cholesterol, mmol/l	AA / AG	2.7 (1.7; 4.0)	0.244	A	2.6 (1.6; 4.0)	0.529
	GG	2.3 (1.7; 3.5)		G	2.3 (1.6; 3.8)	
Fasting glucose, mmol/l	AA /AG	5.1 (4.6; 5.5)	0.322	A	5.1 (4.6; 5.5)	0.352
	GG	5.0 (4.6; 5.446)		G	5 (4.5; 5.5)	
Body weight, kg	AA/AG	59.5 (51.9; 71.7)	0.606	A	59.4 (51.9; 71.6)	0.797
	GG	60.8 (53.8; 70.7)		G	59.8 (53.2; 70.6)	
Body mass index, kg/m^2	AA/AG	23.5 (20.3;27.3)	0.550	A	23.5 (20.3; 27.3)	0.816
	GG	23.6 (21.6; 28.3)		G	23.5 (20.9; 27.5)	
Waist circumference, cm	AA/AG	85.50 (76.2; 96.2)	0.344	A	85.5 (76.2; 96.2)	0.578
	GG	87.2 (79.8; 96.5)		G	85.9 (77.7; 96.2)	
Atherogenic index	AA/AG	1.5 (1.0; 2.2)	0.937	A	1.5 (0.9; 2.2)	0.911
	GG	1.4 (1.0; 1.9)		G	1.4 (1.0; 2.0)	
Fat percentage	AA/AG	15.8 (9.5; 22.6)	0.897	A	15.8 (9.5; 22.6)	0.960
		16.6 (10.2; 22.8)		G	16.4 (9.7; 22.6)	

Note: p is the achieved level of statistical significance of differences when comparing groups using the Mann-Whitney test; data are presented as median and interquartile range in Me format (Q_1; Q_3).

7.2%, respectively (Fisher's exact test, p = 0.017).

For SNP genotyping, we have used TaqMan probes specific to the regions containing the SNPs of interest. Samples and primers were developed using PREMIER Biosof's Beacon Designer 8 software. FAM and R6G dyes were used as reporters, and BHQ-1 was used as a quencher. Real-time polymerase chain reaction was performed in a CFX96 system from Bio-Rad. The volume of the reaction mixture was 25 µl. Each reaction was performed in triplicate. Reaction conditions: activation stage was carried out at 95°C for 3 minutes, course of one cycle consisted of three temperature-time intervals – 95°C (30 sec), 54°C (20 sec) and 72°C (20 sec). The total number of cycles was 40.

The verification of the correspondence of the distribution of genotypes to the Hardy-Weinberg equilibrium state law was carried out using an online calculator at https://wpcalc.com/en/equilibrium-hardy-weinberg/ [11]. Statistical analysis of the data was carried out in the IBM SPSS STATISTICS 22 software suite. When comparing groups depending on the type of data, we used the Mann-Whitney and Pearson χ2 tests. The critical value of the level of statistical significance of differences (p) was taken equal to 5%.

Results and Discussion. The distribution of allelic variants and genotypes of polymorphic markers of the LEPR gene (rs137100) in the group of Yakut people is shown in Table 1. The distribution of genotypes in subgroups is consistent with the Hardy-Weinberg equilibrium state law. In the studied sample, the GG 128 genotype was more common (56.4 %), with the AA 30 genotype being less common (13.2%).

Table 2 shows frequency of polymorphic allelic variant G in different ethnic populations. This variant is significantly more frequent in Asian populations than the European populations. The frequency of allelic variants of G in the Yakut population is similar to other Asian populations [10].

When comparing quartile distribution anthropometric and metabolic until exponent depending on allelic variants and genotypes of rs137100 in LERP gene, we did not detect statistically significant differences (Table 3).

Frequency analysis of metabolic risk factors depending on the presence of allele and genotypes rs137100 of LEPR gene allowed us to detect associative relationship between genotypes AA/AG with hypoalphacholesterolemia (p = 0.021) (Table 4). Also, the frequency of hypoalphacholesterolemia was higher in carriers of the A allele (p = 0.038). There is no statistically significant relationship with other components of the metabolic syndrome. The findings differ from literature data, where allele connected with pathological signs of lipid metabolic imbalance, is the G allele [5, 8, 14, 18], whereas in the present study is the abnormal A allele.

Table 4

Frequency of Metabolic Syndrome and Its Components Depending on the Allelic Variant and Genotypes of the *LEPR* Gene Polymorphic Marker (rs137100)

Genotype	MS Component, n (%)		χ^2, p	Allele	Component MC, n (%)		χ^2, p
	Yes	No			Yes	No	
High blood pressure							
AA/ AG	36 (36.7)	62 (63.3)	χ=0.273 p=0.601	A	36 (36.7)	62 (63.3)	χ=0.029 p=0.865
GG	51 (40.2)	76 (59.8)		G	74 (37.8)	122 (62.2)	
Hypoalphacholesterolemia							
AA/AG	13 (13.3)	85 (86.7)	χ=5.304 **p=0.021**	A	13 (13.3)	85 (86.7)	χ=4.284 **p=0.038**
GG	6 (4.7)	122 (95.3)		G	12 (6.1)	184 (93.9)	
Hypertriglyceridemia							
AA/AG	6 (6.1)	92 (93.9)	χ=0.241 p=0.623	A	6 (6.1)	92 (93.9)	χ=0.107 p=0.743
GG	10 (7.8)	118 (92.2)		G	14 (7.1)	182 (92.9)	
Fasting hyperglycemia							
AA/AG	24 (24.7)	73 (75.3)	χ=0.224 p=0.636	A	24 (24.7)	73 (75.3)	χ=0.154 p=0.695
GG	28 (22)	99 (78)		G	44 (22.7)	150 (77.3)	
Obesity by body mass index							
AA/AG	21 (21.6)	56 (57.7)	χ=0.284 p=0.868	A	21 (21.6)	76 (78.4)	χ=0.07 p=0.791
GG	25 (20.3)	69 (56.1)		G	39 (20.3)	153 (79.7)	
IDF_1 central obesity							
AA/AG	49 (51)	47 (49)	χ=2.542 p=0.11	A	49 (51)	47 (49)	χ=1.213 p=0.314
GG	76 (61.8)	47 (38.2)		G	110 (57.9)	80 (42.1)	
Metabolic syndrome							
AA/AG	16 (16.5)	81 (83.5)	χ=0.243 p=0.622	A	16 (16.5)	81 (83.5)	χ=0.0048 p=0.826
GG	24 (19)	102 (81)		G	34 (17.5)	160 (82.5)	
High and atherogenic index							
AA/AG	6 (6.1)	93 (93.9)	χ=0.210 p=0.647	A	6 (6.1)	93 (93.9)	χ=0.305 p=0.581
GG	6 (4.7)	122 (95.3)		G	9 (4.6)	188 (95.4)	
GG	3 (2.4)	124 (97.6)		G	8 (4.1)	188 (95.9)	

Note. χ2* – Pearson Chi-square test, p – significance level.

Conclusion. At the moment, the world carried on a lot of research devoted to study of the rs1137100 LEPR gene polymorphic variant (K109R) in different ethnic groups. Despite this, the role of the polymorph variants in the development of diseases related to lipid metabolism disorders are contradictory. Thus, when conducting a meta-analysis with the inclusion of 7 studies such as case-control study, we do not establish association of this polymorph marker with type 2 diabetes (type 2 (patients with type 2 diabetes – 3319, healthy – 2844) [16]. Also, a meta-analysis of 7 studies did not establish a clear association of rs1137100 with obesity [10]. A systematic review (analyzed 10 publications, with the included and it in the data analysis of 1989 people with coronary heart disease and 2601 healthy) did not confirm associative connection of this polymorph variants with coronary heart disease [6]. But at the same time there are studies which found associative connection of the polymorphic marker with ischemic stroke [5], obesity [8, 18], the risk of cardiovascular disease [14].

The conflicting results of the studies are probably related not only to the genetic characteristics of ethnic groups, but also with the influence of various factors of the environment, especially climatic conditions, physical activity, and dietary features. Several studies have noted the high level of leptin in the blood of the population living in northern latitudes. We relate this feature to thermoregulation processes [22]. Also, in recent studies it was shown that when respondents (carriers of negative genotype polymorphic marker Lys656Asn/rs8179183 in *LEPR* gene) changed their diets and physical activity, they saw increased sensitivity to insulin and better anthropometric characteristics. In recent years, studies are actively looking at the role of polyunsaturated fatty acids in leptin genes expression, because experiments on animals, humans, and cell cultures showed the impact of fatty acids on the expression of the gene and the concentration of leptin [15] .

In Yakut population there is a high frequency of the G allele, similar to data from other Asian races [10]. Interestingly, in the investigated sample the A allele is negative and is associated with hypoalphacholesterolemia, which does not match the data from other studies, where the G allele is pathological [5, 8, 14, 18]. According to epidemiological studies, the Yakut population, like many other populations of the North, is characterized by a fairly high level of HDL cholesterol [1, 2, 17, 19]. The population of Yakutia has a special genotype formed for centuries, which is adapted to long-term exposure to cold, with diet rich in proteins and fats [3, 20, 21]. Changes in diet and lifestyle are accompanied by an increase in the frequency of metabolic disorders in the population, including a decrease in the concentration of HDL cholesterol, thus hypoalphacholesterolemia is one of the main components of MS in 70% of cases [2, 3]. It is possible that changes in metabolic parameters and an increase in the frequency of their disorders in the population are associated with changes in gene expression under the influence of living conditions and nutrition. In this regard, a further study of the functioning of the genes under the influence of these factors is particularly relevant and has fundamental scientific importance in research. It may reveal more data on the mechanism of human adaptation habitat conditions.

This study was carried out within the framework of the state assignment of the Ministry of Education and Science of the Russian Federation on the topic "Genome-Wide Studies of the Gene Pool of the Indigenous Population of the Arctic Coast of Yakutia" (State Registration Number FSRG-2020-0017).

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D-DIMER LEVEL AS A PREDICTOR OF ADVERSE OUTCOMES IN PATIENTS WITH ISCHEMIC STROKE

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The level of D-dimer is associated with the severity of stroke. The objective of this study was to test the hypothesis that the D-dimer level measured in the first 96 hours of hospitalization has prognostic value for mortality in patients with acute ischemic stroke (AIS) in the acute and early recovery periods.

This retrospective study included 54 patients with AIS. The patients were divided into 2 groups: the survivors after 65 days of observation (n = 37) and those who died during observation (n = 17). The level of D-dimer in blood plasma was determined in the first 96 h after hospitalization and expressed in terms of the number of upper normal levels (UNL). Its prediction of mortality was evaluated.

The D-dimer level was significantly higher in patients with an adverse outcome: 5.53 (2.8-7.84) versus 1.84 (1.2-3.06) UNL, p = 0.00035. Logit regression model of outcomes showed that the probability of death was 30% with 4 UNL, 40% with 5 UNL, and 50% with 7 UNL. Statistically, the lethality was significantly higher in patients with a D-dimer level ≥5 UNL (odds ratio 5.813 [95% CI 1.596-21.174], p = 0.05).

Measured in the first 96 hours from the start of hospitalization, D-dimer level ≥5 UNL was the predictor of an adverse outcome in patients with AIS in the acute and early recovery periods. Therefore, the use of anticoagulants needs to be modified.

Keywords: D-dimer level; acute ischemic stroke; mortality predictor; adverse outcome.

Introduction. Cardiovascular diseases are the main cause of death worldwide [6]. Ischemic stroke, along with ischemic heart disease, is the leading disease of the cardiovascular system and makes a significant contribution to the lethality and disability of the population. In the Russian Federation, the incidence rates of stroke in 2009 and 2010 were 3.5% and 3.27%, respectively, and the mortality rates were 1.19% and 0.96%, respectively [2]. During stroke, the following periods are distinguished: the peracute period (the first 3 days), the acute period (up to 28 days), early recovery (up to 6 months), late recovery (up to 2 years), and the period of residual effects (after 2 years) [1].

In the past two decades, the approach to the treatment and prevention of acute cardiovascular diseases has changed significantly in Russian and worldwide practice [27]. One of the main "extra-cerebral" causes of mortality in stroke is the development of venous thromboembolism (VTE), including deep vein thrombosis (DVT) and pulmonary embolism (PE). The unsatisfactory results of prevention and treatment, as well as the asymptomatic course in the majority of patients [29] are of great importance. Therefore, searching for new methods of anticoagulant therapy is reasonable and relevant [31].

The existing standards and recommendations for the prevention and treatment of thromboembolism of the pulmonary artery in patients with stroke are regularly updated due to the presence of a range of risk factors [1,13,15,22]. However, anticoagulant use is associated with the risk of bleeding, including hemorrhagic transformation (HT) of the ischemic focus [12,29].

A promising predictive index of the coagulogram is the D-dimer level. According to published data, this indicator has prognostic significance in terms of outcomes and mortality in various diseases, including stroke (ischemic [24,32,33] and hemorrhagic [18]), myocardial infarction [17], mesenteric thrombosis [7], and infective endocarditis [9]. D-dimer is one of the few markers of thrombosis that can be determined directly in standard samples of citrated plasma. It does not require specific biomaterial processing, and its determination is quick. D-dimer is also an indicator of the effectiveness of anticoagulant therapy [3,5,8,30].

The results of determining the D-dimer level, in contrast to other markers of activation of the hemostasis system, are practically not affected by the admixture of platelets in the blood plasma [14] but are influenced by factors such as the size of the thrombus, the time from the beginning of clinical signs to the prescription of anticoagulant therapy, the intake of anticoagulants (affected by which the level of D-dimer gradually decreases), and thrombolytic therapy, which causes an increase in its level [11]. Individual differences in the rate of D-dimer increase in some patients can be explained by the different rates of activity of the fibrinolytic system [26]. Thus, in clinical practice, D-dimer can be used as a marker of the body's ability for hypercoagulability and endogenous fibrinolysis, the elevated levels of which are typical for thrombosis [32].

The purpose of this study was to test the hypothesis that the D-dimer level, measured in the first 96 hours of hospitalization, was a prognostically important predictor of mortality in patients with acute ischemic stroke in the acute and early recovery periods.

Methods and Study Design. This study included 54 men and women aged

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40 years and older. An acute ischemic stroke was determined according to the World Health Organization criteria [16]. This study was approved by the Local Ethics Committee.

All patients admitted in the first 40 minutes after hospitalization underwent a Multislice Spiral CT Scan to exclude hemorrhagic stroke or an intracranial malignant neoplasm.

If the patient's age was between 40 and 59 years, the additional criteria for enrolment in the study were: D-dimer level ≥ 2 upper normal levels (UNL) and a history of VTE or cancer (with the exception of non-melanoma skin cancer). If the patient's age was between 60 and 74 years, an additional criterion for enrolment in the study was the D-dimer level ≥ 2 × UNL. Patients aged 75 years and older were included in the study without additional factors.

Exclusion criteria were: high risk of bleeding, inability to undergo a proper bilateral compression ultrasound, contraindication for anticoagulant therapy, pregnancy, intake of oral anticoagulants for 96 hours before the start of therapy with the test drug, heparin therapy lasting longer than 96 hours, and concomitant antiplatelet treatment with two medications [16].

All patients underwent treatment in accordance with the standard for cerebral infarction treatment [25]. The patients received antiplatelet therapy. The drug intake lasted for 14 days, but with continued true immobilization, it was prolonged to 35 days.

The following risk factors were analyzed: age, smoking, diabetes, arterial hypertension, atrial fibrillation, history of an infarction, laboratory indicators, and body mass index. To assess the neurological deficit in the acute period of ischemic stroke, the National Institutes of Health Stroke Scale (NIHSS), the Rivermead mobility index, and the Rankin Scale were used. When assessing the severity of the stroke, the following gradation of the NIHSS was used: mild (0-5 points), medium (6-13 points), and severe (14 points or more) [19,25,28]. For the retrospective analysis, the patients were divided into 2 groups: the survivors during 65 days of observation (n = 37) and those who died during observation (n = 17).

In the early periods (the first 96 hours) after hospitalization, the level of D-dimer in the blood plasma obtained from the cubital vein was determined once. The D-dimer concentration was determined by the immunoturbidimetric method (microlatex agglutination) on a calibrated BCT analyzer (DadeBehring), the INNOVANCE D-Dimer kit (Siemens). In two cases, the analysis was performed on an automatic analyzer ACL TOP 700 (Instrumentation Laboratory, USA) with a reagent D-Dimer (Instrumentation Laboratory, USA). To obtain plasma, the blood was centrifuged at 1500-2000 g for 15 minutes.The results of the D-dimer level determination were reported in mg/l FEU (fibrinogen-equivalent units). The reference range was 0-0.49 mg/L for 52 patients and 0-0.286 mg/L for 2 subjects.

The data were processed using nonparametric tests. Quantitative variables such as age, D-dimer level, body mass index, time interval from hospitalization to D-dimer level determination, NIHSS, Rivermead, and Rankin scales were presented as median, upper and lower quartiles - Interquartile range (IQR). The statistical significance of differences was determined using the Mann-Whitney criterion (U). Correlation analysis was performed using the nonparametric Spearman test (R). Risk factors such as smoking, history of infarction, diabetes, atrial fibrillation, and lower urinary tract infections were presented as odds ratio (OR) with a 95% confidence interval (CI).

Linear and logistic regression analyses of lethality were performed depending on the level of the D-dimer expressed in terms of UNL. The accuracy of the model was verified by analyzing the frequency characteristics (receiver operating characteristic [ROC] curve), and the area under the curve (AUC) was determined to estimate the accuracy of the model. The Yates' correction for multiple comparisons was applied for the comparative analysis of lethality, depending on the level of the D-dimer. Statistical significance was defined as $p<0.05$. The statistical analysis was carried out using the Statistical 6.0 software for Windows.

Results. 3.1 Baseline Characteristics of Study Samples. The mean age of the patients included in the study was 78 years (range: 75-82 years), and 66.7% of the patients were women. The value of the NIHSS at admission was 9 (range: 5-16). Adverse outcome was revealed in 17 patients (31.5%), whose life span was 22 days (range: 14-33 days) of observation.

The two groups did not differ in terms of sex (pF = 1.0) and the presence of risk factors, such as smoking (OR = 0.66, CI 0.1-4.4, pF = 0.65), myocardial infarction in the anamnesis (OR = 0.51, CI 0.1-2.2, pF = 0.44), diabetes (OR = 1.3, CI 0.4-4.5, pF = 0.76), atrial fibrillation (OR = 0.8, CI 0.3-2.95, pF = 1), and lower urinary tract infection (OR = 1, CI 0.3-3.6, pF = 1). However, there was a statistically significant difference in body mass index between the dead and the survivors (23.7 [range: 23.1-26.4] vs. 27.3 [range: 26-29.4] kg/m^2; pU = 0.0097). (Figure 1).

The severity of ischemic stroke, assessed using the Rivermead scale and NIHSS upon hospitalization, was significantly higher in patients with adverse outcomes. A correlation was found between the NIHSS and the D-dimer level (r = 0.41, $p<0.001$).

There was no statistically significant difference in the time between hospital-

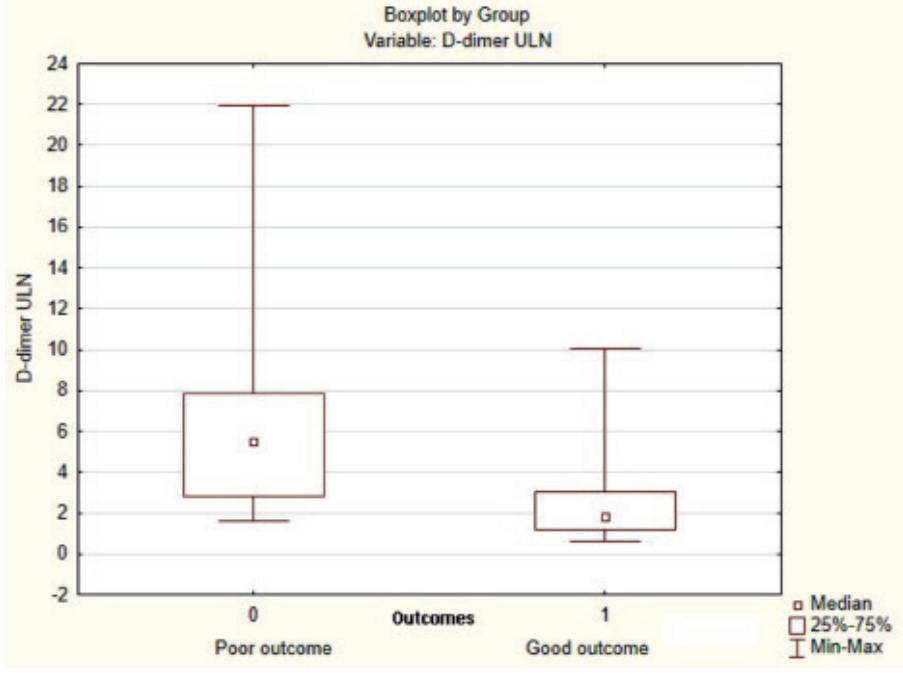

Fig. 1. D-dimer level, expressed in the upper limits of the norm, depending on the outcome

ization and the D-dimer level determination between the two groups. HT of the ischemic site was detected only in two patients from the survivor group; in the group of dead patients, HT was not revealed. In 47.1% of cases, the cause of death was a breakdown of stroke progression—an increase in cerebral edema and the progression of cardiovascular insufficiency. In another 47.1% of cases, the cause was not detected (death occurred in the nursing hospital), and in 5.8% cases (one observation), the cause of death was an acute vascular intestinal disease.

In the group of patients who survived, a severe neurological deficit (NIHSS 14 or more) was present in four patients (10.8%). In three patients, the D-dimer level was lower than 5 UNL, and one patient had a D-dimer level more than 5 UNL. In the group of patients who died, an intense neurological deficit was found in 17 patients (100%). Nine of them (52.9%) had a D-dimer level higher than 5 UNL, and eight of them (47.1%) had a D-dimer level lower than 5 UNL.

A logit regression model based on the obtained data showed that the probability of death was 30% at 4 UNL, 40% at 5 UNL, and 50% at 7 UNL (Figure 2).

The constructed ROC curve with an AUC of 0.79 corresponded to a good quality model with suitability for practical use of the obtained data (Figure 3).

Lethality was significantly higher in patients with a D-dimer level of ≥5 UNL.

Discussion. The level of D-dimer was significantly higher in patients with an adverse outcome: 5.53 (2.8, 7.84) versus 1.84 (1.2, 3.06), p = 0.00035. Logit regression model of outcomes showed that the probability of death is 30% with 4 UNL, 40% with 5 UNL and 50% with 7 UNL. Statistically, the lethality was significantly higher in patients with a D-dimer level ≥5 UNL; OR 5.813 (1.596-21.174), p = 0.05.

Yang et al. showed that a high level of plasma D-dimer is a predictor of mortality in patients with ischemic stroke [24]. Our study has shown the reasonability of the D-dimer level calculation (in UNL), and the criteria of adverse outcome risk, which increases with the level of D-dimer exceeding 5 UNL within 96 hours of hospitalization, have been refined. The informative value of the D-dimer level in predicting lethality during the 65-days observation period in patients with acute ischemic stroke with involuntary immobilization for more than 1 day has also been confirmed. Furthermore, the relationship between the increase in the D-dimer level and the neurological deficiency

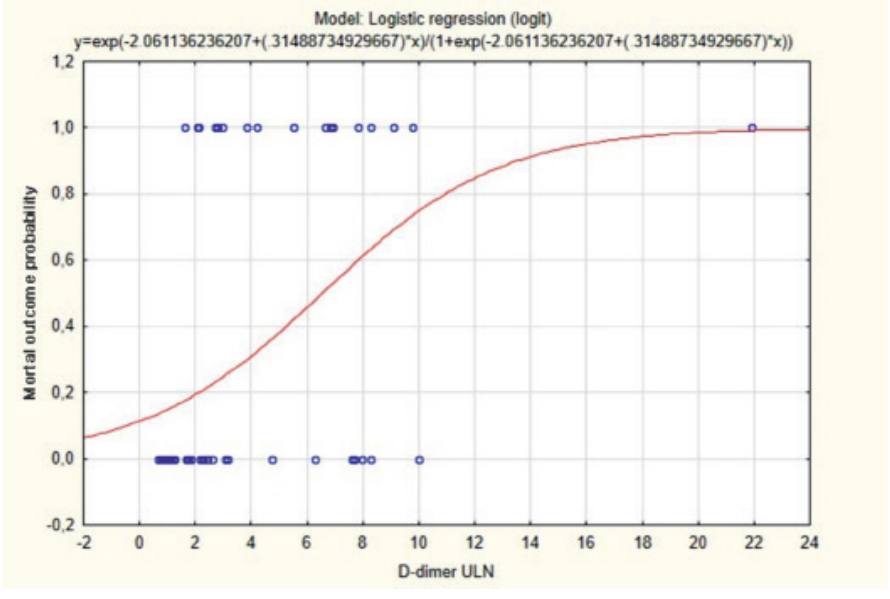

Fig. 2. Logistic regression model of the probability of death depending on the level of D-dimer, expressed in the upper limits of the norm

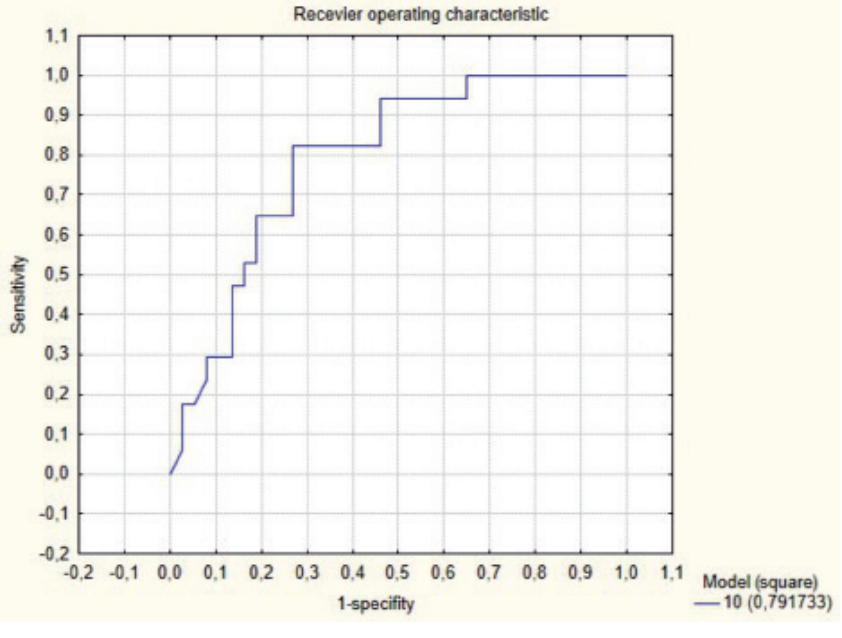

Fig. 3. Receiver operating characteristic (ROC) -curve, showing the adequacy of the model of using the level of D-dimer, expressed in the upper limits of the norm, as a predictor of mortality

level assessed by the NIHSS has been shown. Our results conform to those of other studies, showing that higher D-dimer levels are associated with a high severity of neurological deficits, major ischemic lesions, and poor prognosis in stroke patients [4,23,32].

In accordance with the Russian standard for cerebral infarction treatment [21], all patients hospitalized with a stroke spend 24 hours in the resuscitation department or intensive care unit, i.e., they have strict immobilization, and in the early recovery period of stroke, no more than half of the patients are able to live without outside help [20]. Thus, the results of our study can be applied to the bulk of patients hospitalized with ischemic stroke.

The level of D-dimer not exceeding 1 UNL is a rare phenomenon in patients with thrombosis. This may be due to a small thrombus size and late determination with false-positive results due to a mistake at the pre-analysis stage (such as storage of plasma samples for more than 6 hours), which results in a decrease in fibrinolytic activity due to either deficiency of tissue plasminogen activator (t-PA) or a high level of plasminogen activator inhibitor (PAI-1). A false increase in the level of this indicator can be caused by a violation of the blood sampling technique;

excessive and prolonged application of a tourniquet above the site of venipuncture activates the coagulation system and the release of the tissue plasminogen activator into the bloodstream, which increases the level of D-dimer in the laboratory study [10].

The probability of death, estimated using logistic regression, with a D-dimer level equal to or higher than 5 UNL was 40% (Figure 1). We have confirmed the significance of this value as a cut-off point with respect to the mortality prognosis in patients with acute ischemic stroke. Further, the results of this study may indicate the advisability of applying therapeutic dosages of anticoagulants at a D-dimer level ≥5 UNL.

However, if a high level of D-dimer is a predictor of death, and its decrease during treatment reduces the likelihood of adverse outcomes, then a dynamic control of the D-dimer level in blood plasma should accompany the therapeutic doses of direct anticoagulants. Thus, moving from anticoagulant therapy to preventive dosages is advisable when the D-dimer level is lower than 5 UNL. This hypothesis needs to be verified by further studies.

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ATHEROSCLEROTIC CHANGES IN CEREBRAL VESSELS OF THE ELDERLY AND SENILE PEOPLE: ETHNIC, GENDER AND AGE CHARACTERISTICS

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The study included 522 patients aged 60 to 89 years old, who were divided into three ethnic groups (Evens, Yakuts and Russians) and two age groups (elderly and senile age). Doppler ultrasound examination revealed ethnic features of atherosclerotic changes in cerebral vessels at various stages of chronic cerebral ischemia in elderly and senile patients of the Republic of Sakha (Yakutia). The study revealed that the most pronounced atherosclerotic changes in the extracranial arteries were observed in Russians, then in Yakuts. Evens ranked last in atherosclerotic lesions of the cerebral vascular structure.

Keywords: ethnicity, elderly and senile age, chronic cerebral ischemia, intima-media thickness.

For many years, cerebrovascular pathology remained one of the most pressing problems of medicine, holding one of the first places in the structure of mortality and causes of disability. Ischemic disorders account for about 75% of all cerebrovascular diseases. The observed global aging of the population and the increase in the proportion of elderly and senile people in its structure, associated with an increase in life expectancy, indicates further spread of chronic cerebrovascular insufficiency and a high vulnerability of the brain to ischemia due to involutional development changes. The thickness of the intima-media complex (IMT) in the carotid arteries is one of the independent risk factors for the development of transient ischemic attacks and strokes. The larger it is, the higher the likelihood of developing acute ischemic stroke. Simultaneously, the results of certain studies suggest that the importance of the thickness of this complex as a risk factor for the development of vascular catastrophes requires additional evidence. In this regard, the study of ethnic characteristics of atherosclerotic changes in elderly and senile people is of great importance in planning preventive and therapeutic measures for cerebrovascular diseases.

The aim of the study was to research the ethnic and age-related characteristics of atherosclerotic lesions of the cerebral vessels in elderly and senile patients with chronic cerebral ischemia in the Republic of Sakha (Yakutia).

Materials and methods. The survey was conducted among 522 people aged 60 to 89 years old. All patients were divided into three ethnic groups:

The first group of observations was comprised of 174 patients - representatives of the indigenous small-numbered peoples of the North, living in the Arctic zone. In the subgroup with chronic cerebral ischemia (CCI) I - 87 people, CCI II - 87.

Group II - 177 patients of Yakut nationality (CCI I - 90, CCI II - 87) living in the Vilyuysky district.

Group III - 171 patients of Russian nationality (CCI I - 86, CCI II - 85) living in Yakutsk. These zones differ in their natural and ecological characteristics, and the people who live in them have differences in their lifestyle, food habits, the nature of their main occupations and the level of civilization.

The clinical study included a thorough and in-depth collection of medical history, the study of outpatient records from clinics at the place of residence, archived medical histories, extracts and certificates. During the observation process, all patients were examined by a neurologist, psychiatrist, therapist, and cardiologist.

In order to determine and clarify the stage of chronic cerebral ischemia, the medical histories and complaints of patients were analyzed, and a clinical determination of their neurological status was carried out. To systematize the obtained data on the health status of each patient, a questionnaire was developed.

The diagnosis of chronic cerebral ischemia (CCI) was made in accordance with the classification of vascular brain injuries of the Institute of Neurology of the Russian Academy of Medical Sciences (1985), the diagnosis was made in accordance with ICD-10. The diagnostic criterion was a confirmed lesion of the cerebral vessels with the corresponding clinical picture of the stages of CCI according to the classification of E.V. Schmidt - patients with stage I chronic cerebral ischemia (CCI I) and patients with stage II chronic cerebral ischemia (CCI II). According to the State Statistics Committee of the Republic of Sakha (Yakutia), the average life expectancy for men is 60.6 years, for women - 72.2, the difference is 11.6 years.

The age of the patients ranged from 60 to 89 years (the average age of the examined was 72.7 ± 7.2 years, for men - 72.9 ± 7.2 years, for women - 72.6 ± 7.2 years). As can be seen from Table 2, older people predominate among peers, and in the CCI II subgroup as well; old people are more common among the Yakuts and Russians.

The main method for studying cerebral hemodynamics in this paper was Doppler ultrasound (USG). Doppler study with color scanning and spectral Doppler analysis of the brachiocephalic arteries at the extracranial level was carried out according to the standard technique on ACUSON "Sequoia-512" ultrasound systems using a linear transducer with a frequency of the generated ultrasound signal of 4 and 8 MHz, in continuous wave mode.

To assess the structural and functional state of the arterial blood supply to the brain during duplex scanning, qualitative information on the structural state of the vessels in the form of images of thickening of the intima and the middle membrane complex, atherosclerotic plaques and pathological gyri was collected. IMT was determined at three points of both common carotid arteries in the distal 1.0 cm segment with the calculation of the mean. IMT values of less than 0.9 mm were considered normal.

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The prevalence of atherosclerotic plaques (ASP) in the carotid artery was assessed according to the following principle: grade 0 - no plaque, grade I - one or more plaques with a surface area of less than 10 mm2, grade II - plaques with an area of 10 mm2 or more. Plaque volume was calculated as the difference between vessel and lumen volume.

The statistical processing of the results was carried out using the SPSS 19.0 software package. The mean with standard deviation was calculated to describe the quantitative data. Qualitative characteristics are presented in the form of frequency tables containing absolute values and the relative participation of the characteristic (in percentages). The probability distribution of quantitative characteristics were checked for compliance with the reference distribution using the Kolmogorov-Smirnov test. The test results showed that the distribution of the studied indicators differs significantly from the reference distribution. Based on this, nonparametric tests were used for further statistical analysis. To compare the mean values of the studied indicators, the paired Mann-Whitney test was used. When comparing more than two study groups, the Kruskal-Wallace nonparametric analysis of variance was used. To study the contingency of qualitative characteristics, the classical Pearson chi-square test was calculated, and in cases where the expected frequency in more than 20% of the cells in the contingency tables was less than 5, the Pearson chi-square test was used. Calculated with Yates' correction for continuity. To establish the value of the factor contribution to the overall picture of the contingency tables, we were guided by the value of the normalized remainder in the cells. Provided that the standardized cell remainder was equal to or greater than 2.0, it was concluded that the contribution of this cell was statistically significant with $p < 0.05$. Spearman's rank correlation coefficient was used to assess the relationship between quantitative variables.

Results. It is known that atherosclerosis is a multifocal disease that affects 2-3 vascular basins, and at the same time, the most dangerous location of atherosclerotic plaques is in the vessels of the heart and brain, and especially in the carotid arteries.

Violation of the wall of the main arteries of the head is manifested by thickening of the intima-media, primarily of the carotid arteries, as a result of the progression of atherosclerosis.

In the study of IMT depending on the stage of CCI and ethnicity, we obtained the following results (Table 3). In Evens with CCI stage II, IMT values on the left are significantly higher than in CCI stage I. In Yakuts, statistically significant differences were obtained by comparing the mean values of IMT on the left, which were higher in individuals with stage II CCI. In Russians, we did not obtain significant differences in the main ultrasound characteristics of cerebral vessels between the stages of CCI.

No statistically significant differences were found on the presence of ASP in the cerebral vessels in the three study groups. The characteristics of ASP of the cerebral vessels, depending on the stage of CCI, are shown in Table 4. Both Evens and Russians with stage II CCI were statistically significantly more likely to have hypoheterogeneous than echoheterogeneous ASPs, localized forms were more common and concentric - less common, more often large (> 10 mm) ASPs with a smooth surface were found. In addition, echohomogeneous ASPs were found significantly less frequently among the Evens. In Yakuts with stage II CCI, hypoheterogeneous and, less often, hypoechogeneous ASPs were detected, large (> 10 mm) ASPs were more common.

The study revealed that nationality is one of the factors affecting size ($\chi2 = 18.459$ $p = 0.001$ and $r = 0.181$ $p = 0.042$ for Russians), density ($\chi2 = 30.379$ $p = 0.000$ and $r = - 0.144$ $p = 0.043$ for Yakuts), shape ($\chi2 = 27.159$ $p = 0.000$ and $r = 0.195p = 0.042$ for Evens) and surface of the plaques examined ($\chi2 = 23.146$ $p = 0.000$ and $r = 0.192$ $p = 0.042$ for Evens).

There is also a significant association between age and all characteristics of plaques: density ($\chi2 = 38.050$, $p = 0.000$ $r = -0.228$ $p = 0.041$), size ($\chi2 = 38.067$ $p = 0.000$ $r = 0.247p = 0.042$.), shape ($\chi2 = 42.380$ $p = 0.000$, $r = 0.224$, $p = -.042$) and plaque surface ($\chi2 = 29.571$ $p = 0.000$, $r = 0.223p = 0.041$). The results of statistical analysis also revealed the dependence of plaque properties on gender. At the same time, the statistical relationship is stronger between gender and plaque density ($\chi2 = 9.425$ $p = 0.051$ $r = 0.117$, $p = 0.04$ in men), their size ($\chi2 = 7.570$, $p = 0.032$, $r = - 0.028$, $p = 0.044$ in men) and shape ($\chi2 = 8.109$, $p = 0.044$, $r = -0.092$, $p = 0.044$ in women).

Table 1

Distribution of patients with chronic cerebral ischemia by gender and ethnicity

CCI I	Groups					
	I		II		III	
	n	%	n	%	n	%
Men	27	31.1	28	31.1	25	29.1
Women	60	68.9	62	69.9	61	70.9
	χ^2=25.04; p<0.001		χ^2=25.69; p<0.001		χ^2=30.14; p<0.001	
CCI II						
Men	37	42.5	47	54.1	23	27.1
Women	50	57.5	40	45.9	62	72.9
	χ2=3.89; p=0.049		χ2=1.3; p=0.29		χ2=35.79; p<0.001	

Table 2

Distribution of patients with chronic cerebral ischemia by age

	Groups					
	I		II		III	
CCI I						
Age	n	%	n	%	n	%
60-74	42	48.3	61	67.8	54	62.8
75-89	45	51.7	29	32.2	32	37.2
	χ2=0.21; p=0.65		χ2=22.76; p<0.001		χ2=11.26; p<0.001	
CCI II						
Age	n	%	n	%	n	%
60-74	46	52.9	58	66.7	40	47.1
75-89	41	47.1	29	33.3	45	52.9
	χ2=0.58; p=0.45		χ2=19.33; p<0.001		χ2=0.59; p=0.44	

Table 3

The thickness of the intima-media complex depending on the stage of CCI

Indicator	Group I (n=174)			Group II (n=177)			Group III (n=171)		
	Stage of CCI		p	Stage of CCI		p	Stage of CCI		p
	I (n=87)	II (n=87)		I (n=90)	II (n=87)		I (n=86)	II (n=85)	
IMT on the right, mm (M ± SD)	1.12±0.15	1.07±0.23	0.240	1.19±0.18	1.16±0.18	0.292	1.24±0.19	1.25±0.24	0.492
IMT on the right >0.9 mm, n (%)	79 (90.8%)	66 (75.9%)	**0.008**	84 (93.3%)	80 (92%)	0.725	82 (95.3%)	81 (95.3%)	1.0
IMT on the left, mm (M ± SD)	1.10±0.20	1.15±0.19	0.086	1.13±0.21	1.21±0.22	**0.035**	1.21±0.23	1.27±0.23	0.122
IMT on the left >0.9 mm, n (%)	68 (78.2%)	80 (92%)	**0.011**	80 (88.9%)	80 (92%)	0.489	78 (90.7%)	82 (96.5%)	0.124
IMT >0.9 mm с обеих сторон, n (%)	66 (75.9%)	63 (72.4%)	0.603	80 (88.9%)	77 (88.5%)	0.936	77 (89.5%)	79 (92.9%)	0.431
Presence of ASP, n (%)	33 (37.9%)	37 (42.5%)	0.536	50 (55.6%)	46 (52.9%)	0.720	47 (54.7%)	45 (52.9%)	0.823

Table 4

Characteristics of a sterosclerotic plaque of cerebral vessels with CCI depending on the stage

Indicator, n (%)	Groupe I (n=70)			Groupe II (n=96)			Groupe III (n=92)		
	Stage of CCI		p	Stage of CCI		p	Stage of CCI		p
	I (n=33)	II (n=37)		I (n=50)	II (n=46)		I (n=47)	II (n=45)	
Density of ASP: Hypoechoic Hypogeterogeneous Echoheterogeneous Echohomogeneous	 3 (9.1) 7 (21.2) 16 (48.5) 7 (21.2)	 6 (16.2) 24 (64.9) 7 (18.9) 0	 *0.485* ***<0.001*** ***0.009*** ***0.004***	 18 (36) 10 (20) 11 (22) 11 (22)	 4 (8.7) 22 (47.8) 14 (30.4) 6 (13)	 ***0.001*** ***0.004*** *0.347* *0.251*	 5 (10.6) 10 (21.3) 19 (40.4) 13 (27.7)	 6 (13.3) 23 (51.1) 9 (20) 7 (15.6)	 *0.690* ***0.003*** ***0.033*** *0.159*
The size of ASP: ≤10 мм >10 мм	 25 (75.8) 8 (24.2)	 19 (51.4) 18 (48.6)	 ***0.035***	 46 (92) 4 (8)	 12 (26.1) 34 (73.9)	 ***<0.001***	 37 (78.7) 10 (21.3)	 14 (31.1) 31 (68.9)	 ***<0.001***
The form of ASP: Localized Semi-concentric Concentric	 8 (24.2) 1 (3) 24 (72.7)	 26 (70.3) 5 (13.5) 6 (16.2)	 ***<0.001*** *0.203* ***<0.001***	 22 (44) 9 (18) 19 (38)	 21 (45.7) 9 (19.6) 16 (34.8)	 *0.871* *0.844* *0.744*	 9 (19.1) 6 (12.8) 32 (68.1)	 23 (51.1) 6 (13.3) 16 (35.6)	 ***0.001*** *0.936* ***0.002***
Surface of ASP: Smooth Uneven	 4 (12.1) 29 (87.9)	 26 (70.3) 11 (29.7)	 ***<0.001***	 25 (50) 25 (50)	 22 (47.8) 24 (52.2)	 *0.831*	 12 (25.5) 35 (74.5)	 24 (53.3) 21 (46.7)	 ***0.006***

Violation of the wall of the main arteries of the head is manifested by thickening of the intima-media, primarily of the carotid arteries, as a result of the progression of atherosclerosis.

In the study of hypertrophy and initial atherosclerotic transformation of elastic vessels (Table 5), the mean IMT in group I had a statistically significant difference and was 1.0 ± 0.34 mm in patients with CCI I, CCI II - 1.1 ± 0.28, which was significantly lower than that of other groups. The maximum mean IMT value was found in patients of group III, in whom several main arteries of the head were affected: (in the CCI I subgroup - 1.15 ± 0.33 and in the CCI II subgroup - 1.18 ± 0.34). Group II patients occupy an intermediate place in this indicator (1.13 ± 0.24 and 1.15 ± 0.27, respectively). The study revealed the influence of ethnicity on IMT

Table 5

The thickness of the intima-media complex in patients with chronic cerebral ischemia depending on age and ethnicity (mm)

Category	CCI I			CCI II		
	IMT	χ^2	p	IMT	χ^2	p
Ethnos						
Groupe I	1.0±0.34	228.21	0.001	1.1±0.28	246.29	0.001
Groupe II	1.13±0.24	274.82	0.001	1.15±0.27	262.63	0.001
Groupe III	1.15±0.33	281.58	0.001	1.18±0.34	274.29	0.001
Gender						
Women	1.08±0.31	13.079	0.001	1.14±0.30	13.054	0.001
Men	1.11±0.30	68.949	0.001	1.16±0.31	89.332	0.001
Age						
60-74	0.99±0.30	293.61	0.001	1.00±0.29	298.85	0.001
75->	1.21±0.28	317.93	0.001	1.28±0.30	322.4	0.001

with an average correlation strength ($\chi 2 = 11.374$, $r = 0.118$, $p = 0.044$).

Elderly patients more often have an intima-media thickness of 0.9 cm, and senile patients - more than 0.9 cm thickness. The age distribution showed that the older the subjects are, the higher the thickness of their intima-media is.

Thus, the age factor enhances the degree of remodeling of the vascular wall of the carotid arteries, which is consistent with the works of other authors [3,4].

Comparative analysis of IMT as a function of gender shows that IMT in men is, on average, statistically significantly higher than in women. (1.11 ± 0.31; 1.18 ± 0.31 versus 1.08 ± 0.31 and 1.14 ± 0.30, $p = 0.04$).

Conclusion. Ethnicity-dependent variability of intima-media thickness of the common carotid artery was revealed in elderly and senile residents of the Republic of Sakha (Yakutia). Indirect changes in cerebral blood flow, which influenced the formation and development of cerebrovascular disorders, are due to ethnic, age and gender characteristics.

An analysis of intima-media thickness demonstrated that the Yakuts and Russians have significantly greater IMT than the Evens. In all groups with the second stage of chronic cerebral ischemia, hypoheterogeneous and large atherosclerotic plaques were the most common. When compared by gender, it is significantly higher in men. With age, a remodeling and thickening of this indicator in the form of an increase and hardening of plaques was observed.

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CREATION OF A MODEL OF OVARIAN CANCER IN IMMUNODEFFICIENT MICE OF THE BALB / C NUDE LINE

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The purpose of this study was to create an in vivo model of ovarian cancer allowing control of the dynamics of tumor growth and adequate data on its size. As a model Balb/c Nude mouse lines were used. Removal of the mouse ovary with an implanted tumor fragment under the skin made it easier to visualize and to control the dynamics of xenograft growth.

Keywords: ovarian cancer, PDX model, patient-derived xenograft, Balb/c Nude, xenograft, in vivo models.

Ovarian cancer (OC) is the fifth most common malignant tumor in females aged 55 to 69 years, and the fourth most common cancer in females aged 40-54 years. According to the data on the world morbidity, OC is diagnosed in 9.1 cases per 100 000 female population. According to the Rosstat statistics, 17.8 cases on average were registered per 100 thousand female population in 2016. OC causes more deaths than any other cancer of the female reproductive system in Russia: 7 616 women died from OC in 2018 [6].

OC has several histological and molecular subtypes. Serous carcinomas are the main group of malignant epithelial tumors of the ovaries. Low-grade serous carcinoma consists of cystic, papillary and solid components [11,13]. Early diagnosis of OC is complicated, and its therapy effect is poor, so it is important to study the nature of this disease and develop methods for its treatment using various biological models [7,8].

Today, one of the tasks of researchers creating various animal tumor models is to reproduce the complexity of the tumor

microenvironment. Different experimental models have their own advantages and disadvantages which may influence the subsequent results of translational studies of new chemotherapy and immunotherapy agents [9].

The development of PDX (patient-derived xenograft) models for cancer research is based on the assumption that these models are close to the original donor tumors. Transplantation of cancer cells or tissues can be heterotopic or orthotopic, and each method has its advantages and disadvantages. Heterotopic method is characterized by an easy implantation and accurate monitoring of the tumor size. Orthotopic transplantation is technically more complicated and often requires ultrasound or exploratory laparotomy to monitor the dynamics of the tumor node growth; however, the advantage of this method is the preservation of the microenvironment of human tumors [3]. Similar microenvironment and carcinogenesis of the xenograft and the donor tissue allow assessment of drug effectiveness by determining the degree of inhibition of tumor growth by studying the dynamics of tumor growth [2]. Tumor visualization is one of the main difficulties in creating an adequate PDX model of OC. Being unable to visualize the tumor in vivo, some researchers performed necropsy at the end of the experiment and measured the isolated fragments of xenografts [10,15]. Such method of measurements does not allow registering the individual dynamics of the xenograft growth.

That is why the aim of this study was to create an *in vivo* OC model allowing control of the dynamics of tumor growth and adequate data on its size.

Material and methods. Prior to the experiment, an approval of the ethics committee of National Medical Research Centre for Oncology № 4/82 from 30.06.2020 was obtained.

Tumor material. Patient V. diagnosed with serous ovarian carcinoma Gx T2-3NxM0 was the donor of the tumor material. The patient gave her informed consent for the use of the biological material. The ovarian tumor was surgically removed, and its fragment was placed in nutrient RPMI medium containing gentamicin (5%); the resulting material was purified from elements of necrotic masses and connective tissue and divided into fragments with a volume of 8 mm^3. The time from the moment of resection of the tumor material to the moment of its transplantation did not exceed 20 minutes, which is an important condition for the successful engraftment.

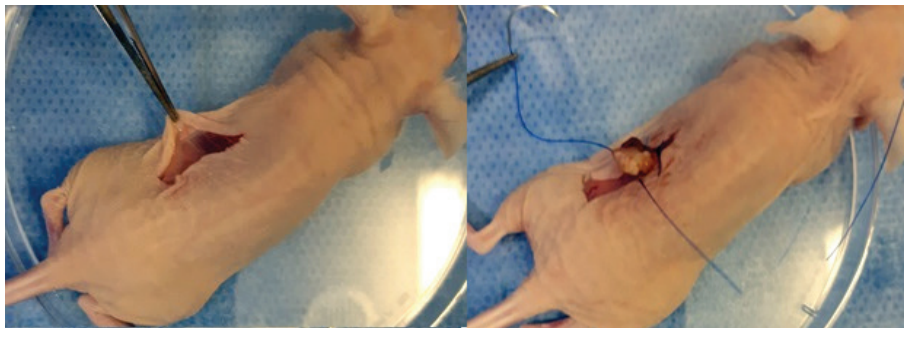
Animals. The experiment was carried out in accordance with the guidelines for the care and use of laboratory animals [1]. An orthotopic model of ovarian cancer was created in 20 female Balb/c Nude mice. The key characteristics of this strain is partial immunodeficiency of animals [5]. The age of the mice was 5 weeks, and their average weight was 22-24 g. The animals were kept in the SPF vivarium of the National Medical Research Centre for Oncology in individually ventilated cages at a temperature of 21-23 °C, with free access to food and water.

Creation of the PDX model of ovarian cancer. The animals were anesthetized prior to the implantation of tumor fragments. This procedure consisted of two stages: premedication with xylazine (injected at a dose of 1.5 ml/kg of body weight of Balb/c Nude mice) and anesthesia with zoletil (injected at a dose of 67.5 mg/kg of body weight of Balb/c Nude mice) [4]. After the mouse entered the surgical stage of anesthesia, it was placed on the operating table in a prone position. The skin of the animal was dissected parallel to the spine, below the costal arch; to make the manipulation easier, a second skin incision was made, 10 mm long, perpendicular to the first one. The skin flap was lifted to visualize the ovary (figure 1A) which was located between the spleen, pancreas (located above the ovary) and the kidney (located closer to the spine).

Then the abdominal wall was dissected parallel to the spine, and the ovary was removed into the surgical wound; the serous membrane was incised with a scalpel along the edge opposite to the vascular pedicle for 2 mm in the implantation area. The ovary was fixed from above to the peritoneum with a ligature, thus leaving it between the abdominal wall and the skin. A tumor fragment was implanted into the ovarian incision, sutured using a 6-0 suture under the control of an operating microscope (figure 1B). The abdominal wall and skin were sutured with a furrier's suture, a 4-0 ligature.

Subcutaneous tumor growth was observed during 8 weeks after xenotransplantation; measurements were performed twice a week using a caliper (GRIFF, Russia). Then necropsy was performed. The resected ovary was examined histologically.

Histological analysis. Tumor fragments were fixed in 10% formalin solu-



A B

Fig. 1. Surgical manipulations in the model creation. A - visualization of the ovary under the peritoneum of a Balb/c Nude mouse; B - implantation of tumor material into the ovary of an immunodeficient mouse

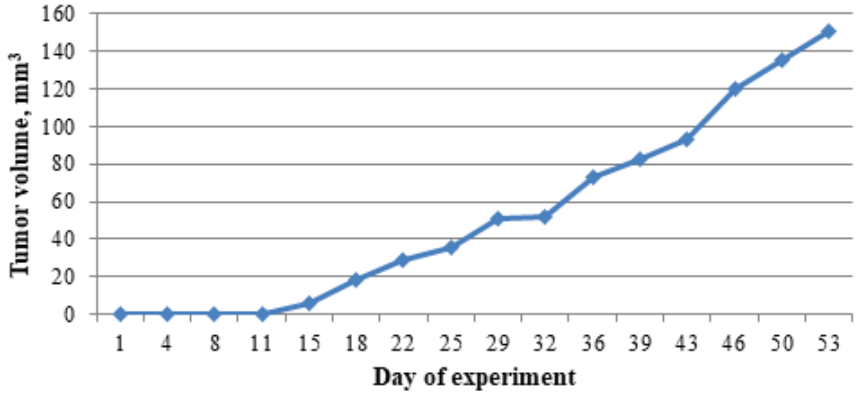


Fig. 2. Dynamics of the growth of an orthotopic xenograft of ovarian cancer during 8 weeks of observation.

tion for 24 h and dehydrated in alcohol and xylene baths. Then the tumor was embedded in paraffin, and micropreparations were prepared. Obtained sections were stained with hematoxylin and eosin. Histological preparations were examined under a light microscope. The primary tumor, PDX and the murine intact ovaries were analyzed histologically.

Results and discussion. During the observation of mice with implanted ovarian cancer, tumor growth was registered in 17 out of 20 operated mice, which accounted for 85% xenograft engraftment. After 8 weeks from the moment of inoculation, tumor nodules could be determined; their volume ranged from 130.43 to 170.1 mm^3. Figure 2 demonstrates the xenorgaft growth dynamics.

Note. Data are presented as averages. At necropsy, the tumor was separated from the ovary to create subsequent passages. Histological examination revealed that the donor tumor had the structure of serous carcinoma with the presence of psammoma bodies. Areas of solid structure were identified in the tumor. No foci of necrosis were found (figure 3 A).

Examination of a xenograft fragment revealed single tumor cells and their accumulations of various sizes and shapes infiltrating the ovarian stroma. Malignant cells were characterized by the presence of distinct small nucleoli and moderate nuclear polymorphism. Foci of cells with karyorrhexis and karyolysis, as well as rather extensive necrotic areas, were noted in the tissue. The data were obtained by light microscopy, and it showed that the tumor reproduced in the created PDX model was identical to the donor one. The histological picture in the experimentally reproduced tumor corresponded to serous carcinoma, and papillary and solid structures were determined in it. In addition, the solid component contained small psammoma bodies (figure 3 B, C).

One of the main disadvantages of creation of an orthotopic model of ovarian cancer is the complexity of visualizing the growth of a tumor node [14]. To solve this problem, we used a new approach to creating an OC model, and the ovary was transposed under the skin of the animal. The access of our laboratory to resected donor tumors ensured the timely delivery of biological material. This allowed xenograft engraftment in 85% cases. According to the literature, the engraftment of an ovarian cancer xenograft in the body of a recipient animal depends on the type of tumor, the transplantation site and the strain of immunodeficient mice; the period averages from 2 to 4 months, and the xenograft engraftment averages about 50% [3,12]. In our experiment, a high engraftment percentage was registered, and after 8 weeks the tumor node reached an average size sufficient for subsequent transplantation, which allowed creation of a model of ovarian cancer with adapted growth for its further use in experimental studies.

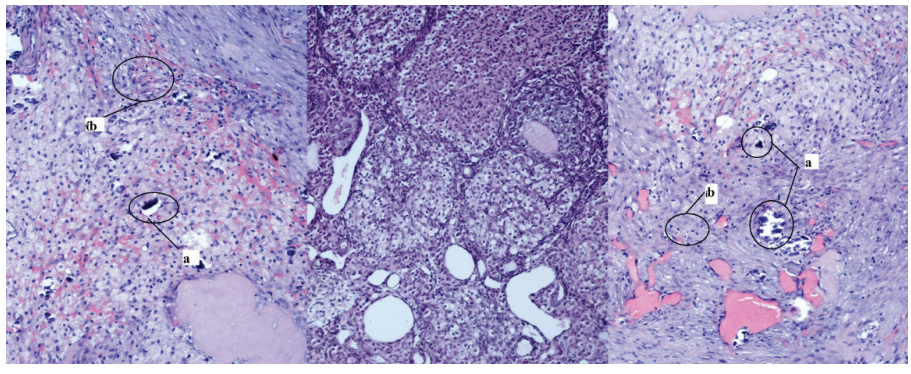


Fig. 3. Histological preparations of the patient's tumor, control ovarian tissue and xenograft. A - serous ovarian carcinoma (donor): a - psammary bodies; b – cancer cells; B - normal tissue of the murine ovary (control); C - tissue of ovarian cancer xenograft after the first passage in the recipient animal with the subcutaneous transposition of the ovary with the tumor: a - psammary bodies; b – cancer cells. Staining with hematoxylin and eosin. Magnification: x200.

Conclusion. A PDX model of ovarian cancer with orthotopic implantation of tumor material was created during the study. The originality of this work consists in a non-standard solution: transposition of the mouse ovary with a tumor fragment under the skin of an experimental animal, which allowed monitoring the development of an orthotopic xenograft without laparotomy or additional expensive equipment. This achievement contributes to the application of such a model in multiple studies.

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INTERRELATION OF THE CONTENT OF TRANSFERRIN, ERYTHROCYTES WITH THE FUNCTIONAL ACTIVITY LEUKOCYTES OF PERIPHERAL VENOUS BLOOD IN RESIDENTS OF THE EUROPEAN NORTH OF THE RUSSIAN FEDERATION

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The aim is establish the relationship between the content of transferrin, erythrocytes and the functional activity of leukocytes of peripheral venous blood in residents of the European North of the Russian Federation. Study included analysis of immunological parameters of 765 people, aged 18 to 89 years, living in the Arkhangelsk, of which 553 women aged 18-89 years and 212 men aged 18-84 years. It was found that increase in 49.21% of the examined individuals, the iron-containing protein increases against the background of a decrease in the content of the membrane transferrin receptor (CD71+). An increase in the content of transferrin is associated with an increase in the concentration of circulating IgG immune complexes in 44.21% of cases. With an increase in the concentration of transferrin in the blood in 27.95% of cases it is associated with a decrease in platelet count and in 24.08% - with a decrease in hemoglobin, mainly in men with increasing age. In 8.06% of the examined individuals, an increase in the level of transferrin is interrelated with an increase in the concentration of reagins. In 5.12% of cases, an increase in the transferrin content is associated with the activation of cell-mediated cytotoxicity, which is mainly supported by the pro-inflammatory cytokine IL-6. No association has been established between the increase in transport protein and the concentration of IL-10.

Keywords: transferrin, erythrocytes, hemoglobin, lymphocytes, cytokines, IgE, circulating immune complexes.

Background. The functions of the iron transport protein are not limited to participation in iron homeostasis [1]. Reference range of transferrin on the serum content is 170-340 mg / dL, in the bloodstream it is in a state of apo-, mono-, or bi-iron transferrin [2] and half-life is 8-12 days [3]. Normally, transferrin is saturated with iron by 20-30% [4,5], therefore, the iron-binding capacity is used only by 1/3. Iron transport protein with partial saturation, being a component of the antioxidant system, binds blood plasma iron and protects cell from disrupting membrane structures and reducing cell energy supply when intensifying lipid peroxidation processes [6]. In addition, iron binds to complexes with albumin, low molecular weight organic compounds, forming a pool of nontransferrin-bound iron [7,8]. The content of iron-binding protein is interrelated with the content of cells with a membrane receptor for transferrin [9,10]. In addition, when binding to receptors on the cell surface, transferrin is involved in the transport of metals: Zn, Co, Ga, Al. It should be taken into account that the content of transport proteins in the inhabitants of the North fluctuates within wide limits due to the pronounced photoperiodicity and intensity of the geomagnetic field [11,12].

The aim of the study was to establish the relationship between the content of transferrin and erythrocytes with the functional activity of leukocytes in peripheral venous blood in residents of the European North of the Russian Federation.

Material and methods. The immunological results of preanalytical and analytical stages of examination of 765 people were analyzed, including 553 women aged 18-89 years and 212 men aged 18-84 years, living in Arkhangelsk, who applied to the center of professional diagnostics "Biolam". The inclusion criteria are residence of the examined persons in Arkhangelsk and voluntary consent to the examination.

All stages of clinical laboratory examination were performed by medical workers of the laboratory of the center "Biolam": 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 examination results. Clinical and

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diagnostic interpretation of the examination results, identification of risk factors and causes of the disease, the formation of recommendation protocols was carried out by the doctor of medical sciences, professor, immunologist Dobrodeyeva Lilia Konstantinovna. The examination results, protocols of recommendations are kept by the attending physician in compliance with the requirements of the legislation of the Russian Federation on the protection of confidential information and personal data. Before the laboratory examination, all volunteers were 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 FECIAR UrB RAS while maintaining the confidentiality of the results.

A database was formed, including the data of the volunteer: date of birth, date of examination, age, sex and indicators of the immune background. The immunological study complex included the study of a hemogram (the number of platelets, red blood cells, white blood cells, the total hemoglobin content in the blood, a leukogram with 5-component differentiation of white blood cells), the phagocytic activity of peripheral blood neutrophil leukocytes in blood smears stained according to the Romanovsky-Gimz method. By flow cytometry using the Epics XL apparatus of Beckman Coulter (USA), Immunotech a Beckman Coulter Company (France) reagents studied lymphocyte phenotypes (CD3+, CD4+, CD8+, CD10+, CD16+, CD71+, CD95+). Transferrin, serum immunoglobulins (IgA, IgM, IgG, IgE) and cytokines (TNF-α, INF-γ, IL-6, IL-10) were determined by enzyme immunoassay. The reactions were evaluated using a Multiskan MS photometer (Labsystems, Finland) and a Bio-RAD Evolis automatic enzyme immunoassay analyzer (Germany). The concentration of circulating immune complexes was determined by a standard precipitation method using 3.5%; 4.0%; 7.5% PEG-6000.

Initial analysis of study results was based on the formation of comparison groups by sex and age (18-50 years and 50-89 years relative to median age). The level of transferrin is stable indicator with less pronounced differences by sex and age [13], so further analysis of results of the study was carried out randomly based on statistical analysis of transferrin concentration, followed by the formation of comparison groups based on the distribution of quantitative values relative to the reference range (170-340 mg/dl): 1st group - transferrin content below the reference limit of content <170 mg/dl (n=68), 2nd group - transferrin content within 170-340 mg/dl (n=441), 3rd group - content transferrin above the reference limit> 340 mg/dl, (n=256). The average age in each group was, respectively: 44.73 ± 1.81; 48.02 ± 0.71 and 48.99 ± 0.92 years. The comparison groups are uneven, which is a consequence of different mechanisms of regulation of decreased and increased levels of transferrin content.

The normality check of the distribution of quantitative indicators was carried out using the Kolmogorov-Smirnov single-sample criterion, both with the full sample volume and when dividing observations into comparison groups, since the results of checking the nature of the distribution using statistical criteria are sensitive to the sample size. In the formed comparison groups, the distribution of the values of most indicators obeyed the law of normal distribution. The values of quantitative indicators are presented as M ± δ, the assessment of differences in the comparison groups (1-2; 2-3; 1-3) was carried out using the Student's t-test and Mann-Whitney U-test in cases of asymmetric distribution of indicator values. To study the relationship between the indicators, the correlation coefficient r-Pearson and Spearman was determined depending on the nature of the distribution of the values of the indicators. Statistical analysis of the data was carried out using the Statistics 21.0 software package.

Findings and discussion. It was found that in 27,95% of the examined individuals an increase in the concentration of transferrin in the blood (from 153,56±1,85 to 422,04±2,94 mg /dl) was associated with a decrease in the platelet count (from 232,54±8,09 up to 209,68±3,98 $x10^9$ cells/l; p=0,008). Evidence appears in the literature on the iron-independent function of transferrin in maintaining coagulation balance. An imbalance in coagulation is associated with an increased level of transferrin, which interacts with thrombin and factor XIIa (FXIIa), enhancing their enzymatic activity, and with clotting inhibitors, blocking the inactivating effect, thereby inducing hypercoagulation. [14,15]. With an increase in transferrin in 24.08% of cases, the concentration of hemoglobin decreases (from 134,76±3,01 to 129,34±1,18 g/l; p=0,048) within the reference limits of the content, without changes in the content of erythrocytes (4,53±0,06 and 4,61±0,13$x10^{12}$ cells/l) and leukocytes (6,40±0,17 and 6,20±0,11 $x10^9$ cells/l). At the same time, the most pronounced decrease in hemoglobin content (from 149.13 ± 1.75 to 135.0 ± 1.98, p = 0.01) occurs in men with an increase in age (Table 1) and no change in women (Table 2). A decrease in the level of hemoglobin is associated with a decrease in the concentration of erythrocytes in the blood (r=0,661, p<0,001).

There is a competitive relationship between erythropoiesis and leukopoiesis in the red bone marrow [16], which has become fundamental in establishing the relationship between the transferrin content with leukocytic row. Despite the fact that, depending on the level of transferrin content, no change in the total content of leukocytes in the blood was established, a decrease in the level of lymphocytes was revealed in the structure of the hemogram. So, with an increase in the concentration of iron transport protein in the blood, the content of lymphocytes decreases (from 2,15±0,06 to 1,87±0,03 $\times10^9$ cells/l, p=0,001; r = -0,137, p<0,001), mainly for the count of mature T-lymphocytes (CD3 +) (from 1,03±0,02 to 0,92±0,01, p=0,001; r= -0,160, p <0,001), activated T-lymphocytes to the transferrin receptor (CD71+) (from 0,62±0,01 to 0,37±0,01, p=0,001; r= - 0,644, p <0,001) and cells to programmed cell death (CD95+) (from 0,50±0,01 to 0,41±0,01, p=0,001; r= -0,202, p <0,001). Pays attention that in 49,21% of the examined individuals, an increase in the concentration of iron-containing protein against the background of a decrease in the content of cells with a membrane receptor for transferrin (CD71+) reflects a known pattern of changes in the activity of membrane receptors and a substrate for it by changing expression or shedding into the intercellular environment [17]. Shedding CD71 ensures elimination of excessive accumulation of Fe3 + and reactive oxygen species (ROS) during reticulocyte maturation [18]. Regeneration of superoxide radical in cells increases under hypoxic conditions [19].

Expression of CD71 + receptors depends on the presence of iron in the cell [13] and provides further differentiation and maturation of T-lymphocytes, since CD71 + provides the activated cell with iron. With an increased demand for intracellular iron or iron deficiency, the expression of the receptor for the iron-containing protein increases. In various lymphoproliferative processes, lymphocytes begin autonomous synthesis of CD71 + or utilize iron in a transferrin-independent way [20].

The expression of iron transport protein receptors is negatively affected by proinflammatory cytokines. With an increase in the content of transferrin in the blood, the concentration of pro-inflammatory cyto-

Взаимосвязь содержания трансферрина и параметров гемограммы в зависимости от возраста у мужчин и женщин

Indicators	Reference content limits	Возраст		p – validity of difference
		18-50 years old	50-84 years old	
		n=106	n=106	
Male gender				
Transferrin, mg/dl	170-340	284.06±9.78	299.75±10.01	
Erythrocytes, ×1012 cells/l	4.0-5.1	4.98±0.05	4.59±0.06	
Platelets, ×109 cells/l	180-320	198.02±5.01	197.0±8.24	
Hemoglobin, g/l	130-160	149.13±1.75	135.0±1.98**	0.01
Leukocytes, ×109 cells/l	4.0-8.8	6.30±0.16	6.32±0.16	
Monocytes, ×109 cells/l	0.09-0.6	0.46±0.02	0.50±0.02	
Lymphocytes, ×109 cells/l	1.5-4.0	1.97±0.05	1.98±0.06	
Neutrophils, ×109 cells/l	2.0-5.2	3.68±0.14	3.64±0.11	
Eosinophils, ×109 cells/l	0.02-0.3	0.19±0.01	0.18±0.01	
Female gender				
Transferrin, mg/dl	170-340	296.15±5.84	298.01±6.31	
Erythrocytes, ×1012 cells/l	4.0-5.1	4.35±0.02	4.39±0.03	
Platelets, ×109 cells/l	180-320	228.13±3.53	218.64±4.39	
Hemoglobin, g/l	130-160	124.21±0.97	127.48±0.91	
Leukocytes, ×109 cells/l	4.0-8.8	6.42±0.22	6.21±0.12	
Monocytes, ×109 cells/l	0.09-0.6	0.46±0.01	0.47±0.01	
Lymphocytes, ×109 cells/l	1.5-4.0	1.98±0.03	2.04±0.04	
Neutrophils, ×109 cells/l	2.0-5.2	3.61±0.07	3.53±0.09	
Eosinophils, ×109 cells/l	0.02-0.3	0.17±0.01	0.17±0.01	

kines increases: TNF-α (from 11,23±0,67 to 17,93±1,36 pg/ml, p=0,002), IL-6 (from 9,11±0,76 to 15,28±0,89 pg/ml, p=0,001), INF-γ (from 11,22±0,82 to 13,62±0,53 pg/ml, p=0,026). The strongest correlation is observed between the content of transferrin and IL-6 (r-Spearman = 0,222, p<0,001) and between the content of IL-6 and cells with a membrane receptor for the iron transport protein (r-Spearman = -0,197, p<0,001). The relationship between the iron-containing protein and the anti-inflammatory cytokine IL-10 has not been established, the concentration of which does not change (7,05±0,29 and 6,45±0,26 pg/ml) with an increase in transferrin content.

An increase in the content of iron-containing protein is associated with an increase in the concentration of circulating IgG immune complexes (CIC) in 44,21% of cases. It is known that the formation of CEC is aimed at removing genetically foreign agents from the body [21]. The mechanism for increasing the CEC is the activation of the kinin system, as well as exocytosis of biologically active substances of granulocytes and macrophages. Increasing the formation of various proteolytic enzymes and reactive oxygen species, acid hydrolases, katahepseins and collagenases contributes to strengthening the processes of damage, cell destruction, followed by an increase in components, substances in the intravascular medium, requiring binding and transport [22].

In 8,06% of the examined individuals, an increase in the transferrin concentration was associated with an increase in the IgE content (from 45,58±4,92 to 57,54±5,07 IU/ml, p=0,011) without changes in the IgA content (2,10±0,31 and 2,34±0,56 g/l), IgM (2,70±0.84 and 1,85±0,07 g/l) and IgG (17,93±0,56 and 18,00±0,24 g/l). This pattern is explained by the fact that IgE are the most sensitive and react with very low antigen concentrations, recognizing conformational epitopes, in contrast to immunoglobulins of other classes, which are able to recognize only linear epitopes of proteins [23].

In 5,12% of the examined individuals, an increase in the concentration of transferrin (from 153,56±1,85 to 422,04±2,94 mg/dl) is associated with an increase in the activation of cell-mediated cytotoxicity (CD8+), which is mainly supported by the pro-inflammatory cytokine IL-6 (r-Spearman=0,145, p=0,001).

Conclusion. It was found that in 49.21% of the individuals examined, the content of transferrin increases against the background of a decrease in the level of cells with a membrane receptor to transferrin (CD71+), which reflects the known competing relationships of the receptor and its level of shedding and substrate (transport). It is known that transferrin receptors can react with Fc Ig and even with IgA [24]. Thus, an increase in transferrin content is associated with an increase in the concentration of CIC containing IgG in 44.21% of cases. In 27.95% of the examined individuals, an increase in the concentration of iron-containing protein in the blood is associated with a decrease in platelet content (r = -0.073; p = 0.05), possibly indicating the involvement of transferrin in maintaining coagulation balance. In 24.08% of people, when the level of transferrin increases, the concentration of hemoglobin decreases (r = -0.275; p = 0.043), which confirms the increased iron requirement for erythropoiesis. The most pronounced decrease in hemoglobin was found in men with an increase in age. The remaining relationships given are of little importance and can be random. Thus, in 8.06% of the examined persons, the increase in the level of transferrin is interconnected with an increase in the concentration of reagents (r = 0.084; p=0.011). In 5,12% of cases, an increase in the content of the transferrin transport protein is associated with the activation of cell-mediated cytotoxicity, which is mainly supported by the pro-inflammatory cytokine IL-6 (r=0,195; p<0,001). The relationship between the increase in transport protein and the concentration of IL-10 has not been established. An increase in cytotoxicity enhances the processes of damage, destruction of cells with a subsequent increase in components, substances in the intravascular environment that require binding and transport. An increase in the concentration of not only circulating immune complexes, but also concentration transferrin is aimed at achieving this goal.

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DIAGNOSTIC AND TREATMENT METHODS

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STUDY OF THE COGNITIVE PROFILE IN PATIENTS WITH ESSENTIAL TREMOR

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The clinical picture of essential tremor, in addition to motor symptoms, includes a wide range of non-motor manifestations, which includes cognitive disorders, psychiatric and other disorders. This article presents the results of a study of the cognitive status in patients with essential tremor using the 3-CT scale. As a result of the study, it was shown that patients with essential tremor are diagnosed with cognitive impairment of the dysregulatory profile.

Keywords: essential tremor, trembling hyperkinesis, non-motor symptoms, cognitive disorder.

Introduction. Essential tremor (ET) is a chronic, slowly progressive disease of the extrapyramidal nervous system, the main manifestation of which is trembling hyperkinesis [2]. The initial view that ET is a relatively "simple" and monosymptomatic disease is erroneous. Currently, ET is increasingly considered in the aspect of a neurodegenerative disease with a heterogeneous clinical picture and a wide range of non-motor symptoms (NMS) [10]. Among them, cognitive, psychiatric, sensory and other disorders are actively discussed [7,8,9]. NMS of essential tremor, along with obligate symptoms, constitute a general, rather complex phenotype of the disease [14]. Numerous studies conducted in North America, Europe and Asia demonstrate cognitive deficits in ET patients ranging from mild to moderate cognitive disorder to dementia [8]. Moreover, cognitive disorder can be observed not only in the elderly, but also in young patients. In some cases, it has been shown that changes in cognitive status may precede motor manifestations of the disease [12]. Research shows that mild cognitive deficits primarily affect executive function. Such as fluency of speech, the ability to solve problems and inhibit reactions [4, 6]. In the development of cognitive disorder, the role of the neurodegenerative process in the cerebellum is also considered. In particular, dysfunction of the connection with the prefrontal cortex [11]. In addition, in patients with rapid progression of cognitive disorder, the spread of the neurodegenerative process outside the cerebellum is assumed [15].

Purpose of the work: to assess the frequency and characteristics of cognitive disorder in patients with essential tremor.

Materials and methods. The study included patients with a confirmed diagnosis of essential tremor according to the diagnostic criteria of the International Society for the Study of Parkinson's Disease and Movement Disorders (MDS, 2017), observed at the Center for Extrapyrmidal Disorders and Botulinum Therapy at the NEFU's Clinic. Classical ET was shown when bilateral kinetic-postural tremor of the hands was detected in combination with tremor of another localization, in the case of a combination of classical ET with mild cerebellar signs, ET-plus was diagnosed with other extrapyramidal manifestations.

The 3-CT scale was used to assess the neuropsychological profile of patients with ET and people of the control group [1].

The following cognitive domains were assessed:

1) Test of drawing a clock [3]. The subjects were given the task to draw a dial, arrange numbers and show the specified time with arrows. The result was evaluated on a 3-point scale, 1 point was deducted for each error.

2) Memory was assessed using the visual memory test from the SKT scale [5]. The patient was instructed to memorize 12 familiar drawings. Immediate and delayed reproduction, as well as recognition of previously seen patterns of 48 images, were assessed. Each correct answer was scored 1 point. An indicator of a decrease in short-term memory was considered to be the reproduction of less than 5 drawings.

3) Research of semantic and phonetic speech activity [13]. For 1 minute, the subjects were asked to name animals (semantic speech) and words with the letter "L" (phonetic speech) in 1 minute. Each correct answer was scored 1 point. Statistical processing of the research results was carried out using the SPSS Statistics 22 software. Descriptive statistics for quantitative data are given as the median and the 25th and 75th quantiles (Me [Q25; Q75]). To compare two independent groups, the analysis was performed using the Mann-Whitney U-test. $\chi 2$ and Fisher's exact test were used to compare qualitative data. The critical level of statistical significance for the two groups was determined at $p \leq 0.05$.

Research results. The main group included 53 patients with ET aged 21 to 89 years, the median age was 67 [55.5; 72.5] years. The distribution by gender was as follows: 19 (35.8%) men and 34 (64.2%) women. Classic ET was detected in 8 (15.1%) patients, and ET-plus variant was detected in 45 (84.9%) patients. ET patients were divided into 2 subgroups. The first subgroup consisted of 22 (41.5%) patients with ET of the Yakut ethnicity. The second subgroup included 31 (58.5%) patients with ET of the Russian ethnicity. The control group consisted of 34 individuals without ET, as well as burdened heredity for extrapyramidal diseases. The age in the control group varied from 30 to 86 years, the average age was 63.4 ± 0.93 years, the median age was 65.0 [59.0; 69.0] years. The main and control groups did not statistically differ in age and gender distribution.

All subjects passed the clock drawing test. Difficulties in copying the cube were found in 6/53 (11.3%) patients with ET, who were associated with writing disorder due to tremor. Statistically significant differences in the study groups were obtained when assessing speech activity and short-term memory ($p < 0.05$). The structure of cognitive disorders accord-

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ing to individual tests 3-CT is presented in table 1.

The results of a comparative analysis of visual memory are presented in Figure 2. At the same time, all patients coped with the recognition of the previously seen images - 12.0 [11.0; 12.0] and 12.0 [11.7; 12.0], respectively, without statistical differences between groups (U = 824; Z = –0.84; p = 0.4). There were no false recognitions in the ET group and the control group.

A comparative assessment of the results of the 3-CT test was carried out between the first (the Yakut ethnic) and the second (the Russian ethnic) main groups of patients with ET. However, there were no statistically significant differences in the structure of cognitive disorders in the ethnic aspect (p> 0.05).

When analyzing the severity of cognitive disorder depending on the clinical variant and the form of ET, there was shown no statistical difference between the groups of patients with ET (Table 2).

Table 1

The structure of cognitive disorder on the 3-CT scale

Parameter	The main group, n = 53	The control group, n = 34	p
Clock drawing test	3.0	3.0	-
Copying a cube	49 (92.5%)	34 (100%)	0.15
Semantic speech activity	16.0 [13.5; 19.5]	19.5 [16.0; 25.0]	**0.001**
Phonetic speech activity	9.0 [6.5; 10.5]	9.5 [7.7; 14.0]	**0.03**
Instant playback	6.0 [6.0; 8.5]	9.0 [6.7; 10.0]	**0.002**
Delayed playback	5.0 [5.0; 8.0]	7.0 [6.0; 9.0]	**0.01**
True recognitions	12.0 [11.0; 12.0]	12.0 [11.7; 12.0]	0.4
False recognitions	0	0	-

As a result of the analysis of the test results, it was shown that in patients with ET in the studied groups, the frequency and severity of cognitive disorder did not differ depending on ethnicity, form, variant of the disease, and also on the age of ET debut. Evaluation of tests for visual-spatial orientation, drawing of a clock,

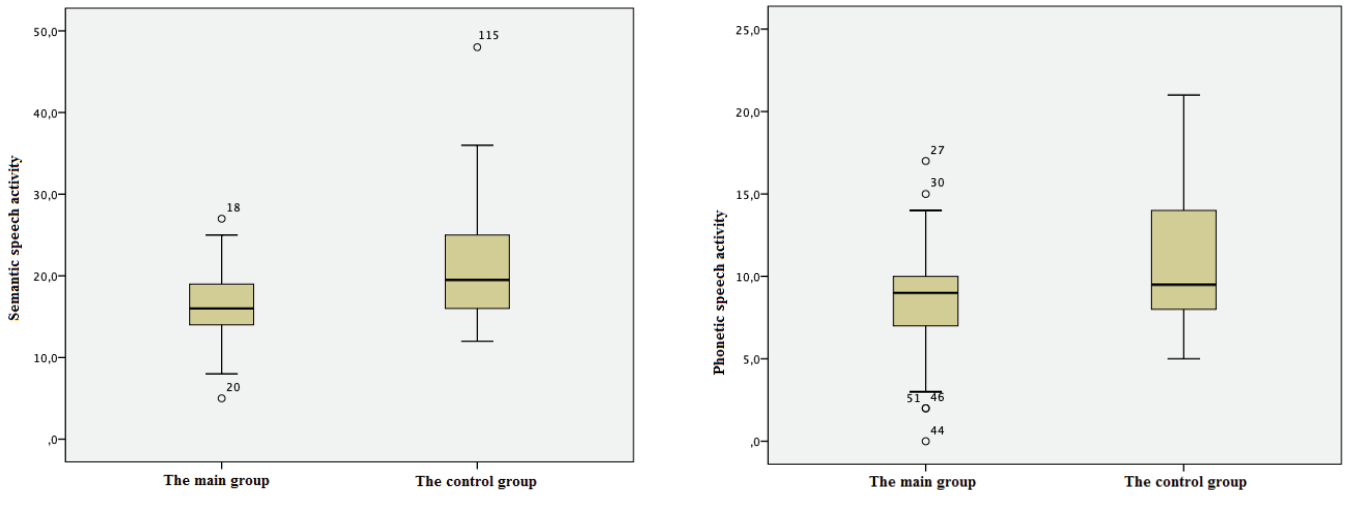

Fig. 1. Assessment of speech activity in patients with ET and the control group

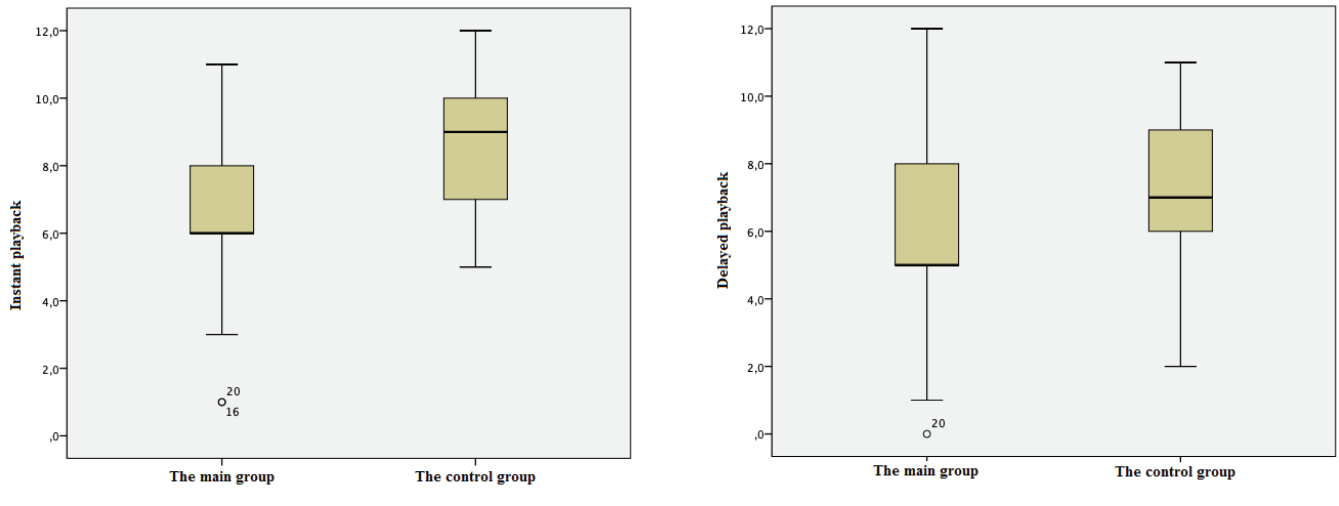

Fig. 2. Comparative assessment of visual memory in the group of patients with ET and persons in the control group.

Table 2

The structure of cognitive disorders by 3-CT subscales depending on the clinical variant and the form of ET, Me [Q25; Q75] points

Parameter	Форма заболевания					
	Classic ET, n = 8	ET-plus, n = 45	p	Familial form, n=28	Sporadic form, n=25	p
Semantic speech activity	16.0 [13.0; 16.0]	16.0 [13.8; 20.3]	0.59	16.0 [14.25; 22.0]	15.0 [11.5; 18.0]	0.08
Phonetic speech activity	9.0 [9.0; 14.0]	9.0 [6.0; 10.0]	0.1	9.0 [8.0; 10.75]	9.0 [6.0; 10.5]	0.48
Instant playback	6.0 [6.0; 7.0]	6.0 [6.0; 9.0]	0.82	6.0 [6.0; 8.75]	6.0 [6.0; 8.5]	0.83
Delayed playback	5.0 [4.0; 5.0]	5.0 [5.0; 8.0]	0.35	5.0 [5.0; 7.75]	5.0 [5.0; 8.0]	0.89
True recognitions	12.0 [12.0; 12.0]	12.0 [11.0; 12.0]	0.25	12.0 [11.0; 12.0]	12.0 [11.0; 12.0]	0.42
False recognitions	0	0	-	0	0	0.55

speech activity and visual memory revealed a decrease in speech activity and short-term memory in patients with ET. However, the results did not show any ethnic differences. ET revealed a dysregulatory profile of cognitive disorders.

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IMMUNOMODULATORS IN THE TREATMENT OF ACUTE RESPIRATORY VIRAL INFECTIONS

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The scientific review presents a research analysis of acute respiratory viral infections (ARVI) treating methods using drugs available in Russia that have immunomodulatory effect. The article describes the rational usage of this sort of medicine according to the evidence-based medicine methods.

Keywords: immunomodulatory, ARVI, flu, children, adults.

Introduction. Acute respiratory viral infection (ARVI) is a widespread group of inflammatory diseases of the upper respiratory tract (URT) in people of all ages and clinical forms. At the moment, ARVI are one of the urgent and priority problems of world health for doctors of different specialties [24]. Despite a large number of studies proving the effectiveness and / or the choice of a particular drug for treatment, ARVI continues to occupy a leading position in the structure of the population morbidity. The choice of drugs for treatment is a subject of debate in the medical community, since ARVI is a nosological group with similar clinical signs, but with a wide range of probable pathogens [8].

According to the WHO, acute respiratory viral infections are one of the most common reasons for seeking medical attention. However, the current data of the epidemiological surveillance of ARVI (as of the 9th week of 2021, the incidence rate of ARVI and influenza in the whole of the Russian Federation was 61.8 per 10,000 population) should be interpreted with caution, due to the increased demand for medical care, as a result of the ongoing pandemic of COVID-19. By comparison, at the 9th week of 2019, the incidence rate in the country was 82.6 per 10,000 population. Consideration should be given to the fact that hygiene and physical distancing measures adopted by states have played a significant role in reducing the transmission of influenza virus [9, 10]. Nevertheless, given the high proportion of patients who do not seek medical help during the period of active illness, confirm the data that the incidence of ARVI is quite widespread [35].

Immunomodulators - are therapeutic drugs that eliminate the imbalance of the immune system's various parts. These drugs are aimed at normalizing the parameters of immunity. To classify a drug into a group of therapeutic immunomodulatory agents, during preclinical and clinical studies, the ability to change the immunological reactivity of the organism, depending on its initial state, must be proven [11]. These drugs are actively prescribed for the treatment of acute respiratory viral infections in the territory of the post-Soviet space, by doctors of many specialties. Accordingly, the number of articles devoted to the study of the activity of immunomodulators written by Russian-speaking authors is significant. It should be noted that according to foreign researchers, the concept of "immunomodulators" is rather vague. Many drugs have potential, but often virtual, immunomodulatory properties. There are more drugs in the Russian Federation, they can be freely purchased in pharmacies. The role of immunomodulators is not fully understood and their use may be unsafe [25, 30].

The aim of this work is to analyze the literature data on the study of the drug activity of immunomodulators in the treatment of ARVI.

Assigned tasks:

1. Study the literature data on the features and effectiveness use of immunomodulators in acute respiratory infections.
2. Evaluate research results from the perspective of evidence-based medicine.

The review includes publications from the following databases: eLibrary, CyberLeninka, PubMed. Search queries were formed with the following criteria: publication date last 10 years, keywords used for search: immunomodulator, ARVI, influenza, children, adults.

Modern medicine has an abundance of pharmacological drugs used to treat viral infections. A significant difference in the treatment of ARVI in many countries, including the Russian Federation, is the uncontrolled use and sale of immunomodulatory drugs in the Russian Federation without taking into account the principles of evidence-based medicine. International (European and American) clinical guidelines describe the inappropriateness of treatment with nonspecific antiviral drugs due to the lack of proven data indicating a better effect and safety of such drugs as compared to placebo. For example, preparations with the active ingredient imidazolylethanamide pentanedioic acid have not been subjected to randomized placebo-controlled clinical trials; their use does not go beyond Russia. There is an opinion that the early prescription of antibiotics for ARVI reduces the risk of activation of the opportunistic bacterial flora of the upper respiratory tract. But, in this case, the prescription of antibiotics is not only unjustified, but also entails a decrease in nonspecific immunity, the body's ability to produce endogenous IFN, which is directly related to the course and outcome of the disease [31]. Moreover, prolonged use of interferon inducers can lead to IFN-mediated cytokine release syndrome. The doctor should make the decision on the advisability of prescribing systemic antibiotic therapy based on the severity of the disease and the risk of complications.

In the practice of a doctor, the issue of prescribing immunomodulators remains the most controversial. A large number of immunomodulatory drugs have been registered, the pharmacodynamic effect of which, according to pharmacological companies, is aimed not only for the treatment of ARVI, but also for its prevention. Numerous works on the complex treatment of diseases contain contradictory conclusions from calls to completely abandon this group of drugs to their unjustifiably frequent prescription [2]. Most often, prescribing by doctors and

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recommendations by pharmaceutical specialists of medicines (drugs) for the treatment of acute respiratory viral infections and influenza is dictated mainly by a positive assessment of their advertising effectiveness and safety [4]. To confirm the effectiveness and safety of immunomodulators, it is necessary to conduct multicenter clinical trials [15]. Clinical studies to assess the effectiveness and safety of immunomodulators, conducted in Russia, are most often carried out without adherence to generally accepted world standards, principles of randomization and clinical treatment protocols [19].

According to some studies, the appointment of immunotropic drugs is justified in the development of secondary immune deficiency (SIN) URT. One of the manifestations of VIN is frequent illnesses [2, 7].

Polyoxidonium (Azoximer bromide) is one of the drugs that are widespread in the Russian Federation and are classified as synthetic immunomodulators. This drug is devoted to a large number of articles describing the research conducted, while the sample of patients is small, some articles are accompanied by a description of one clinical case [2, 7]. It is worth mentioning that according to the basic norms of evidence-based medicine, works in which one drug is investigated cannot be considered and have a high level of evidence, especially when they contain direct advertising of a particular drug [2, 3, 6, 7, 16 , 22, 27, 28].

The mechanism of action of such drugs is described incompletely, or rather it is practically absent, in the pharmacodynamic description only a brief description of the effects is indicated, which, already at this stage, casts doubt on the advisability of these therapeutic agents [34].

A more serious study is presented in the article by S.M. Kharit and A.N. Galustyan, which reflects the results of a double-blind, placebo-controlled, randomized clinical trial of II and III phases of the drug Polyoxidonium. A total of 228 children from 3 to 14 years old took part. The data obtained showed that the complex therapy of ARI significantly reduces the period of normalization of body temperature in comparison with taking a placebo and contributes to the normalization of immunity parameters [29].

The article by T.I. Garashchenko, O.V. Karneev, G.D. Tarasov, I.V. Kim, R.A. Khanferyan describes a multicenter double placebo-controlled study. It includes 155 children aged 1 to 12 years. According to the results of the study, the authors stated the superiority of the use of Azoximer bromide (AB) in comparison with placebo in complex therapy, and the inclusion of Polyoxidonium in the complex therapy of acute respiratory viral infections in children makes it possible to better control the symptoms of intoxication, reduce the severity of symptoms by the 5th day of therapy, and increase it by 2 times. the number of patients with no symptoms of "Nasal discharge" by the 3rd and 5th days of therapy, to reduce the severity of the infectious-inflammatory process [5]. The use of Polyoxidonium was accompanied by the use of antipyretic drugs. There was no control of the onset of ARVI disease [5].

In the meta-analysis conducted by A.V. Karaulov and A.V. Gorelov selected 5 studies comparing the effectiveness of complex therapy with the use of AB in the treatment of viral diseases and standard symptomatic therapy; in total, 542 children, aged 3 to 18 years old, took part in the study. According to the results of the work, the authors concluded that the addition of AB to ARVI therapy from the first day of treatment makes it possible to reduce the period of temperature normalization, shortens the duration of symptoms of fever and intoxication, and the disappearance of headache, muscle and joint pain. At the same time, the duration of the clinical symptoms of acute inflammation of the upper respiratory tract decreases in general by 1.23 days [13].

The study of the use of Polyoxidonium in foreign literature is described in a single article by P. Pruzinec, N. Chirun, A. Sveikata. The sample consisted of 502 patients, the results confirm the safety of use and no effect on the kidneys, however, studies of the effectiveness of the pharmacological action have not been described [33].

Another quite popular drug is Cycloferon. There is much less research on its effectiveness. One of these is the study of V.A. Isakov and D.V. Isakov: in which the effectiveness of the tablet form of cycloferon is studied in the complex therapy of viral respiratory tract infections in adults. The duration of fever was 1.8 times and intoxication 1.4 times shorter than in the comparison group. The catarrhal syndrome and the general duration of the disease turned out to be less prolonged, complications developed less frequently [12].

Systematic review and meta-analysis of N.K. Mazin, I.V. Sheshunov, P.V. Mazin, V.P. Mazin, A.L. Kovalenko, V.A.Zaplutanova: based on the results, they claim a milder course ARVI, when using tableted Cycloferon as a prophylactic and therapeutic agent in both adults and children [18].

In the article by A.V. Karaulova, with the study of the less popular immunomodulatory drug Groprinosin, it is concluded that it is an effective immunomodulator even in the case of allergic diseases. The goal of immunotherapy is to eliminate the pathological focus, reduce the severity of the inflammatory response, improve the clinical picture of the underlying disease, and reduce the need for antibacterial and anti-inflammatory therapy. [fourteen]

In the work of M.S. Savenkov, A.A. Afanasyev, G.M. Balakirev, the medicinal effect of the drug Groprinosin (inosine pranobex), advertising of which occupies a significant part of the entire article, on respiratory tract diseases is also investigated. However, the emphasis is on the concomitant herpesvirus infection, and as the authors note, the treatment of such diseases is multifaceted and requires further study. [26]

When assessing the effectiveness of Groprinosin in the study by T.A. Kryuchkova, the duration of admission is described for at least 7 days, while the drug was taken for another 2 days, even after the symptoms disappeared. Given the average duration of ARVI, which is less than a week, it makes no sense to expose the child to excessive drug load. [17]

The work of O.Yu. Filatov, O.V. Kashaev, M.A. Gordeev, O.A. Paevskaya describes the therapy of 220 patients with moderate acute respiratory infections with the immunomodulatory drug Derinat. It is noted that the injected drug is successfully used both in patients with an active inflammatory process and in complications after suffering a cold [28].

In the articles of Yu.V. Marushko and co-authors, A.P. Babkin. with a team of authors, the use of Derinat is effective in the complex treatment of acute respiratory viral infections, while the proven effectiveness of the drug is only 5 days, when, according to the assurances of the authors, increases the secretion of immunoglobulins A in the mucous membranes of the nasal passages by 3 times. [1, 20]

The meta-analysis of the effectiveness of Derinat carried out by V.I. Moerchuk, V.S. Bortnitsky and co-authors describes several studies conducted in the period from 2010 to 2015, a sample of patients, their number does not allow us to call the article fully a meta-analysis. [21] There are no new studies proving the effectiveness of the drug against diseases of the upper respiratory tract.

Priority in the treatment of ARVI is given to symptomatic therapy, the effect of which is aimed at relieving symptoms and reducing patient discomfort [32]. The

predominant symptoms of ARI are fever, nasal congestion, and coughing. In order to reduce swelling of the nasal mucosa and, consequently, improve respiratory function, the use of decongestants is recommended, in a short course of up to 5-7 days [19]. The pathogenesis of cough in ARI is due to mechanical irritation of the mucous membrane of the posterior pharyngeal wall and larynx by nasal secretions. It should be remembered that antitussive therapy in children should affect the improvement of bronchial drainage, therefore, it is advisable to prescribe mucolytics (acetylcysteine, carbocysteine) [23].

Conclusion. For the Republic of Sakha (Yakutia), which has harsh climatic conditions, as well as a high seasonal incidence of ARVI, the selection of adequate immunomodulatory therapy is important. The search for adequate ways to treat ARVI remains relevant today, despite the large number of available drugs sold in pharmacy chains. The literature review confirmed the relevance of randomized scientific research on the search for effective immunomodulators in ARVI, the demand for immunomodulators and adaptogens from local northern raw materials on the market.

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THE METHOD OF INDIVIDUALIZED REGIONAL LYMPHOTROPIC THERAPY WITH PHOTOPHORESIS IN PATIENTS WITH MULTIDRUG – RESISTANT PULMONARY TUBERCULOSIS

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We propose a method of individualized regional lymphotropic therapy (RLTT) with photophoresis, as part of multimodality treatment for newly identified destructive multidrug-resistant pulmonary tuberculosis (MDR-TB). The method was developed to meet local specifics of the causative agent, M.tuberculosis (MTB), and metabolic characteristics of the indigenous population of the north, which had been elucidated in earlier studies: low level of drug resistance to isoniazid, predominance of slow isoniazid acetylation rate irrespective of administration routes, leading to accumulation of high bactericidal concentrations of isoniazid in blood. The treatment method is suited for patients with low MTB resistance to isoniazid (1 mcg/mL), and slow or medium acetylation rate. Indications and contraindications are specified, as well. The method is protected by patent RU2650633 'Method for treating infiltrative pulmonary tuberculosis with multiple drug resistance' [12]. The proposed method requires administration of anti-tuberculosis drugs standardly recommended for chemotherapy of MDR-TB, market-authorized medical device for laser therapy, and standard equipment for procedure room and laser therapy room. The article describes administration sites, dosages, routes of administration and laser irradiation (photophoresis), drug regimens, and timings for x-ray check-ups. Use of the proposed method resulted in improved treatment efficiency per 6-month period: time to eradication of clinical manifestations shortened by a factor 1.3 (OR 6.31; 95% CI 1.31-30.37); time to culture conversion shortened by a factor of 1.4 (OR 5.1; 95% CI 1.85-14.09); time to cavity healing reduced by a factor of 1.6 (OR 3.42; 95% CI 1.51-7.72); duration of hospitalization needed for intensive phase shortened by a factor 1.2 ($p<0.01$).

Keywords: tuberculosis, levels of drug resistance to isoniazid, isoniazid inactivation, regional lymphotropic therapy, photophoresis, efficiency.

Introduction. The idea of personalized or individualized approach to patient treatment has been existing since the early days of medicine.

Key to treatment efficiency in tuberculosis (TB) are drug circulation lifetime, and concentrations of medications in blood and in the TB lesions. As a rule, treatment is prescribed based on local bacteriological specifics of the causative agent (MTB), and taking into account metabolic characteristics of the indigenous population of the north [2, 5, 11]. According to findings reported by Vinokurova M.K. (2010), multidrug-resistant MTB in the Sakha Republic (Yakutia) showed minor level of resistance to isoniazid (1 mcg/mL) in 92.4% of cases, minor and high levels of resistance to rifampicin (40 and 80 mcg/mL, respectively), while high levels of resistance to isoniazid (10 mcg/mL) and rifampicin (80 mcg/mL) were observed in 7.6% of cases [11].

As is known, one of the biological properties of MTB is its lymphotropism [2, 3, 15]. As far back as the 30s of the 20th century, Shtefko V.G. established bactericidal action of the lymph on the MTB as it enters the lymphatic bed. Later, evidence has been obtained supporting the view, that the lymphatic system of the lungs is not merely the route of infection spread, but also the area of the lungs where healing starts [3, 13]. Prof Y.M. Levin developed the method of regional lymphotropic therapy (RLTT), a method of drug delivery to the lymphatic system, and patients have been treated with it for more than 30 years in all sorts of health facilities. As certain parts of the lymphatic system have been shown to be interconnected with certain organs/tissues, drug administration to lymphatic vessels and nodes at certain, specified body regions enables preservation of optimal drug concentration in the target lesion for 24 hours, allowing achievement of high therapeutic efficacy [4, 9].

Gavriliev S.S. and colleagues have established that the majority of the population of Yakutia were weak isoniazid acetylators irrespective of administration route, and because of it, accumulated high bactericidal isoniazid concentrations in their blood [5]. Lower therapeutic effect in rapid acetylators, and respectively, higher therapeutic effect in weak acetylators, was observed by number of researchers [16]. The method of photophoresis (low-level laser therapy) has been successfully used to accelerate healing process, as it was shown to have beneficial effect on biochemical reactions, metabolism, microcirculation, and antioxidant activity [10, 6, 7, 8]. Further studies by Gavriljev S.S. and colleagues (2004) demonstrated that intercostal intramuscular administration of isoniazid followed by local photophoresis resulted in increased diffusion of isoniazid in the abnormally altered lesion, and was associated with higher rates of healed destructions in the lungs, and faster culture conversion. Based on the above-mentioned, we developed a method of RLTT for pulmonary MDR-TB, wherein lymphotropic administration of isoniazid with photophoresis is used on top of standard chemotherapy regimen for MDR-TB (regimen IV).

Statistical processing of results was performed using IBM SPSS Statistics 22 software suite. Due to absence of normal distributions for most of the parameters, and due to small study group sizes, measure of central tendency and measure

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of spreading are presented as medians (Me) and quartile distributions (Q1; Q3). Comparisons of quantitative variables and qualitative characteristics between groups were done using Kruskall-Wallis test, and Pearson's chi-squared test, respectively.

Material and methods. Drug susceptibility to isoniazid was determined based on urine samples, using absolute concentration method and solid medium, in the presence of 1 mcg/mL and 10 mcg/mL of isoniazid.

Urine samples were tested for isoniazid acetylation using L.I. Grebennik's modification of Wollenberg's test (1961, 1965) in 189 patients. According to procedure description, patients can be referred to one of the following types: slow, intermediate, or rapid inactivators (acetylators). Test result was determined based on amount of active isoniazid excreted in urine. Amounts below 10%, between 11 and 15%, or above 16% were interpreted correspondingly, as rapid, intermediate, or slow acetylators.

Serum isoniazid levels and dynamics were estimated using HPLC (high-performance liquid chromatography) in 96 slow and intermediate acetylators, using different drug administration routes: regional lymphotropic (n=32), intravenous drip (n=28), intramuscular (n=19), and oral (n=17). Blood samples were collected at specified time after drug administration via different routes (regional lymphotropic (1), intravenous drip (2), intramuscular (3), oral (4)): 1.5 hours later (1st sample), 6 hours later (2nd sample), 9 hours later (3rd sample).

Resources required for the proposed treatment include medications for anti-TB chemotherapy, market-authorized medical equipment, and standard equipment for procedure room and for laser therapy room.

1. Laser therapeutic device Uzor-A-2K, 2-channel, supplied with magnet applicator tips (Technical Specification TU TУ ТВО.290.001; Manufacturer: State-owned enterprise 'Voskhod', Kaluga; Reg. no. 94271-122). Light wavelength: 0.89 μm; number of channels: 2; pulse power per one channel: 4 W; pulse repetition rate: 80-300 Hz; weight: 5.5 kg; pulse duration: 300 nsec.

2. Medications: 10% isoniazid solution (amp): 5.0 mL; 0.5% novocaine injection solution (amp); heparin injection solution (amp): 5000 U/mL.

Treatment method is protected by patent RU2650633 'Method for treating infiltrative pulmonary tuberculosis with multiple drug resistance' [12].

Results and discussion. Cultures of MTB in all patients exhibited low levels of resistance to isoniazid (1 mcg/mL). As is seen in Table 1, rapid acetylators prevailed mostly among non-indigenous population (43.7%), intermediate acetylators were found at equal proportions among non-indigenous and indigenous population (47.9% and 48.2%), while the proportion of slow acetylators was reliably larger among indigenous population of Yakutia (45.4%).

As it is seen in Table 2, statistically meaningful differences were apparent in serum isoniazid concentrations after 1.5 hours: in group 1 (regional lymphotropic) compared to groups 3 (intramuscular) and 4 (oral); in group 2 (intravenous drip) versus group 4 (oral). Lowest serum isoniazid concentrations were observed in patients treated via lymphotropic route, and the highest concentrations – in patients who received oral treatment.

After 6 hours, no differences in serum concentrations between groups with different administration routes were noted. Concentrations remained at therapeutic levels irrespective of administration route, but tended to decrease in groups with intravenous drip, intramuscular, and oral administration, compared to lymphotropic route.

After 9 hours, group 4 (lymphotropic route) showed statistically reliable differences in serum isoniazid concentrations, compared to the rest of groups. The sus-

Table 1

Isoniazid acetylation types based on urine tests, among indigenous and non-indigenous population of extreme north

Population	Isoniazid acetylation type						Total	
	Rapid		Intermediate		Slow			
	абс.	%	абс.	%	абс.	%	абс.	%
Indigenous	9	6.4	68	48.2	64	45.4	141	100
Non-indigenous	21	43.7	23	47.9	4	8.4	48	100
Total	30	15.9	91	48.1	68	36.0	189	100
p*	$\chi^2 = 31.87$ df = 1 p = 0.001		$\chi^2 = 0.01$ df = 1 p = 0.894		$\chi^2 = 18.29$ df = 1 p = 0.001			

Note: *p – Pearson's χ2 test.

Table 2

Serum isoniazid concentrations for different administration routes, mg/L (Me (Q1; Q3))

Collection time	Isoniazid concentration (mg/L) for different administration routes:				p*
	1	2	3	4	
1st sample	4.2 (3.4; 5.0)	8.0 (5.3; 10.7)	12.5 (9.4; 15.6)	17.1 (23.8; 10.3)	$p_{1-3} < 0.001$ $p_{1-4} < 0.001$ $p_{2-4} = 0.030$
2nd sample	3.1 (2.0; 4.2)	2.2 (0.9; 3.5)	2.4 (1.0; 3.8)	2.2 (1.0; 3.4)	p = 0.321
3rd sample	2.2 (1.7; 2.7)	0.8 (0.2; 1.4)	0.8 (0.4; 1.2)	0.8 (0.6; 1.0)	$p_{1-2} = 0.003$ $p_{1-3} = 0.005$ $p_{1-4} = 0.008$
Isoniazid concentration decrease, %					
After 6 hours	27.5 (13.6; 41.4)	74.0 (63.7; 84.3)	79.0 (67.7; 90.3)	78.0 (66.3; 89.7)	$p_{1-2} = 0.012$ $p_{1-3} < 0.001$ $p_{1-4} = 0.002$
After 9 hours	48.5 (39.6; 57.4)	84.3 (74.7; 93.9)	92.6 (88.6; 96.6)	93.7 (89.2; 98.2)	$p_{1-3} < 0.001$ $p_{1-4} < 0.001$

Note: * Kruskall-Wallis test

tained therapeutic serum concentrations were observed in lymphotropic group, compared to decreased concentrations (below 1 mg/L) in groups with intramuscular and oral drug administration.

RLTT with photophoresis is indicated for patients with newly detected extensive destructive pulmonary MDR-TB, with minimally allowed level of resistance to isoniazid equal to 1 mcg/mL, and who are either slow (above 16%) or intermediate (11-15%) isoniazid acetylators.

Contraindications to RLTT include: intolerance/allergy to any agents used in treatment; pyoinflammatory diseases of skin and subcutaneous tissue; pronounced pain syndrome in response to drug administration.

Contraindications to photophoresis include: bleeding or hemoptysis; advanced concurrent diseases or complications (stage IIB-III heart failure, neoplasms, organic injury of central nervous system, stage II-III thyrotoxicosis, diseases of hematopoietic system); pregnancy; diseases associated with pronounced metabolic disturbances (decompensated diabetes mellitus) or dystrophy; disorders of blood.

RLTT with photophoresis was used on top of intensive phase of the chemotherapy regimen for pulmonary MDR-TB (regimen IV). RLTT is performed in combination with isoniazid, a highly lymphotropic anti-TB drug with remarkable capacity to reach and reversibly bind with cell structures of lymphatic tissue. Lymph-stimulating permeator agent is needed to ensure delivery of isoniazid to lymphatic bed. As permeator, we used heparin (1.0 mL, 5000 MU) dissolved in 4.0 mL of 0.5% novocaine solution.

Daily dose (10 mg/kg) of isoniazid solution is administered to subcutaneous tissue, alternating injection sites each time (axillary space; 5th intercostal space near the sternum edge; infraclavicular space in the projection of the joint between the first rib and sternum) 5 times a week, followed by photophoresis, during 25 days.

RLTT is performed in the procedure room, strictly adhering to the standards of aseptics and antiseptics.

Injection sites (axillary space, 5th intercostal space, infraclavicular space) are alternated every day, based on the extent of disease. Also, daily alternation of injection sites helps prevent injection site induration in subcutaneous tissue.

The 1st syringe is filled with 1.0 mL (5000 MU) of heparin and 4.0 mL of 0.5% novocaine solution; the 2nd syringe is filled with daily dose of 10% isoniazid solution (10 mg/kg) and 0.5% novocaine solution till a total amount of 10.0 mL is reached. Then the injection site is swabbed with alcohol swab, and a subcutaneous injection of heparin is performed, followed by injection of isoniazid solution using the same needle.

Injection to axillary space is performed in sitting position. The patient is sitting on the chair, the arm at the side of injection is placed behind the patient's head. Needle is inserted horizontally, parallel to costal surface, for two thirds of needle's length, stepping 4-5 cm. away from the axillary fossa and 1.5-2 cm. away from the edge of greater pectoral muscle (*musculus pectoralis major*).

Injection to the 5th intercostal space near the edge of the sternum is performed in supine position. The injection site is palpated, then a needle is inserted, stepping 1-1.5 cm. away from the edge of the sternum, subcutaneously, at an acute angle, parallel to the rib, for the half of the needle's length.

Injection to infraclavicular space is performed likewise in supine position, with the patient's head turned the opposite way to the injection side. The joint between the first rib and the manubrium of sternum is palpated, and stepping 1-1.5 cm. away, the needle is inserted subcutaneously, at acute angle, parallel to the rib, for the half of needle's length.

The injection site is covered with alcohol wipe and adhesive strip.

15-20 min. after the injection, body area where isoniazid was injected is further exposed to photophoresis. Photophoresis is performed in laser therapy room (pulse frequency 150 Hz, pulse power 2 W, exposure duration 256 sec.), 5 days a week, during a course of 25 seances.

Additionally, in the evening (18.30), patients received 0.3 g. of isoniazid, depending on their body weight.

On weekends, and after the completion of the course of RLTT with photophoresis, isoniazid was administered intramuscularly (daily dose of 10 mg/kg) in the morning (10.00), and orally (0.3 mg) in the evening (18.30), depending on patient's body weight.

X-ray and CT checkup was performed 21 days after the completion of RLTT with photophoresis. Based on indications, a course of RLTT with photophoresis was repeated.

The efficiency of the proposed treatment in newly detected infiltrative destructive pulmonary MDR-TB in terms of culture conversion estimated after 6 months of treatment was 89.1% (experiment group) vs. 61.5% (control group) ($p<0.05$).

Radiological assessment after 6 months of treatment among slow or intermediate acetylators with low levels of resistance to isoniazid (1 mcg/mL) showed reliably higher proportion of healed cavities in group treated with RLTT with photophoresis on top of standard chemotherapy (regimen IV): 74.5% (experiment) vs. 46.2% (control) ($p<0.05$).

Conclusions.

1. Bacteriological examination of *M.tuberculosis* isolated from patients participating in the study showed that all patients had low levels of resistance to isoniazid (1 mcg/mL).

2. Indigenous residents of Yakutia, in the majority, were either slow (45.4%) or intermediate (48.2%) isoniazid acetylators ($\chi^2 = 18.29$ df = 1 p = 0.001).

3. Regional lymphotropic administration of isoniazid in combination with photophoresis allowed more long-lasting bactericidal concentration of isoniazid in serum, assessed 9 hours after drug administration (2.2 (1.7; 2.7) mg/L) vs. baseline (4.2 (3.4; 5.0) mg/L)), compared to other administration routes ($p<0.001$).

4. Lymphotropic administration of isoniazid with photophoresis on top of intensive phase of standard chemotherapy regimen IV, in patients with newly detected infiltrative pulmonary MDR-TB with low resistance to isoniazid, substantially improved treatment efficiency after 6 months of treatment: time to eradication of clinical manifestations shortened by a factor 1.3 (OR 6.31; 95% CI 1.31-30.37); time to culture conversion shortened by a factor of 1.4 (OR 5.1; 95% CI 1.85-14.09); time to cavity healing reduced by a factor of 1.6 (OR 3.42; 95% CI 1.51-7.72); duration of hospitalization needed for intensive phase shortened by a factor 1.2 ($p<0.01$).

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THE PRINCIPLE OF TREATMENT OF FRACTURES OF DISTAL METAEPIPHYSIS OF THE FOREARM BONES AND THEIR CONSEQUENCES IN PERSONS OF GERONTOLOGICAL PROFILE

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The paper presents data on the treatment of distal metaepiphysis of the forearm bones (beam in the "typical place") and their consequences in persons of gerontological profile. A retrospective analysis of 500 medical records of elderly and senile patients with fractures of the radius in the "typical place", who sought emergency medical care, was also used in the work of clinical material for the treatment of 57 patients with developed neuroischemic disorders after fracture of this localization. A significant amount of clinical material revealed the main errors and complications in the treatment of fractures of this localization in this category of patients. The reasons of their occurrence and ways of overcoming are analyzed.

Keywords: distal metaepiphysis bones of the forearm, persons of elderly and senile age, neuroischemic complications.

According to the UN, people over the age of 60 will make up one third of the world's population [7]. According to leading sociologists, Russia is one of the countries where the share of people of older age groups is increasing and approaching 20% of the total population; the number of older people (60 years and older) today is 29 million people and is projected to continue to increase, reaching 39.5 million people by 2021 (i.e. more than a quarter-26.7% - of the total population of the country) [2]. At the same time, it becomes relevant to create conditions and preserve the potential of older generations, the leading dominants of which are health, independent living and the possibility of professional activity.

It should be noted that injuries among elderly and senile citizens do not decrease. According to European researchers, the proportion of people in this age group is 40% of all those seeking emergency trauma care [9].

Injuries in the area of the distal metaepiphysis of the radius and forearm bones (the so-called "beam fracture in a typical place") are the most common among all injuries of the adult population: the proportion of this damage in older age groups is more than 25% [6]. At the same time, the percentage of unsatisfactory treatment results, exceeding 50%, remains high [4]. If we take into account the presence of orthopedic chronic pathology in this population group in the form of osteoporosis and osteoarthritis, osteochondrosis, which accompany the aging process of the body, then improving the care of elderly and senile people with fractures of the distal metaepiphysis of the radius and forearm bones is the most important task of the traumatological and orthopedic service.

The aim of the study: a comprehensive analysis of the conditions for optimal treatment of fractures of the distal metaepiphysis of the radius and forearm bones in elderly and senile people. To achieve this goal, the following tasks are solved in the work: 1) to identify age-related features that determine the course of the traumatic process in fractures of the distal metaepiphysis in elderly and senile people; 2) to consider the best ways to treat fractures of this localization in people over 60 years of age; 3) to determine the specifics and causes of adverse outcomes in the treatment of fractures of the distal metaepiphysis of the radius in persons over 60 years of age; 4) based on the analysis of the data obtained, to clarify and summarize the clinical recommendations for the treatment of the distal metaepiphysis of the radius and forearm bones in elderly and senile people.

Materials and methods: the study was conducted on the basis of the city Clinical Hospital No. 1 named after N. I. Pirogov and the Russian Gerontological Research and Clinical Center in Moscow. The data of 240 computed tomographic studies of the distal metaepiphysis of the radius and forearm bones in persons over 60 years of age were analyzed; a retrospective analysis of 500 case histories of patients of the specified age with fractures of the radius in a "typical location", who sought emergency medical care in and 57 patients treated in for neuroischemic complications after a fracture of the distal metaepiphysis of the radius in the period 2015-2018. The study included patients over 65 years of age, the average age was 76.6±0.5, the majority of 81.5% were female.

The results of the study and their discussion: according to the data of a large hospital of the megalopolis of the N. I. Pirogov State Clinical Hospital No. 1, Moscow, in 2015-2018, the number of elderly and senile people was more than 41.9% of all those seeking emergency trauma care. Among them with fractures of the distal metaepiphysis of the radius and bones of the forearm - 21.4%, which accounted for 43.7% of all fractures of the upper limb. In 81.5% of cases, they were female. Analysis of the data of 240 tomograms of the distal metaepiphysis of the forearm bones in elderly and senile individuals indicates a bone-destructive reconstruction of this part of the forearm, expressed in sclerotic changes in the trabeculae of the bone matrix, which was revealed in 98% of cases, in 32% of cases with the formation of "voids" in the form of cystic rearrangement of the bone tissue

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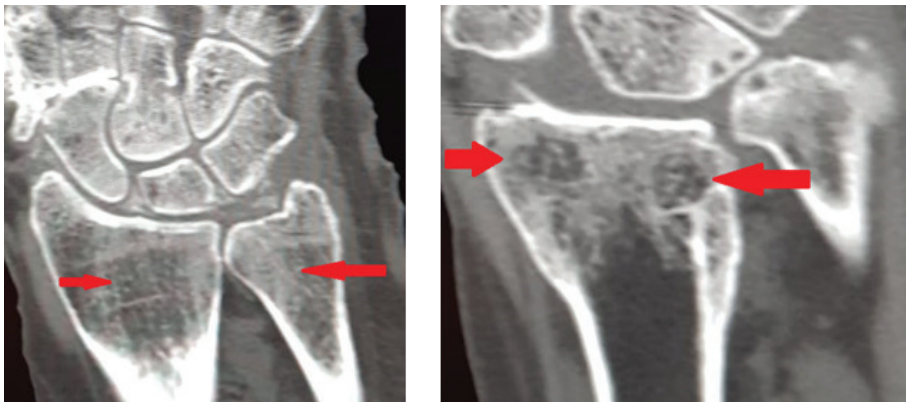

a b

Fig. 1. Spiral computed tomography of the wrist joint in persons over 70 years of age, bone-destructive reconstruction of the distal metaepiphysis of the forearm bones, sclerotic changes in the bone matrix trabeculae (a), the formation of "voids" in the form of cystic bone tissue rearrangement (b) - indicated by arrows

(Fig. 1 a, b), as a result of which, in fractures of the distal metaepiphysis, the articular surface is "crumpled" and its subsidence even after adequate traction and reposition [1]. Kneading of the spongy bone is the cause of shortening of the radius during the fusion of fragments. As a result, the protruding head of the ulna is located distal to the articular surface of the radius, and the wrist joint expands significantly against the background of this displacement (Fig.2). Often, in addition to damage to bone structures, there is a complete rupture of the ligaments of the lower radiolocular joint and, as a result, displacement of the distal end of the ulna, which leads to repeated displacement of fragments and deformation of the forearm [5].

A retrospective analysis of 500 medical records of patients over 60 years of age with fractures of the distal metaepiphysis of the radius and forearm bones indicates that the main method of treatment for this injury is conservative. However, in 246 (48.9%) cases, the occurrence of secondary displacement of fragments was noted, which in 142 cases (28.4%) was accompanied by the development of persistent pain syndrome and in 64 cases (12.8%) by neuroischemic complications of varying severity. The main reasons for the secondary displacement of fragments, as already noted above, are not only the rough compression of the spongy bone tissue against the background of osteosclerosis, which leads to the destruction of bone beams, causing the" subsidence "of the distal fragment in the process of regeneration, but also a violation of the algorithm of conservative treatment, which was revealed in 83 cases (16.6%): refusal of primary reposition in the presence of a slight displacement

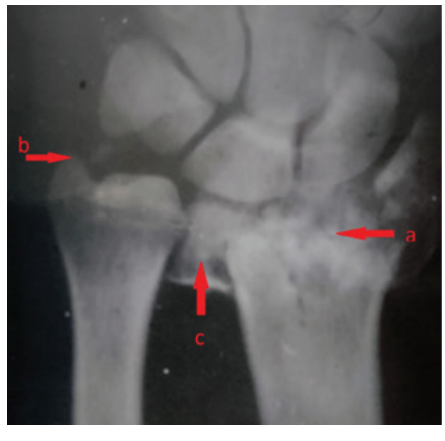

Fig. 2. Features of changes in fractures of the distal metaepiphysis in individuals of the gerontological profile: a- "creasing" of the articular surface; b – standing of the head of the radius; c-increase in radioulnar diastasis-indicated by arrows

of fragments, the use of a "bespodkladochnoy" plaster splint during primary immobilization, refusal to "band up" the longuette as the edema subsides and the plaster longuette is replaced with a circular plaster cast, and, finally, the untimely termination of immobilization. Analysis of the results of treatment of patients with advanced neurotrophic disorders after fractures of the distal metaepiphysis of the radius and forearm bones allowed us to determine the main causes of these terrible complications: significant displacement of fragments; pronounced edema in the fracture area; rough reduction of fragments during reposition; the use of a "non-lining" plaster splint, its tight bandaging; compression of soft tissues with a circular plaster cast; exceeding the terms of immobilization by more than 2 times; violation of the patient's management in the rehabilitation period. We have identified and determined the indications for surgical treatment of fractures of the distal metaepiphysis of the radius and forearm bones in elderly and senile people, the main of which are: open nature of the damage, pronounced displacement of fragments (including secondary), accompanied or threatened by a violation of the function of the hand, compression of nerve trunks, damage to tendons. At the same time, it is important to strictly control the operational risk, since almost 100% have severe somatic diseases that can worsen in the process of both injury and treatment. Surgical treatment for osteoporosis requires a special approach to the choice of the method and technique of surgery in order to avoid the development of instability, non-fusion of the fracture, as well as drug therapy using drugs that accelerate the consolidation processes [3]. Taking into account the above, 23 patients (4.6%) were operated on for emergency indications: 7 of them had open injuries, 20 had a rough displacement of the fragments during manual reposition. At the same time, as the primary method of immobilization, an external fixation device was used, on two half-rings, which allowed not only to adequately treat the wound in the case of open damage, but also to carry out the traction of fragments in order to reduce the "creasing" of the articular surface. Subsequently, pre-modeled plates with angular stability were used.

With the development of neutrophic complications, the most acceptable option is a step-by-step surgical treatment: the first stage is performed neurolysis, then after the rehabilitation period (4-6 weeks) - surgery on bone structures. In this way, 57 patients over 65 years of age with a good clinical result of treatment were treated at the Research Institute of Gerontology in Moscow.

Preoperative planning was carried out on the basis of clinical and anamnestic examination and additional diagnostic methods: computed tomography (to clarify and objectify the degree of displacement of bone structures) and ultrasound (ultrasound) for the study of soft tissue structures (tendons and nerve trunks [9].

The analysis of the results showed that in 25 cases (43.8%), the operated patients had polypocal combined nerve compression in several zones. Carpal tunnel syndrome was most common – in 45 cases (78.9%). Guyon canal syndrome was detected in 28 patients (49.1%), of which isolated Guyon canal syndrome was detected in only 5 (8.8%) cases. In 22 (38.9%) cases, a combination of carpal canal and Guyon canal

syndrome was observed, and in 3 (5.3%) - a combination of the cubital canal and Guyon canal. Relatively rarely, cubital canal syndrome was detected – in 11 cases (19.3%), Wartenberg syndrome (compression of the superficial branch of the radial nerve) – in 4 cases (7%).

Clinical example: patient P., 74 years old, who continues to work as a jeweler, as a result of a fall, received a fracture of the flexor distal metaepiphysis of the right radius with a displacement of fragments (according to the Smith type) (Fig.

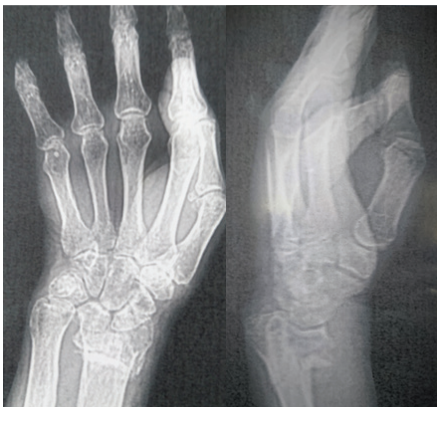

Fig. 3. Radiographs of patient P., 74 years old at admission

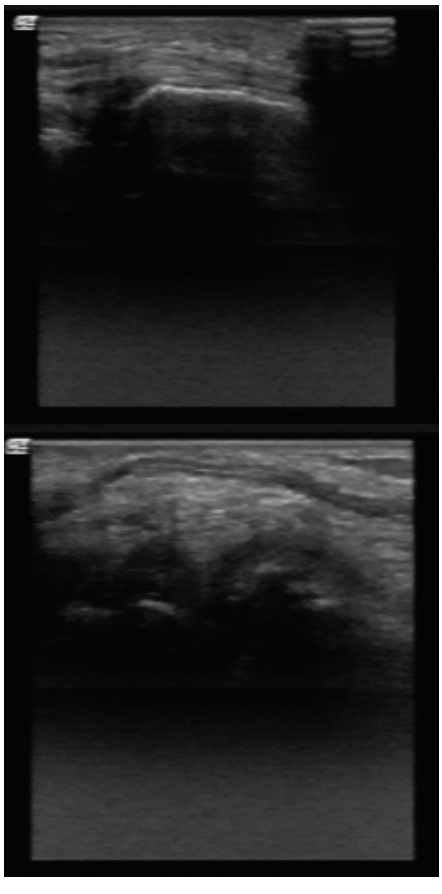

Fig. 4. Ultrasound of the right wrist joint of patient P., 74 years old: carpal tunnel syndrome. Synovitis

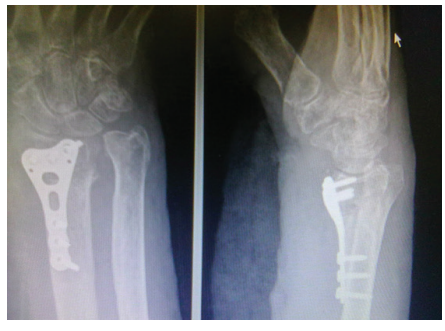

Fig. 5. Radiographs of patient P., 74 years after surgical treatment

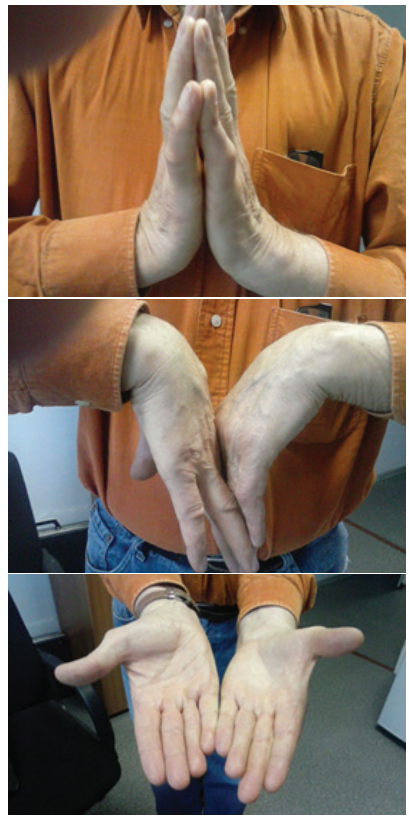

Fig. 6. Functional result 6 months after the surgical treatment

3). Within 6 months after removing the plaster immobilization, he was treated conservatively for severe pain and limited movement in the fingers of the hand. He turned to the Research Institute of Gerontology with clinical signs of neuroischemic complications in the form of neuropathy of the median nerve. Ultrasonography revealed signs of compression of the median nerve in the area of the carpal canal, which were expressed in the unevenness of its diameter: an increase in the diameter of the nerve at the entrance to the bone-fibrous canal (before compression) and a decrease inside the canal (after compression) (Fig.4). At the same time, the nerve section with an increased diameter (for 0.5 cm) had an undifferentiated hypoechoic structure. In addition, in the proximal part of the canal (before the compression zone), a thickening of the nerve was determined by the type of edema syndrome of the nerve trunk and a decrease in its diameter in the canal (the place of compression). Surgical treatment included 2 stages. At the first stage, neurolysis of the median nerve was performed. In the postoperative period, the patient received drug therapy aimed at improving microcirculation and stimulating the restoration of nerve function. A month later, the 2nd stage of surgical treatment was performed: osteotomy, bone osteosynthesis of the radius with bone autoplasty (bone graft from the iliac crest) (Fig. 5). The functional result was evaluated as excellent after 6 months (Fig.6).

Conclusions: the analysis of the conducted studies suggests that in patients over 60 years of age, it is necessary to take into account the type of fracture of the distal part of the forearm bones and all components of the damage, the features of the structure of bone structures associated with age-related changes. Thus, in open fractures, as the primary method of stabilization, it is advisable to use an external fixation device, followed by submerged osteosynthesis; in case of incorrectly fused fractures – osteotomy with the use of a premodeled plate and bone-plastic material, in case of incorrectly fused with the development of neuroischemic complications-step-by-step treatment of neurolysis with the imposition of an external fixation device, followed by bone osteosynthesis and the use of bone-plastic material.

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MANDIBULAR ANESTHESIA BY THE GOW-GATES METHOD

Despite the extensive study of local anesthesia in dentistry, problems of safe and anaesthesia quality improving remain unsolved. At the same time, one of the important aspects of adequate local anesthesia is knowledge of the anatomical-topographic features of the maxillofacial area, which have their own age and gender differences. In this regard, researches improving local anesthesia with anatomical features is of important theoretical, scientific and practical importance. **The research aim** is to increase the accuracy of determining the topography of the target point in condylar process neck by Gow-Gates mandibular anesthesia method. **Materials and methods.** A total of 91 lower jaws (49 males, 42 females) were studied, and 108 CT scans were analyzed. Statistical processing was carried out with the SPSS software package, version 22. Correlation and factor (by Varimax method) analyses were performed with Pearson coefficient (r). **Results.** Individual anatomical and topographic features of the mandibular ramus were obtained, which influence on the exact determination of the target point and needle immersion depth during Gow-Gates mandibular anesthesia. Thus, we have developed Ushnitsky-Chakhov's device for mandibular anesthesia using the Gow-Gates method. Discussion. The advantages of this device are the precise determination of the injection needle direction in the area of the mandibular condylar process outer surface, which eliminates the use of complex and difficult-to-remember anatomo-topographic points for the dentist, which are used in the standard mandibular anesthesia by Gow-Gates method. **Conclusion.** Determination of target point topography by Gow-Gates mandibular anesthesia method is carried out by the device that promotes more accurate delivery of the injection needle tip to the inner surface of the condylar process neck of mandibular ramus, which increases the safety and effectiveness of anesthesia. Traumatic injuries of maxillary artery, tissues of temporomandibular joint, mandibular neuro-vascular bundle and lateral pterygoid muscle are excluded or minimized as much as possible.

Keywords: anatomy and topography, mandible, local anesthesia, safety, effectiveness.

Introduction. Today the priority tasks of health care development are the quality improvement of medical and preventive care with the introduction and application of innovative technologies, which will directly have a positive impact on the preservation and improvement of public health [18, 32]. In practical dentistry, one of the important factors is qualitative anesthesia in the medical care provision, which is accompanied by effective methods search [1, 4, 12, 17, 19, 20]. It should be noted that the effectiveness and safety of anesthesia conductor methods and quality of medical interventions depend on anatomy and topography knowledge of the maxillofacial region, which have age and gender features [2, 3, 5-17, 21-27, 29, 30]. In Researches improving the quality of local anesthesia, taking into account the anatomical and topographic features of the maxillofacial area, have important scientific, theoretical and practical significance [17, 28, 31, 33].

The research aim is to increase the accuracy of determining the target point topography in condylar process neck by Gow-Gates mandibular anesthesia method.

Materials and methods. A craniometric study by V.P. Alekseev, G.F. Debets methods was conducted to identify the anatomical and topographic variability of the lower jaw data (1964). Determina-

tion by the examined anatomical material gender for the female and male types was carried out according to V.I. Pashkova's method (1958). There were no destruction and deformations of anatomical material. Some of them had single minor destruction of teeth, crowned processes, which did not have a negative effect in obtaining reliable results during craniometric measurements. The research was carried out at the departments of therapeutic, orthopedic, surgical dentistry and pediatric dentistry, normal and pathological anatomy, operative surgery with topographic anatomy and forensic medicine of the Medical Institute of the Federal State Educational Institution " M.K. Ammosov North-Eastern Federal University". Our craniometric research included 91 lower jaws, 42 females and 49 males, 108 CT images of "KaVo OP300 Maxio" tomograph for 3D diagnostics (Germany) with the OnDemand 3D™ program.

The following craniometric points of the mandible were measured:

March. 71a. Biom. rb', ramus smallest width; distance starting from the mandibular canal opening to the front edge of the lower jaw ramus; distance from the front edge of the lower jaw ramus to the target point; the distance between the temporal crest to the anterior edge of the mandible ramus; the distance between the target point of temporal crest of the mandible ramus; thickness of the lower jaw ramus in the region of the front edge at the level of the target point. A caliper was used to measure condylar and angular width, smallest ramus width, symphysis and body height, anterior width, mandibular opening, width and height of the tongue of the lower jaw ramus. At the same time, we additionally proposed a L point located at the level of the condylar crest above the inner surface lingula of the lower jaw ramus to determine the dimensions: 1) from the front edge of the ramus to the point L; 2) from the trailing edge of the ramus to the point L; 3) from the lower jaw notch to point L; 4) from the base of the lower jaw to the point L and dimensions: the angle in the lingula projection located on the inner surface of the jaw ramus; distance from the anterior edge of the ramus and temporal crest of the lower jaw; bone thickness of the jaw ramus within the target point; distance from the temporal crest to the target point of the mandible branch. The obtained results from the study of craniometric points and additional dimensions of the mandible gave us the basis for the device development for performing mandibular anesthesia by Gow Gates method [14], consisting of 4 main components, which include a guiding cylinder (oral part), fixing ring for thumb of left hand, arched forming parts of structure, point of needle direction (inside part) with opening for fixation by means of middle finger of left hand.

Statistical processing of clinical material was carried out by standard methods with "SPSS" programs, version 22. At the same time, factor (according to the Varimax method) and correlation analyses were carried out with the Pearson coefficient determination (r).

Results and discussion. There are confirmed individual features of the index of mandible ramus width, which influence the needle immersion depth during mandibular anesthesia. In general, there are absolute dimensions of the smallest ramus width of the lower jaw (March. 71a. Biom. rb'). So, according to V.P. Alekseev, G.F. Debets (1964), after the final process of growth of the facial skeleton bones, the lower jaw ramus width in men with its very small and very large size is 24.8-29.5 and 37.9-42.6 mm, and 23.2-27.6 and 35.4-39.8 mm in women, respectively,.

The craniometric results show the variability presence of ramus width and mandibular opening, as well as target point. At the same time, the difference between the minimum and maximum ramus width indicators in men was at the level of 17.89 + 0.56 mm, and in women - 18.99 + 0.68 mm ($p > 0.05$), where the overall average difference between the minimum and maximum data in men and women was 18.44 + 0.39 mm. A comparative analysis of the obtained average values of the smallest ramus width in men (31.65 + 0.32 mm) and women (29.32 + 0.33 mm) revealed significant differences ($p < 0.05$), where the total average indicator of women and men was 30.83 + 0.23 mm, which indicates significant differences compared to the average indicators of women and men ($p < 0.05$).

The obtained craniometric indicators of the difference between the minimum and maximum values of the distance between the opening and front edge of the lower jaw ramus in women are at the level of 12.41 + 0.45 mm, and 13.02 + 0.41 mm in men ($p > 0.05$), where the overall average indicator between the minimum and maximum values in men and women was at the level of 12.71 + 0.28 mm. A comparative analysis of mean values of the distance between the opening and anterior edge of the mandibular ramus in men (18.21 + 0.19 mm) and women (17.20 + 0.20 mm) revealed significant differences ($p < 0.05$). Meanwhile, the overall average between the minimum and maximum values for men and women was within the numerical values of 17.71 + 0, 37mm. At the same time, a comparative analysis of the total average indicator (17.66 + 0.15) and the average indicators of men and women characterizes the presence of significant differences ($p < 0.05$).

It should be emphasized that the mandible craniometric data related to the study of the distance between the ramus anterior edge and the target point characterize certain differences between the minimum and maximum values in men and women, which are respectively 11.89 + 0.40 mm and 12.19 + 0.43 mm ($p > 0.05$). At the same time, the overall average difference between the minimum and maximum values for men and women is 12.04 + 0.25 mm. Meanwhile, the obtained average values in men (16.86 + 0.18 mm) and women (15.67 + 0.21 mm) characterize the presence of significant differences ($p < 0.05$). The data analysis of the average values of men, women and the total average (men, women) is 16.44 + 0.14 mm, which determines the presence of significant differences ($p < 0.05$).

It should be noted that for the device development and mandibular anesthesia method, when conducting craniometric studies, we took into account the indicators of the lower jaw ramus, which are associated with the distance from the temporal crest to the target point, from the ramus front edge to the temporal crest, as well as with the indicator of the ramus thickness in the area of the anterior edge of the lower jaw at the level of the target point. At the same time, the minimum and maximum values of the distance between the ramus anterior edge and the temporal crest in women and men ranged from 1.60 + 0.10 to 12.0 + 0.22 mm. At the same time, a comparative assessment of the average data of men and women did not reveal significant differences ($p > 0.05$), where their average value was 6.40 + 0.19 mm. Meanwhile, the average distance between the temporal crest and the target point for men and women is 10.5 + 0.07. Meanwhile, a similar situation is determined in values of the lower jaw ramus thickness in the anterior edge region at the level of the target point, where the data were respectively from 3.80 ± 0.07 to 11.40 ± 0.16, as well as 7.38 ± 0.08 mm.

Pearson's correlation analysis revealed a marked association of the smallest ramus width of the mandible with the distances between the ramus anterior edge and the target point ($r = 0.69$), the ramus anterior edge and the temporal crest ($r = 0.51$), the temporal crest and the

Anatomical and topographic characteristics of the lower jaw for conducting mandibular anesthesia (mm)

Name of indicators	Men (l/j, n=49); (CT, n=57)			Women (l/j, n=42); (CT, n=51)			Men and women (l/j, n=91); (CT, n=108)		
	min	max	average	min	max	average	min	max	average
March. 71a. Biome. rb′, smallest branch width	21.5 ±0.57	39.0 ±0.25	31.65 ±0.32	20.0 ±0.67[1]	39.4 ±0.34	29.32 ±0.33[2]	20.0 ±0.41	39.4 ±0.18	30.83 ±0.23[3]
Distance from the front edge of the branch to the opening of the lower jaw	12.0 ±0.41	25.0 ±0.22	18.21 ±0.19	10.5 ±0.44[1]	23.0 ±0.24[1]	17.20 ±0.20[2]	10.5 ±0.31	25.0 ±0.16	17.66 ±0.15[3]
Distance from the front edge of the branch to the target point	11.0 ±0.34	23.0 ±0.25	16.86 ±0.18	9.7 ±0.43[1]	21.9 ±0.22[1]	15.67 ±0.21[2]	9.7 ±0.28	23.0 ±0.14	16.44 ±0.14[3]
Distance between the front edge of the branch and the temporal ridge	1.60 ±0.16	11.0 ±0.30	6.58 ±0.14	3.0 ±0.11[1]	12.0 ±0.32	6.23 ±0.20	1.60 ±0.10	12.0 ±0.22	6.40 ±0.19
Distance between the temporal ridge and the target point	6.5 ±0.09	16.0 ±0.19	11.01 ±0.10	6.3 ±0.08	14.5 ±0.18	9.99 ±0.10	6.3 ±0.07	16.0 ±0.13	10.5 ±0.07
Thickness of the lower jaw branch in the region of the anterior edge at the level of the target point	3.8 ±0.11	10.0 ±0.20	7.36 ±0.08	4.4 ±0.11[1]	11.4 ±0.25[1]	7.41 ±0.14	3.8 ±0.07	11.4 ±0.16	7.38 ±0.08

Note. 1 - statistically significant differences between the minimum and maximum indicators of men and women; 2 - statistically significant differences in the average statistical indicators of men and women; 3 - statistically significant differences between the average indicators of men, women and general average indicators (men and women).

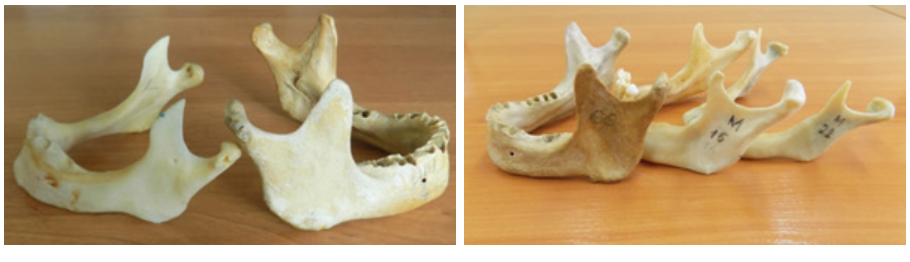

Fig. 1. Anatomical and topographic features of the variability of the width of the lower jaw ramus

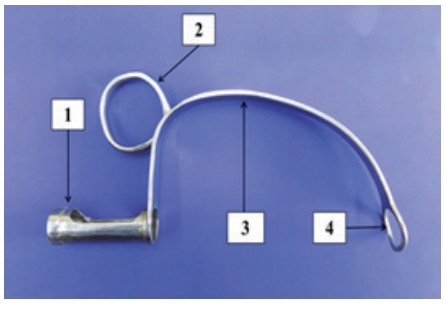

Fig. 2. Ushnitsky-Chakhov device for mandibular anesthesia according to the Go-Gates method (metal structure for reusable use)

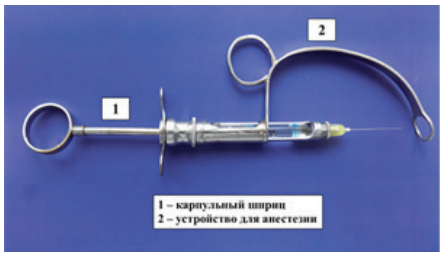

Fig. 3. Technological integration of the device with a fixed standard cartridge syringe located in the channel of the guide cylinder

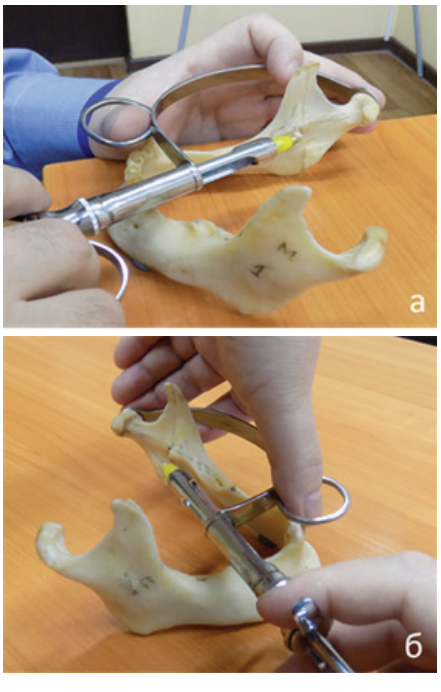

Fig. 4. Application of the device for conducting mandibular anesthesia according to the Go-Gates method: a - on the right; b - left

target point (r = 0.54) and characterizes that the needle immersion depth during mandibular anesthesia depends on individual width sizes. This trend is also confirmed by the results of a factor analysis of effect of the mandibular ramus width on craniometric indicators taken into account when performing mandibular anesthesia by the Varimax method with Kaiser normalization.

Taking into account the craniometric research results, we have developed a device characterized by design simplicity and technological implementation. This device used for Gow Gates mandibular anesthesia consists of all-metal body with 4 main components, including a fixing ring, a cylinder guiding a carpool syringe, end part with a fixing hole for guiding a needle with an arch (Figures 2, 3). The device guide cylinder has a diameter corresponding to the diameter of the carpel syringe (12 mm). At the same time, the cylinder length is 45 mm, which provides optimal limitation of excessive movement of the injection needle outside the target point and into soft tissues. Also, the cylinder allows to shift the mouth angle when choosing the device arrangement in the oral cavity in the region of premolars and molars of the mandible from the opposite injection side and promotes smooth needle insertion in the tissue, which is important for high-quality anesthesia due to unimpeded sliding of the syringe inside the cylinder. In addition, it has a wide window located centrally to control the aspiration sample, volume and rate of anesthetic administration. The left hand thumb fixing ring 2 is a metal ring with a 15 mm diam-

eter, which is located on the arch outside and allows the device to be securely fixed during anesthesia. The arcuate forming part of the construction 3 takes into account the face average dimensions, connects the guiding cylinder 1 and direction point of the needle 4, which allows using the device for patients with different facial types and directing the needle to the target point. The direction point of the needle 4 with the opening for fixing the middle finger of the left hand is located in the end part of the forming arch and serves for fixation in the area of the outer surface of the condylar process of the mandible, which is the direction point of the injection needle to the target point (the condylar process inner surface). To improve the device fixation, the metal rim of the fixing hole has a concavity that takes into account the shape of the condylar process from the outside, which makes it possible to use the device from both the left and right sides of the lower jaw (Figure 4 - a, b). The device parts are made of carbon steel - hardened stainless steel and are sterilized by standard methods.

Thus, the advantage of anesthesia device use is the precise determination of the direction of the injection needle, which is carried out by the guiding cylinder and needle direction point (fixing hole), which is installed in the area of the external surface of the condylar process of the lower jaw using the middle finger of the left hand, which eliminates the use of complex and difficult-to-remember anato-topographic points for the dentist, which are used by Gow-Gates standard method of mandibular anesthesia.

Conclusion. Determination of target point topography during mandibular anesthesia by Gow-Gates method will be carried out by the device that promotes more accurate delivery of the injection needle tip to the inner surface of the condylar process mandibular ramus neck. Such approach creates prerequisites for improving the safety and effectiveness of anesthesia associated with the elimination of maxillary artery injury, temporomandibular joint tissues, mandibular nervous-vascular bundle and lateral pterygoid muscle. In addition, the device is used for mandibular anesthesia on both sides and is easy to use, which is very important for young doctors.

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HEALTHY LIFESTYLE. PREVENTION

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ASSOCIATIONS OF RECURRENT CEPHALGIA WITH VARIOUS ONLINE BEHAVIOR OF TEENAGERS

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The aim of the study is to study the frequency and structure of recurrent cephalalgias in adolescents with various types of online behavior. *Materials and methods:* 2992 adolescents in Krasnoyarsk at the age of 12-18 years, 1374 (45.9%) - boys and 1618 (54.1%) - girls were examined by the method of random sampling. Online behavior was assessed using the Chen Internet Addiction Scale (CIAS). The type of recurrent cephalalgias was assessed using a screening questionnaire. The indices were compared in 3 groups - with adaptive (API), non-adaptive (NPI) and pathological (PPI) Internet use. The data were processed in the program "Statistica 12". *Results:* The frequency of API was 50.6%, NPI - 42.6% and PPI - 6.8% of the total sample of the surveyed. A distinctive feature of the comorbidity of PPI in adolescents is its more pronounced association with cephalgias (both frequent and rare), more frequent absence of cephalgic episodes, and greater pain intensity. *Discussion:* Comparison of API with other types of addiction (mixed, undifferentiated, and dependence on social networks) revealed a more pronounced association of the latter with rare and frequent cephalgias and greater pain intensity, and similar comparisons of gambling addiction with rare cephalgias and more severe pain. *Conclusions:* A more pronounced association of recurrent cephalgias (especially frequent episodes) in adolescents with maladaptive types of online behavior (PPI and NPI) indicates the adverse effects of computers and the Internet and substantiates the need for a personalized approach to these contingents of adolescents to prevent the development of chronic types of psychosomatic pathology in them.

Keywords: adolescents, online behavior, Internet addiction, recurrent cephalalgias.

Introduction. The problem of dependence on the Internet is extremely relevant in modern society [9,11,17]. The role of the Internet is great as the main channel of communication, a source of information and entertainment, which has both positive and negative effects on the body of users [20,24,27]. The most susceptible to addiction to the Internet are adolescents [15,25,29]. The consequence of problematic use of the Internet (PIP) often becomes a violation of the mental and somatic health of a teenager [1,2,8], a violation of his mental status [14,16], the likelihood of personal, interpersonal [21] and social problems [28] increases, the risk is higher suicidal behavior [3,5,18,23,30] and socio-psychological maladjustment [22].

An increasing problem in all countries of the world is the growth of comorbid diseases and conditions associated with the pathological use of the Internet, including functional somatic disorders, a significant proportion of which are recurrent cephalalgias [8,12,13,26]. The prevalence of cephalalgias in children varies widely depending on the region of residence, methodology, genetic differences, and diagnostic criteria used [6]. The overwhelming majority of all types of cephalgia (95-98%) are primary forms of headache, secondary forms are quite rare (no more than 5% of all cases of cephalgia).

Studies devoted to the pathological use of the Internet in adolescents and functional somatic disorders associated with PPI (including recurrent cephalalgias) are extremely few and require an urgent solution to this problem [19,31]. The presence of episodes of headache (especially frequent) negatively affects the quality of life [7], negatively affects school performance, behavior and social adaptation of adolescents.

All of the above served as a rationale for the need for our study, the *purpose* of which was to study the frequency and structure of recurrent cephalgia in adolescents with various types of online behavior.

Materials and research methods. The object of the research was random samples of adolescents 12-18 years old - students of 10 general educational institutions of Krasnoyarsk in 5 districts of the city. The primary screening program included a questionnaire survey of schoolchildren using 4 questionnaires. The total number of those surveyed was 3055 people. Incorrectly filled out questionnaires were excluded from the analysis, in which there were no answers to some questions, as a result, 2992 questionnaires were subject to statistical processing. Of these, 1,374 (45.9%) are boys and 1,618 (54.1%) are girls.

The following observation groups were distinguished: by type of online behavior: 1 gr. - with API, 2 gr. - with NPI, 3 gr. - with PPI (with IZ). Allocated the following subgroups: 1 gr. - with gambling addiction, 2 gr. - with addiction to social networks, 3 gr. - with mixed IZ (presence of both gambling addiction and dependence on social networks), 4 gr. - with undifferentiated Internet addiction (adolescents with IZ, confirmed by a total score on the Chen scale ≥ 65, but at the same time they do not have gambling addiction and do not depend on social networks). The type of online behavior of adolescents was verified using the internationally accepted Chen Internet Addiction Scale (CIAS) [10], adapted by VL Malygin and KA Feklisov [4]. Chen's total CIAS score ≥ 65 points was taken as the criteria for the presence of PID or Internet addiction; if the value of this indicator corresponded to the range of 27-42 points, the absence of Internet addiction (or TIP) was stated, the total CIAS score included in the range of 43-64 points, indicated the presence of maladaptive Internet use (NPI).

The study was approved by the Ethics Committee of the Scientific Research Institute of the Ministry of Railways and supported by a grant from the Russian Foundation for Basic Research (No. 18-29-22032 / 20). The collection of information on the presence of cephalalgias was carried out by the method of questioning according to the author's screening questionnaire, developed by prof. S.Yu.

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Tereshchenko. The type of headaches was verified taking into account the criteria for the frequency of episodes of cephalalgia set out in the international classification of headaches (ICGB-3b, 2013). The criteria for the type of RSL were the presence and frequency of cephalalgia over the past 3 months: with a headache frequency of no more than 1 time per month (or during this time the head did not hurt at all), it was concluded that there was no RSL, with a headache frequency of 1 to 15 days per month - RSL was considered rare, with a headache frequency of more than 15 days a month - RSL was regarded as frequent. The intensity of pain (in points) was assessed on a VAS scale, graded from 0 to 5, where item "0" corresponds to the answer "pain did not bother", item "5" corresponds to the answer "pain bothered very much, interfered with the usual activities." This scale was included as a section of the questionnaire used in the study. The criterion for the presence of RHL was the intensity of headache ≥ 4 points.

When processing the obtained data, the module of nonparametric statistics of the program "Statistica 12" (USA) was used. Confidence intervals for percentages were calculated using the Wilson method using an online calculator. The level of significance of differences (p) for binary features in pairwise comparison of the two groups was assessed using Pearson's chi-squrae test. The form of presentation of the obtained results were:% share, limits of the confidence interval (CI), the absolute value of Pearson's χ2 test, the degree of freedom (df) for the χ2 test and the statistical significance of the differences (p). Quantitative characteristics are represented by indicators of the sample mean (M), median (Me) and interquartile range (Q25 – Q75). The statistical significance of differences in quantitative traits was determined by the Mann – Whitney U-test. The critical level of statistical significance when testing the null hypothesis was taken at the 95% significance level (p≤0.05).

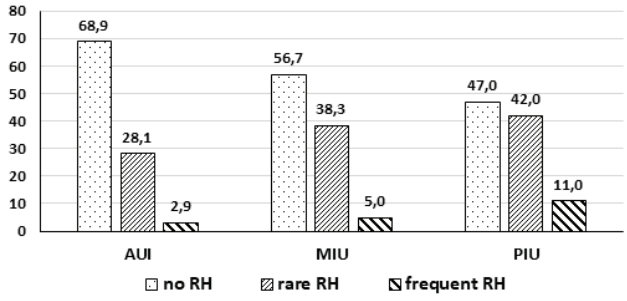

Fig. 1. The structure of RH in groups of adolescents with different types of online behavior, in %. Statistical significance of differences according to Pearson's χ2 test (p) for the "no RH" indicator: p1-2 <0.0001, p1-3 <0.0001, p2-3 = 0.0106; for the indicator "rare RH": p1-2 <0.0001, p1-3 = 0.0001, p2-3 = 0.3205; for the indicator "frequent RH": p1-2 = 0.0047, p1-3 <0.0001, p2-3 = 0.0008.

Results and discussion. Of the 2992 surveyed, 1515 (50.6%) were characterized by adaptive use of the Internet (API), 1274 (42.6%) adolescents had non-adaptive use of the Internet (IPI) verified and 203 (6.8%) of the surveyed had pathological use of the Internet (IPI)) or Internet addiction (FROM). The frequen-

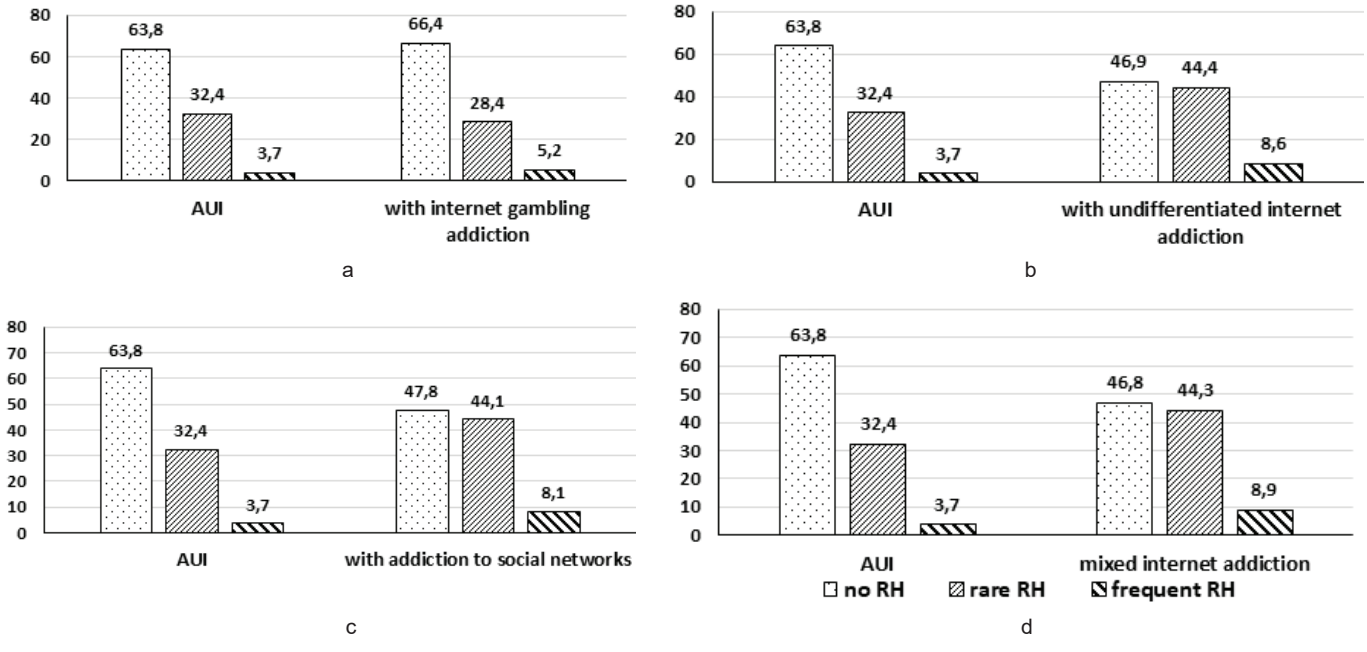

Fig. 2. The structure of recurrent headache (RH) in adolescents with various types of Internet-dependent behavior (in %)
a – The structure of RH in adolescents with IPA and with play IA, in %. Note: statistical significance of the differences according to Pearson's χ2 test (p) for the "no RH" indicator: p1-2 = 0.4217; for the indicator "rare RH": p1-2 = 0.1926; for the indicator "frequent RH": p1-2 = 0.2480;
b – The structure of RH in adolescents with API and undifferentiated IA, in %. Note: statistical significance of differences according to Pearson's χ2 test (p) for the "no RH" indicator: p1-2 = 0.0019; for the indicator "rare RH": p1-2 = 0.0237; for the indicator "frequent RH": p1-2 = 0.0241;
c – The structure of RH in adolescents with API and addiction from social networks, in %. Note: statistical significance of differences according to Pearson's χ2 test (p) for the "no RH" indicator: p1-2 <0.0001; for the indicator "rare RH": p1-2 = 0.0024; for the indicator "frequent RH": p1-2 = 0.0063;
d – The structure of RH in adolescents with API and mixed Internet addiction, in %. Note: statistical significance of the differences according to Pearson's χ2 test (p) for the "no RH" indicator: p1-2 = 0.0020; for the indicator "rare RH": p1-2 = 0.0272; for the indicator "frequent RH": p1-2 = 0.0220

cy of occurrence of PPI (IZ) according to the results of our study turned out to be comparable with the results obtained by other researchers.

The presence of recurrent headache and its structure in groups with various types of online behavior is illustrated in Figure 1.

The highest prevalence of rare RSL was observed in adolescents with NPI (38.3%) and PPI (42.0%), this type of RSL was recorded much less frequently in those surveyed with IPI (Fig. 1).

The percentage of frequent RSL was the highest in the group of Internet-addicted adolescents (with PPI) - 11.0%, this type of RSL was found much more often (5.0%) among those surveyed with NPI.

From 1/2 to 2/3 of all adolescents examined by us did not suffer from recurrent cephalalgias. More than 2/3 of those surveyed with IPI did not have a history of repeated headache episodes, there were fewer such persons in the group with IPI, and even less was the number of this contingent among adolescents with Internet addiction (with IPI) (Fig. 1).

We carried out a comparative analysis of pain intensity by quantitative values of the headache intensity scale in groups with different types of online behavior. It was found that adolescents with PPI had the highest headache intensity indicators, the second rank was occupied by adolescents with NPI, and those with IPI were characterized by less headache intensity (Table 1).

We carried out a comparative analysis of the frequency and types of GBL in adolescents with different content structures of online behavior (with gambling addiction, addiction to social networks, mixed and undifferentiated Internet addiction) in comparison with a group of adolescents without Internet addiction (group with API).

We failed to identify statistically significant differences in the incidence and structure of RSL in adolescents with gambling addiction and without IZ behavior (Fig. 2a).

The severity of headache, assessed by the visual analogue scale of pain intensity VAS, did not have statistically significant differences in the groups with and without Internet gaming addiction (Table 2).

The group of examinees with gambling addiction differed in a large% of adolescents with no history of headache episodes, while the presence of undifferentiated Internet addiction was associated with a more frequent occurrence of both rare and frequent RSL in comparison with the group of adolescents with adaptive Internet use (Fig. 2b).

There were no statistically significant differences in the scores for the intensity of headache in the groups with and without undifferentiated Internet addiction.

In the group with IPA, there were more adolescents without RSL in comparison with those surveyed, characterized by the presence of dependence on social networks, while the latter were distinguished by a greater occurrence of both rare and frequent RSL (Fig. 2c).

Adolescents with addiction to social networks were characterized by a higher intensity of headaches, which was confirmed by the presence of higher VAS scores in them in comparison with similar indicators of the group without dependence on social networks (Table 2).

Adolescents with IPA were significantly more often characterized by the absence of recurrent cephalalgia in comparison with those surveyed with mixed Internet addiction. At the same time, they were distinguished by a higher occurrence of rare and frequent RHLs (Fig. 2d).

Adolescents with mixed Internet addiction were distinguished by a greater intensity of headache, which was demonstrated by higher VAS scores in comparison with similar indicators of adolescents without mixed Internet addiction (Table 2).

We conducted a comparative analysis of the frequency and structure of RSL in

Table 1

Intensity of headache in points of visual analog scales (VAS) in adolescents with various types of online behavior (M; Me, Q25-Q75)

Indicators	Examined groups			The statistical significance of the differences "p" (by Mann-Whitney U-test)
	1 gr. - teenagers with adaptive internet use	2 gr. - teenagers with maladaptive internet use	3 gr. - adolescents with pathological Internet use	
Headache intensity (mean VAS score)	1.2 1.0 0.0-2.0	1.6 1.0 0.0-3.0	2.0 2.0 1.0-3.0	1-2 < 0.0001 1-3 < 0.0001 2-3 < 0.0001

Table 2

Intensity of headache in points of visual analog scales (VAS) in groups of adolescents with various types of Internet-dependent behavior (M; Me, Q25-Q75)

Groups	N (number of surveyed)	M	Me	25‰	75‰	The statistical significance of the differences "p" (by Mann-Whitney U-test)
1 gr. - without gambling internet addiction 2 gr. - with internet gambling addiction	2673 331	1.43 1.48	1.00 1.00	0.00 0.00	2.00 2.00	0.7828
1 gr. - without dependence on social networks 2 gr. - with addiction to social networks	2764 239	1.40 1.90	1.00 2.00	0.00 1.00	2.00 3.00	< 0.0001
1 gr. - no mixed internet addiction 2 gr. - with mixed internet addiction	2924 79	1.43 1.85	1.00 2.00	0.00 1.00	2.00 3.00	0.0055
1 gr. - without undifferentiated internet addiction 2 gr. - with undifferentiated internet addiction	144 84	1.87 2.07	2.00 2.00	1.00 1.00	3.00 3.00	0.2074

adolescents with gambling addiction and other types of Internet addiction (dependence on social networks, mixed, undifferentiated).

It was found that among those surveyed with gambling addiction there was a greater number of adolescents without recurrent cephalalgia, while the group with undifferentiated Internet addiction was distinguished by a high frequency of occurrence of both rare and frequent RSL (Fig. 3a).

Comparative analysis of the presence and structure of RSL in groups with gambling addiction and dependence on social networks revealed a statistically significant predominance of rare RSL and a lower incidence of the absence of RSL among adolescents with dependence on social networks, the differences in frequent RSL in the compared groups did not reach statistical significance, although in general it is possible note the high incidence of frequent RSL in adolescents with social addiction. networks (Fig. 3b).

Mixed Internet addiction was significantly more often associated with the presence of rare PCD in adolescents, a more frequent absence of recurrent cephalalgia, with some prevalence of frequent PCD in the absence of statistically significant differences between groups for this indicator (Fig. 3c).

The intensity of pain, assessed in terms of the VAS scale, in the groups with API and undifferentiated Internet addiction was comparable (Table 2).

Based on the results of the study, the following **findings** can be drawn:

1. The structure of types of online behavior was established in 2992 adolescents aged 12-18 years in Krasnoyarsk: API was noted in 50.6%, NPI - in 42.6% and PPI (IZ) - in 6.8%.

2. A distinctive feature of PPI comorbidity in adolescents is its more pronounced association with cephalgias (both frequent and rare), and a high intensity of pain.

3. In comparison with the IPA, the presence in adolescents of addiction to social networks, mixed and undifferentiated Internet addiction is more associated with both rare and frequent recurrent cephalgias and a greater intensity of pain.

4. Comparative analysis of gambling addiction with other types of addiction (addiction to social networks, mixed and undifferentiated) showed a more pronounced association of the latter with rare cephalgias, comparability of the occurrence of frequent cephalgias and more frequent absence of cephalgic episodes.

Conclusion. Thus, the presence of functional somatic disorders (in our study, these are recurrent cephalalgias) in adolescents with maladaptive types of online behavior (PPI and NPI) indicates an unfavorable effect of the computer and the Internet on the health of adolescents - Internet users, and in the case of untimely diagnosis and lack of correction of pathological changes can lead to the formation of chronic psychosomatic pathology. All this substantiates the expediency and necessity of a personalized approach to adolescents with maladaptive and pathological Internet use for early diagnosis and prevention of functional somatic disorders and their transformation into chronic forms of psychosomatic pathology.

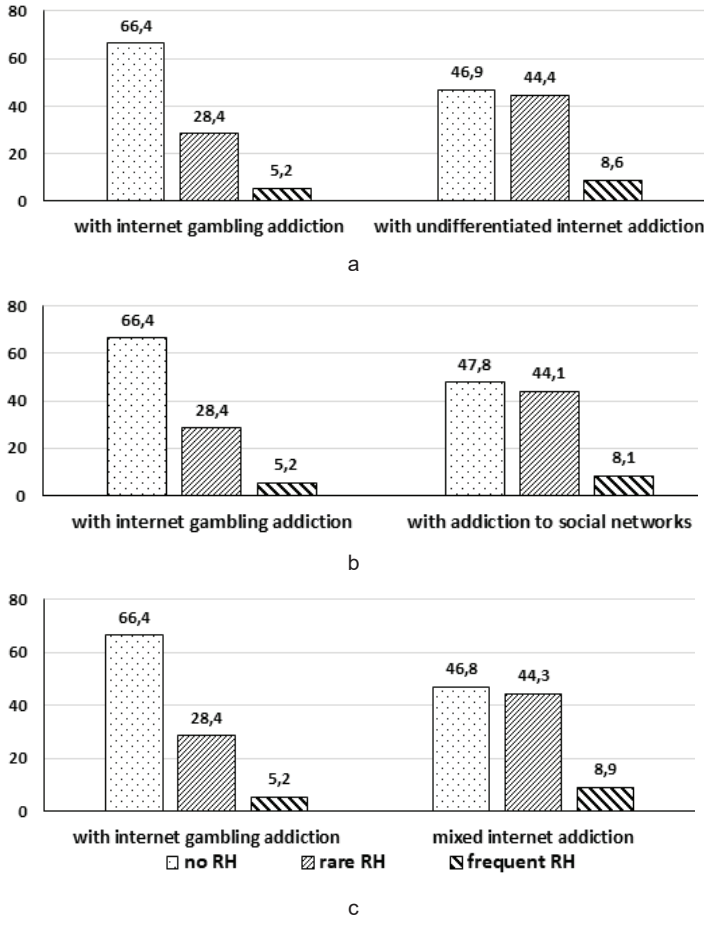

Fig. 3 - The structure of RH in adolescents (in %):
a - with gambling and undifferentiated Internet addiction. Note: statistical significance of differences according to Pearson's χ2 test (p) for the "no RH" indicator: p2-3 = 0.0017; for the indicator "rare RH": p2-3 = 0.0073; for the indicator "frequent RH": p2-3 = 0.2585.
b - with gambling addiction and addiction to social networks. Note: statistical significance of differences according to Pearson's χ2 test (p) for the "no RH" indicator: p2-4 = 0.0002; for the indicator "rare RH": p2-4 = 0.0011; for the indicator "frequent RH": p2-4 = 0.2426.
c - Structure of RH in adolescents with gambling and mixed Internet addiction. Note: statistical significance of differences according to Pearson's χ2 test (p) for the "no RH" indicator: p2-5 = 0.0008; for the indicator "rare RH": p2-5 = 0.0084;
for the indicator "frequent RH": p2-5 = 0.2352.

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ADHERENCE OF PARENTS AND PHYSICIANS OF IRKUTSK TO PERTUSSIS VACCINATION

DOI 10.25789/YMJ.2021.74.12

Background. Mass pertussis vaccination has significantly reduced morbidity and mortality among young children, however, despite the high coverage of primary vaccination against pertussis, there is an increase in pertussis morbidity and mortality in Russia.

Aim of the study. Assessment of knowledge and attitudes regarding pertussis preventive vaccination among parents and physicians in Irkutsk.

Materials and methods. To study the level of knowledge and attitudes regarding pertussis cocoon vaccination, we carried out a descriptive retrospective epidemiological study which included a voluntary anonymous survey of 1620 parents and 324 physicians of various specialties. Statistical data processing was performed using MS Excel 2010 (Microsoft Corporation, USA).

Results. Our study showed that 39.5 % of physicians are not sufficiently informed of cocoon vaccination strategy; 26.6 % of physicians consider it necessary to recommend cocoon vaccination; 29.1 % of physicians do not consider it necessary to recommend this strategy; 4.8 % of physicians consider vaccination to be contraindicated for pregnant women. Secondary vaccination against pertussis in carried out in 8.4 % of children of preschool, school age and in adolescence. 33.5 % of women were informed of the possibility to get vaccinated against pertussis during pregnancy, and 8.5 % of the respondents answered that they didn't understand the importance of pertussis vaccination before and during pregnancy.

Conclusion. We revealed a low level of awareness of the necessity for secondary vaccination at school age, and especially of cocoon vaccination strategy among parents and physicians in Irkutsk.

Keywords: vaccination, immunization, prevention, children, parents, physicians, whooping cough, pertussis, *Bordetella pertussis*

Introduction. Nowadays pertussis remains a common infectious disease, often with the development of complications, despite the achieved WHO-recommended vaccination coverage [5]. It has been earlier found out that pertussis makes the course of the disease more severe for those children, affected with premorbid metabolic or neurological diseases [13, 7]. Globally, since 2010, DTP (Diphtheria, Tetanus, Pertussis) vaccine coverage has remained insufficient - 86% [2]. Pertussis is a heavy burden for the healthcare system in many countries, including Russia. In recent years, there has been a significant increase in the incidence of pertussis, registered on the territory of the Russian Federation; and this increase occurs regardless of what vaccines have been used [10, 1, 11, 14]. Order No 125n of 21 March 2014 "On the approval of the national calendar of preventive vaccinations and the calendar of preventive vaccinations for epidemic indications (NCPV)" provides only one revaccination against pertussis at the age of 18 months.

In the Irkutsk region, there has been an increase in incidence rates since 2016, which exceeds the national level by 1.5-2 times [9, 8].

Aim of the study. To provide clinical and epidemiological characteristics of pertussis to justify measures for optimization of the pertussis vaccination strategy in the Irkutsk region and to analyze the commitment of parents and physicians to pertussis vaccination, the degree of awareness of revaccination necessity in preschoolers, schoolers, and adolescents, and of the cocoon strategy of vaccination.

Materials and methods. A descriptive retrospective epidemiologic study was carried out. The long-term trends in the incidence of pertussis among the total population and children of different age groups were analyzed for the period from 2000 to 2019, according to the State Federal Statistical Monitoring Forms (Form 2 "Information on Infectious and Parasitic Diseases"). We compared the incidence rates among children of different age groups in the year with the minimum level (2012) during the analyzed period and then in 2019.

To study the level of awareness and attitude to the cocoon strategy of vaccination against pertussis, a voluntary anonymous survey of physicians of various specialties (n = 324) was carried out. From this sample of physicians, 3 groups were selected: group 1 (n = 18) – physicians, prescribing vaccinations for children (pediatricians, neonatologists); group 2 (n = 54) – physicians, prescribing vaccinations for adults (therapists, obstetricians-gynecologists); group 3 (n = 52) – physicians, who can advise on vaccination issues (neurologists, surgeons, and other specialists).

Parents also participated in the study. These were the parents of children, aged 0 to 18 years inclusive, who came for the outpatient appointments in 12 city children's polyclinics in Irkutsk (n = 930); the parents of children, who underwent treatment in the pediatric hospital of the Clinic of the Scientific Centre for Family Health and Human Reproduction Problems (n = 104); expectant mothers, attending antenatal clinics (n = 339); female patients of the city perinatal center of the III level (n = 247). Our own questionnaire included 15 questions to let us know more about: the degree of awareness of the vaccination

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necessity against infections, according to the NCPV; the degree of awareness of the vaccination possibility against pertussis of a pregnant woman and her environment to protect her baby from pertussis; adherence to revaccination against pertussis in preschoolers, schoolers and adolescents.

To assess the statistical significance of differences in relative indicators, confidence intervals were calculated with a significance level of 95% (95% CI). The statistical significance of intergroup differences in terms of qualitative characteristics was assessed using the χ2 criterion: with Rabs. <10 - with Yates' correction, with Rabs. <5 - using two-sided Fisher's exact test. The 95% CI for frequencies and fractions was calculated using an online calculator, proposed by the Vassar Stats: Web Site for Statistical Computation (http://vassarstats.net). Graphical data processing was performed using MS Excel 2010 (Microsoft Corporation, USA).

Results and discussion. Long-term trends in the incidence of pertussis among the total population of the Irkutsk region were characterized by uneven distributions of indicators over the years with a slight upward trend, the average annual growth rate (Tgr.) was 2% for the analyzed period (2000–2019). Until 2015, there was a pronounced downward trend with a decline rate of –5.8%, with an average annual value of 4.8 per 100 thousand people of the population. In 2016, a significant increase in the incidence rate was registered: for example, the indicator was 4.4 per 100 thousand people of the population, followed by an increase (Tgr. = 17.5%).

During the analyzed period, the incidence among children of 0-14 years old was significantly higher than that among adults and among the population, the long-run annual average value is 28.03 per 100 thousand people of this age group, the average annual growth rate for the period 2015-2019 is 31.6%. Incidence among adults was registered at a sporadic level, exceeding 1.0 per 100 thousand people of the corresponding group since 2018 (Fig. 1).

The highest incidence rates were registered among children in the age group under 1 year of age with an the long-run annual average value of 53.6 per 100 thousand people of the corresponding group, significantly exceeding similar indicators in the age groups of children of 1–2 years (1.9 times), 3–6 years (in 2.5 times), 7-14 years (3.1 times) and 15-17 years (8.1 times). Moreover, children under 1 year of age were the most affected

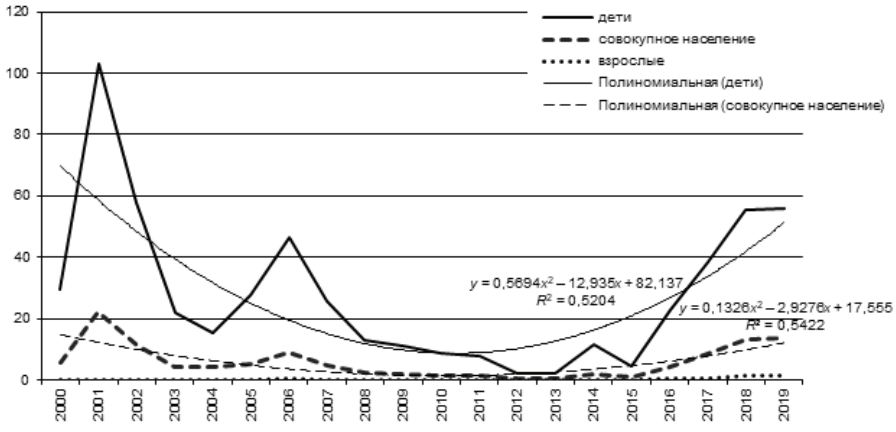

Fig. 1. Long-term trends in the pertussis incidence in adults and 0–14 y.-o. children in the Irkutsk region in 2000–2019 (per 100 000 people of the population)

Table 1

Pertussis incidence (per 100 000 people of the population. 95% CI) and the proportion (%) of children from various age groups during the periods of high and low incidence

Age groups	Years				χ^2	*p*
	2012		2019			
	%	Per 100 000 people	%	Per 100 000 people		
under 1 year	18.2	5.4 [0÷12.9]	11.3	121.1 [82.2÷160.0]	38.4	$p < 0.001$
1–2 year-olds	27.3	4.3 [0÷9.1]	14.6	69.3 [41.6÷97.0]	41.1	$p < 0.001$
3–6 year-olds	18.2	1.6 [0.9÷2.3]	16.8	37.5 [27.7÷47.3]	40.6	$p < 0.001$
7–14 year-olds	36.4	1.8 [0.1÷3.5]	41.8	52.8 [43.9÷61.7]	106.2	$p < 0.001$
15–17 year-olds	0	0	7.9	31.0 [18.9÷43.2]	26.7	$p < 0.001$

age group during periods of high and low incidence rates (Table 1).

Even though the region has achieved and is maintaining the normative indicators of preventive vaccination coverage against pertussis in the decreed groups of the child population (vaccination coverage of children at the age of 12 months was 97.03%, revaccination coverage of children at the age of 24 months was 97.08%) [8], in 2019 there was an increase in the incidence in all age groups (p <0.001).

Over the analyzed period, the proportion of children under 14 years of age among those, who became sick, was 89.5%, adolescents - 5.1%, adults - 5.4%.

The proportion of children of different age groups has changed (Fig. 2, 3) since 2016, which was affected by the excess incidence. There was a statistically significant decrease in the propor-

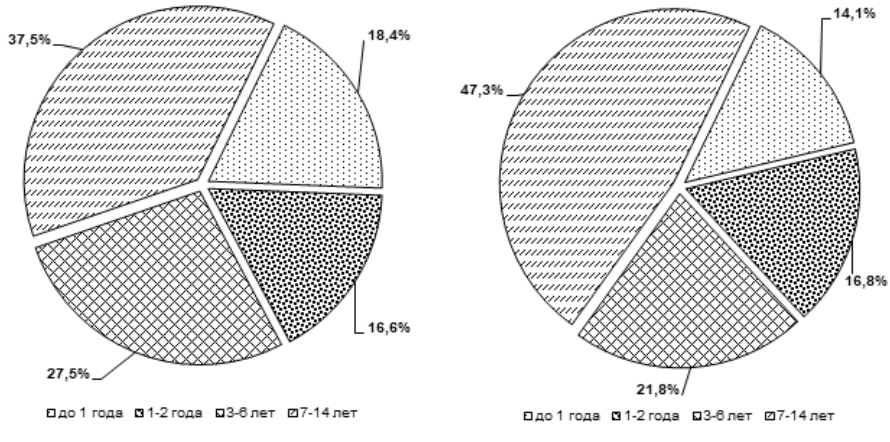

Fig. 2. The structure of pertussis incidence (%) in children of various age groups in 2000–2015 (па), 2016–2019 (б)

Table 2

Attitudes of physicians of different specialties to the cocoon strategy of vaccination (n = 324)

Respondents' answers	Group 1 (n = 218)			Group 2 (n = 54)			Group 3 (n = 52)			The total number (n = 324)		
	abs.	%	[95% CI]	abs.	%	[95% CI]	abs.	%	[95% CI]	abs.	%	[95% CI]
Yes, I consider iYes, I consider it necessary to recommend the cocoon strategy of vaccination for pregnant women	57	23.8	[18.6÷29]	20	35.1	[22.8÷47.4]	17	29.3	[17.6÷41]	94	26.6	[22.1÷31.1]
No, I do not consider it necessary to recommend the cocoon strategy of vaccination for pregnant women.	75	31.3	[25.7÷36.9]	14	24.5	[13.5÷35.5]	14	24.1	[13.1÷35.1]	103	29.1	[24.4÷33.8]
Insufficiently informed about the cocoon strategy of vaccination	94	39.3	[33.3÷45.3]	21	36.8	[24.3÷49.3]	25	43.1	[30.4÷55.8]	140	39.5	[34.6÷44.4]
Vaccination is contraindicated for pregnant women	13	5.6	[3.1÷8.3]	2	3.6	[0÷8.3]	2	3.5	[0÷8.2]	17	4.8	[2.7÷6.9]

Note. Some physicians gave several answers, % was calculated on the number of answers (group 1 - 239 answers; group 2 - 57 answers, group 3 - 58 answers).

tion of children under 1 year of age ($\chi 2 = 6.3$; $p < 0.05$); the proportion of children of 1–2 years old did not change; the proportion of children of 3–6 years old and 7–14 years old increased significantly ($\chi 2 = 7.9$; $p < 0.01$ and $\chi 2 = 18.2$; $p < 0.001$, respectively).

Several European countries use the cocoon strategy of vaccination, which is vaccination of family members surrounded by a newborn, quite successfully. Our research has shown that among the total number of respondents, 39.5% of physicians are not sufficiently informed about the cocoon strategy of vaccination; 26.6% consider it necessary to recommend the cocoon strategy of vaccination; 29.1% do not consider it necessary to recommend the cocoon strategy of vaccination; 4.8% consider vaccination to be the contraindication for pregnant women (intergroup differences according to the $\chi 2$ criterion are not statistically significant ($p > 0.05$)) (Table 2).

The analysis of the parents' questionnaires showed that 98% [97.3 ÷ 98.7%] of parents (1590 out of 1620) vaccinate their children according to the NCPV, including additional vaccinations. Revaccination against pertussis in preschoolers, schoolers and adolescents is carried out by 8.4% [7.1 ÷ 9.8%] of respondents (136 out of 1620). Refusal to vaccinate was registered in 2% of the parents surveyed. At the same time, as our earlier works demonstrated, the level of education (incomplete secondary), the financial situation in the family (average) and the acquisition of information about vaccinations through the mass media affect the refusal to vaccinate [3].

33.5% [31.2 ÷ 35.8%] (543 out of 1620) women were informed about the possibility of getting vaccinated against this infection during pregnancy, and 8.5% [7.2 ÷ 10.0%] of the respondents (138 of 1620) told they did not understand the importance of pertussis vaccination before and during pregnancy.

29.4% [27.4 ÷ 31.7%] of the parents (477 out of 1620) know and agree that before entering school it is necessary to revaccinate against pertussis, diphtheria, and tetanus. Revaccination against diphtheria, tetanus, and pertussis before entering school was carried out by 33.5% [31.1 ÷ 35.8%] of respondents (542 out of 1620), and 24% [22.0 ÷ 26.4%] (390 of 1620) replied that they did not do the revaccination.

Discovered trends are traced in presence of epidemiological problems with pertussis. At the present stage, the epidemic process of pertussis is characterized by a high incidence rate of children under one year of age, fading away of post-vaccination immunity, and a high susceptibility of adolescents and adults [12].

Several works [6, 4] demonstrated that among children, affected by pertussis, previously vaccinated children of the first 2 years of life predominated, which is due to insufficient coverage with preventive vaccinations due to parents' refusal to vaccinate children, unreasonable medical exemption, non-adherence of vaccination and revaccination schemes, as well as no domestic vaccine for age-related revaccinations for children over 5 years old.

Conclusion. There has been an increase in the incidence of pertussis in children of all age groups. Low coverage with revaccination against pertussis in schoolers and adolescents has been registered. There is an objective necessity to introduce age-related revaccinations into the vaccination calendar for children, aged 6 and 14 years. In addition, it is necessary to do vaccinations of people in contact with an unvaccinated child under 1 year of age.

Among different groups of the population (parents and physicians) in Irkutsk city, there is low awareness of the revaccination necessity at school age, especially in relation to the cocoon strategy of vaccination. What is need is to conduct training seminars, lectures for physicians and to hold events to raise awareness of the population.

Conflict of interests. The authors declare neither direct nor potential conflicts of interest related to the publication of this article.

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HYGIENE, SANITATION, EPIDEMIOLOGY AND MEDICAL ECOLOGY

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ELIMINATION OF THE EPIDEMIC OF VILYUISK ENCEPHALOMYELITIS

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The article presents data on the termination of the epidemic of Viliuisk encephalomyelitis (VEM) in the Republic of Sakha (Yakutia), Russian Federation. VEM is a fatal chronic central nervous system disease that has claimed hundreds of lives. Originally found only in a small population living in the Viliuisk ulus (a Siberian administrative district), VEM later spread to densely populated areas of Central Yakutia. The occurrence of secondary cases among previously unaffected populations indicates that VEM was a transmissible disease with a mode of transmission characteristic of other chronic infections.

Decrease in close contacts between patients during acute and subacute phases of VEM with the surrounding population through prolonged hospitalization of patients in specialized medical institutions and improved social and hygienic living conditions in Yakut villages was followed by a slow decline in new cases of VEM; incidence slowly decreased in the 1980s and 1990s, reaching zero in the 2010s. No new cases of VEM have been reported since 2012. The elimination of the VEM epidemic prevented the disease from spreading into unaffected areas of Yakutia and other parts of the Russian Federation and might well have kept it from introducing to the world yet another emerging infection.

Keywords: Viliuisk encephalomyelitis, territorial distribution, disease prevention, Eastern Siberia, Republic of Sakha (Yakutia).

Introduction. VEM was first observed about 170 years ago in several villages around *Lake Mastakh* of the Viliuisk ulus (a Siberian administrative district) [4]. Its presence in the area was later confirmed by a medical team [3]. Most likely, the disease had smoldered for centuries in small nomadic Siberian tribes. In the 1930s, the population of such tribes was forcibly relocated to larger Yakut villages on the other side of Viliui River in order to organize collective farms. Doctors began to notice that a fatal neurological disease was occurring among the new settlers. Contacts with VEM patients expanded in the villages and farms, and, by the 1950s, the number of new cases had skyrocketed. In the 1960s, VEM spread to neighboring areas along the Viliui River, and then to densely populated industrial areas of Central Yakutia, resulting in an epidemic that was difficult to control [18]. The greatest spread of VEM occurred during the period of intense and well-documented migration of people after the end of the Second World War.

Systematic studies to understand the nature of the VEM epidemic and prevent its further spread were undertaken in the 1950s by Prokopiy Andreevich Petrov [7, 8], who would turn 100 this year. P.A. Petrov's research was supported and continued by other investigators in the following decades [6, 10, 15]. As part of these studies, an unusual virus, termed *Viliuisk virus,* was isolated from patients [9]. Viliuisk virus cross-reacts with *Theiler's murine encephalomyelitis virus* (TMEV) in serologic tests [12], however, the nucleotide sequences of the Viliuisk virus differ from the classical representatives of TMEV, therefore the Viliuisk virus was renamed the *Viliuisk human encephalomyelitis virus (VHEV)* and recognized as a separate Theilovirus clade, the first of a new group of *human TMEV* viruses within the *Theiler's virus* group [15]. The etiologic relationship between VHEV and VEM has never been established [13].

The ***objective of this study*** was to analyze long-term epidemiological data on the incidence and territorial distribution of VEM and to evaluate the hypothesis that the elimination of the VEM epidemic resulted from preventing close contacts between patients in the acute and subacute phases of VEM and the surrounding population. Opportunities for close contacts were reduced by introduction of prolonged hospitalization of VEM patients in specialized medical institutions as well as by general improvement of social and hygienic living conditions in Yakut villages.

Materials and methods. Systematic documentation and registration of VEM cases began in 1951. Particular attention was paid to the early identification of patients with suspected VEM. Complaints of high fever and headache by a resident of an endemic village were sufficient reason to consult a neurologist, and patients with neurological symptoms were sent to one of two specialized hospitals: the *Neurological Department of the Viliuisk Regional Hospital* (later the *Viliuisk Psychoneurological Hospital*) or the *Department of Encephalitis of the Yakutsk Republican Hospital* (until 1998). Expeditions of neurologists and epidemiologists from Yakutsk and Moscow periodically visited each settlement in the endemic zone to identify new patients. In 1992, a new State Program "*Biology of Viliuisk Encephalomyelitis*" was adopted; it was later closed, but, by order of the Ministry of Health of the Sakha (Yakut) Republic since 2000, all neurologists in the ulus are required to submit reports on monitoring of patients with chronic VEM. All patients with suspected VEM still undergo inpatient examination in the *Neurological Department of the Republican Hospital No. 2, Emergency Medical Aid Center* (directed by Dr. L.T. Okoneshnikova). Diagnosis of VEM is based on clinical examination and investigations, including testing of the cerebrospinal fluid, computed tomography or magnetic resonance imaging of the brain, and postmortem pathological examination.

Clinical variants. *Acute VEM* is characterized by prolonged fever (up to several months), meningeal signs, lymphocytic pleocytosis in the cerebrospinal fluid (CSF), and persistent neurological signs, including ophthalmoparesis, spastic tetraparesis and, in some cases, generalized tonic-clonic seizures. *Subacute VEM* develops in patients who have survived the acute phase of the disease. It

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is a slowly progressive neurological syndrome with dementia, dysphagia, dysarthria, spastic tetraparesis or paraparesis of the lower extremities, bradykinesia, postural instability and muscle stiffness. CSF pleocytosis with increased total protein concentration persists for months or years. Magnetic resonance imaging (MRI) shows signs of significant brain atrophy [5]. Patients usually die within one to six years as disease progresses.

In some patients, the disease stopped progressing at some point during the subacute phase; in others the chronic disease began gradually, without a recognized acute onset. Some such patients lived with chronic VEM for the next 20, 30, or even 40 years in a state of profound disability with cognitive impairment and difficulty speaking and walking. The VEM patient's appearance and behavior took on characteristic features: abnormal behavior with inappropriate laughter or tears, a hunched posture, slow movements. Residents of the affected villages call this condition "*bokhoror*" meaning "stiffness" in the Sakha language. Patients with chronic VEM eventually die, most often from trauma, pneumonia, or kidney failure.

The ratio between acute, subacute and chronic forms VEM changed during the epidemic; acute-onset cases predominated in the 1950s and 1960s [7, 8, 11]; in the 1970s, there were about the same numbers of cases with acute and insidious onsets of the disease. By the 1980s and 1990s, 80% of cases had insidious onset, developing directly into a chronic form of VEM [6].

In the CSF of patients with subacute and chronic VEM, oligoclonal immunoglobulins were detected by isoelectric focusing and subsequent immunoblotting with antibodies against human *IgG*, a technique used as a useful diagnostic test to differentiate VEM from non-inflammatory syndromes. Detection of *IgG* bands had a diagnostic sensitivity of 93% with a specificity of 80% [14]. Oligoclonal *IgG* bands persisted in CSF up to three decades after the onset of the disease.

Postmortem studies of VEM patients showed multiple micronecrotic lesions surrounded by T- and B-lymphocytes and reactive astrocytes in brains. Such lesions were present in the cerebral cortex, basal ganglia, cerebellum and brainstem; massive loss of neurons (by lysis) and dystrophic changes in the preserved nerve cells were found inside and outside the necrotic foci [17]. Small blood vessels are surrounded by inflammatory cuffs consisting of activated T-lymphocytes, natural killer cells, and killer-like cytotoxic T-lymphocytes [1]. With the transition to a chronic course, fibrous meninges and adhesions appeared, impeding the circulation of cerebrospinal fluid and leading to hydrocephalus and cerebral atrophy. Acute, subacute, and chronic VEM were clearly phases of the same underlying disease with differences corresponding to the rate of progression. Inflammatory changes or their consequences can be observed at each phase.

Thus, we have developed strict criteria for the diagnosis of VEM, excluded diseases of a different nature, and created an electronic database containing 356 cases that met the diagnostic criteria for definite VEM.

Statistical analysis. The average incidence rates of VEM were determined based on the number of new cases recognized within each decade: 1940-49, 1950-59, 1960-69, and so on. The population size was estimated by interpolating the census data of the Republic of Sakha (Yakutia) for 1949, 1954, 1959, 1970, 1979, 1989, 2002 and 2010. The territorial distribution of VEM was determined using Google Earth (http://earth.google.com/) by measuring the distance between the patient's place of residence and the epicenter (*Lake Mastakh* in the Viliuisk ulus). Student's t-test was used to determine the significance of differences between observed estimates of epidemiological parameters.

Results and discussion. During the course of this long-term study, we found that patients with definite VEM were almost all ethnic Sakha (Yakut), with the exception of six Evenk and eleven patients born of Sakha-Evenk marriages. All patients came from small villages. The ages at disease onset ranged from 11 to 68 years. The average age at disease onset increased from 30.2 (CI 27.5–33.0) years at the beginning of the epidemic to 37.1 (CI 35.1–39.1) years at the peak of the epidemic and remained at this level. The ratio of affected women to men changed from 2:1 in the 1950s and 1960s to about 1:1 in the following decades. The average incidence of VEM in the Viliuisk ulus reached the level of 286-840 cases per 100 thousand population per decade in 1940–1980. In other regions of the Republic of Sakha (Yakutia) taken together it was 14 to 37 per 100 thousand per decade. The peak incidence of VEM in the Viliuisk ulus occurred in 1954, while in other regions of the Republic it peaked in 1976 ($p < 0.05$). In the 1980s, VEM incidence began to decline first, and somewhat faster, in the other regions of the Republic of Sakha (Yakutia), followed later by a decline in the original endemic villages of Viliuisk ulus (Fig. 1). The decline continued into the 1990s and the 2000s.

Since 2012, no new cases of VEM have been reported in any part of the Republic. As of January 2020, only 24 patients from the VEM database remained alive, all in advanced stages of chronic VEM.

Trends in territorial spread. The total number of villages affected by VEM increased from four in the 1940s to 18 in the 1950s and 52 in the 1970s. During that time, the area of VEM distribution increased 15 times. After the discovery of very rich deposits of coal, metals, oil and natural gas in other parts of the Republic, people began to move from Viliui villages, further impoverished during the war, to industrial townships in Central Yakutia. VEM then appeared among local residents of Central Yakutia, most of whom had never been to Viliui. By the 1970s, people throughout almost the entire territory of Central Yakutia were affected (Fig. 2).

The circles delineate areas of concentration of 90% of new cases; the blue dots in the centers of the circles represent the geographic centers.

Mechanisms of transmission. P.A. Petrov in early studies [7] noted that in some affected families there were as many as five patients with VEM. We investigated this phenomenon. Complete family histories were obtained for 194 families of which 27 had more than one VEM case, two per family in 24 and three per family in three. Multiple cases of VEM in the same household were observed more often than expected based on random distribution for both genetically related family members ($p < 0.0001$), and adopted family members ($p < 0.001$). The median incubation time between the onset of disease in the original case and that in the secondary case was 14.1 years for genetically related and 4.6 years for adopted family members [13].

The sequence of events in these families supports the view that VEM was transmitted through prolonged intra-household contact with a patient manifesting the disease, sometimes passing to unrelated persons in non-endemic regions. However, the exact transmission mechanisms remain undetermined.

Prevention of further spread. Reliable records of hospitalization were available for 180 patients with diagnoses of definite VEM from 1965-1980. Of these, 66 patients were first hospitalized in the acute phase of the disease. Patients remained in the hospital during the entire period of the acute phase, showing manifestations of illness such as low-grade

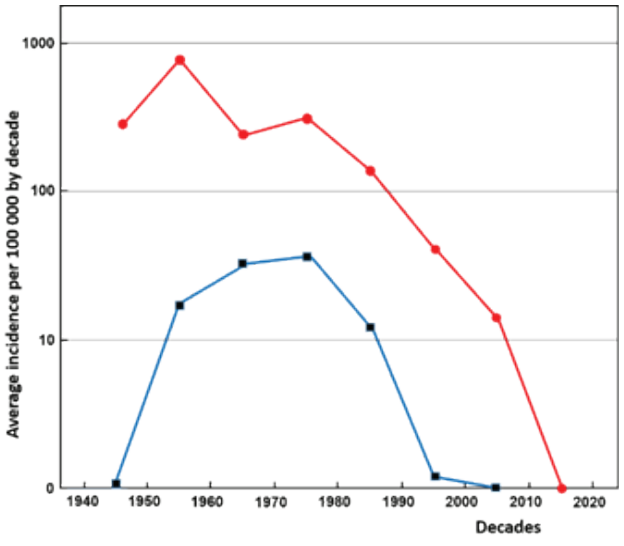

Fig. 1. The incidence of Viliuisk encephalomyelitis by decade as cases per 100 thousand inhabitants of the Viliuisk ulus (red curve) and other regions of the Republic of Sakha (Yakutia) combined (blue curve)

fever of 37.1-38°C and pleocytosis in the CSF exceeding 30 cells per 1 mm^3, evidence for an ongoing inflammatory process. The duration of the first hospitalization of patients in the acute phase of illness varied from 17 to 518 days, on average 114 days, comprising 80 to 100% of the total duration of the acute phase (Fig. 3, left panel).

Patients with *subacute VEM* repeatedly returned to the encephalitis ward to confirm the diagnosis, determine the rate of disease progression, adjust the treatment, and confirm disability. The total times patients with subacute VEM spent in hospitals ranged from 19 to 2074 days, on average 288 days, comprising 20% of the average total duration of the disease (Fig. 3, middle panel). In addition, many patients with subacute VEM, on average about 40% of them, depending on family circumstances, ended up in the Sosnovka Nursing Home (a selected special long-term care facility in the village of Sosnovka), Viliuisk ulus. Thus, patients with subacute VEM were in either a hospital or a skilled nursing home for an average of about 60% of the total duration of the disease.

Of the patients with *chronic VEM*, disabled people who had no other means of subsistence, more than 60% of the total time of their illness were kept in hospitals or remained in the Sosnovka Nursing Home (Fig. 3, right panel). Prolonged hospitalization and nursing home care helped avoid or postpone appearance of complications such as pneumonia or kidney failure, while keeping patients away from close contact with susceptible family members and neighbors.

Patients with subacute and chronic VEM who stayed with their families either temporarily or permanently were eligible for the highest level of disability insurance and were not required to work. A law passed in the mid-1960s limited contacts between patients and fellow villagers during summer fieldwork, when workers lived together in overcrowded agricultural camps away from home. In the 1980-1990s, life in Yakut villages became more comfortable; most residents began to build standard wooden houses. Improved living conditions led to fewer close contacts between VEM patients and vulnerable family and community members.

We conducted a retrospective socio-economic study of families in regions with a high incidence of VEM [2, 16]. Using a standard prepared questionnaire, we interviewed 30 patients with definite chronic VEM, 69 members of their families who were in long-term contact with patients, and 39 people living in the same settlements but not in physical contact with patients (control group). Patients with VEM were raised in families with an average of six children. Close relatives (grandparents, uncles and aunts) lived

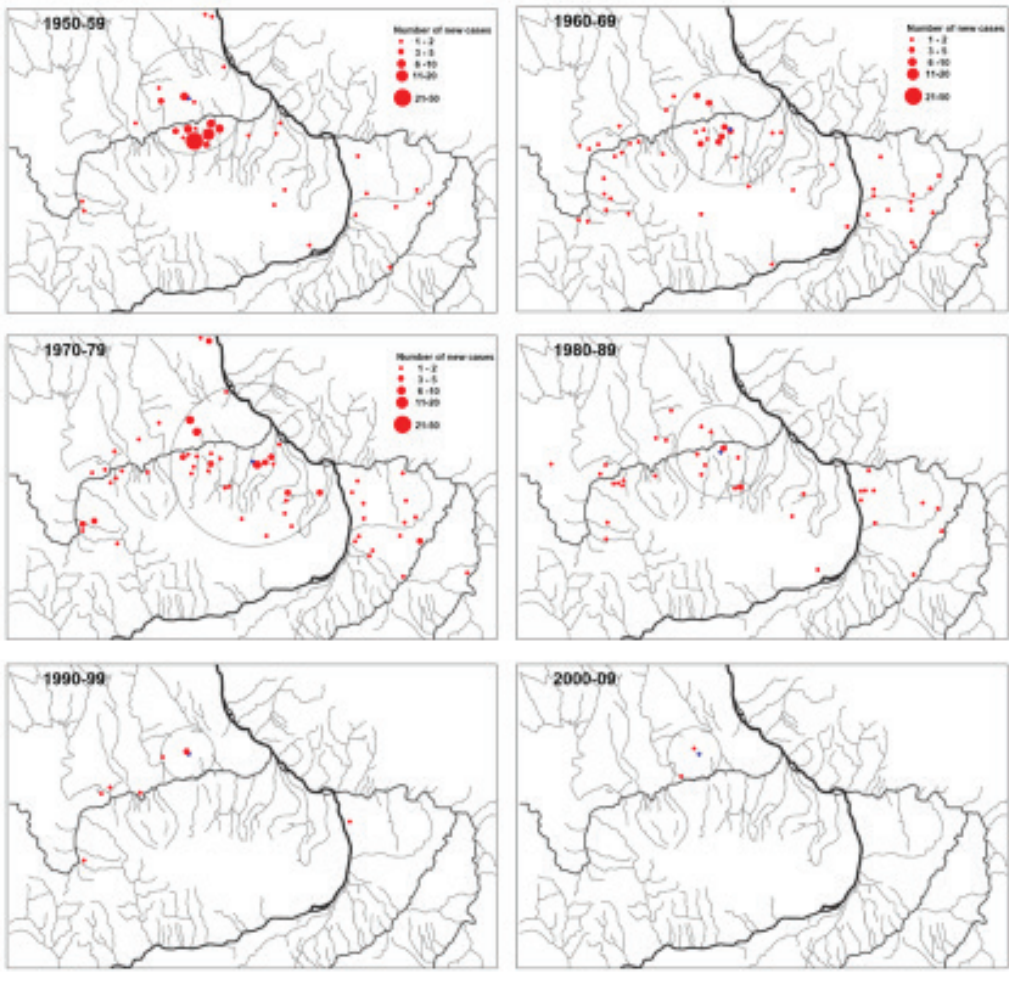

Fig. 2. Geographic location of settlements (red dots) in which new patients with Viliuisk encephalomyelitis were registered, by decades

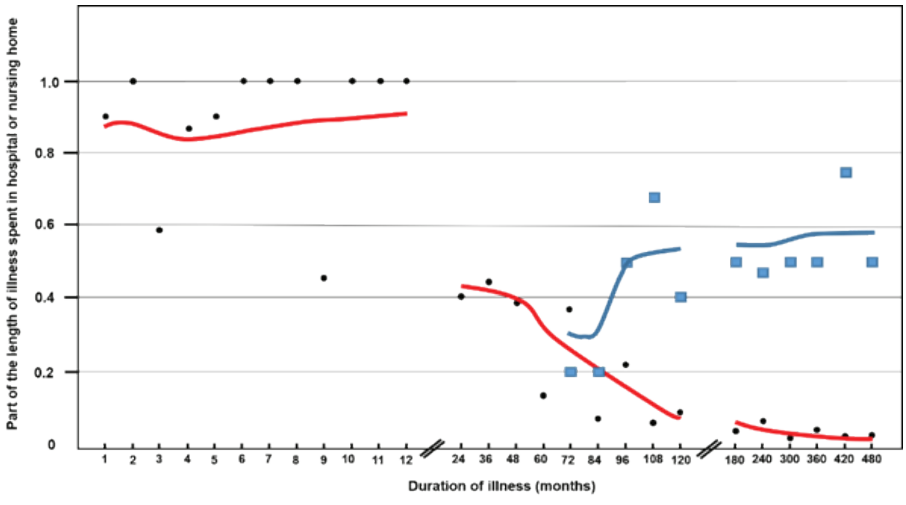

Fig. 3. Duration of hospitalization of patients with Viliuisk encephalomyelitis. Left panel: the curve represents the duration of hospitalization of VEM patients in the acute phase of illness in relation to the total length of the acute phase (n = 66). Middle panel: the red curve corresponds to the duration of hospitalization of patients with subacute VEM in specialized hospitals (including follow-up admissions); the blue curve reflects the length of stay in the Sosnovka Nursing Home in relation to the total length of their illness (n = 66). Right panel: the red curve shows the time patients with chronic VEM spent in specialized hospitals (including follow-up admissions); the blue curve represents the length of stay in the nursing home in relation to the total duration of illness (n = 111). Notes:1. Some patients who survived the acute phase of VEM who went on to develop subacute or chronic VEM status may have been counted more than once. 2. The dots mark the duration of hospitalization; the squares – the length of stay in the Sosnovka Nursing Home.

in the same household with the patient in 42% of cases but in only 29% of control families (p <0.05). Thirty-two percent of patients with VEM lived in a room of a house belonging to another family, in the same room with another family, or in a dormitory room, compared with only 13% of control families (p <0.01). In remote villages of the Viliuisk ulus, where the highest incidence of VEM was observed, people lived in houses that were much smaller than those in villages near the city of Viliuisk (p = 0.03). In the 1950s, there was only one public bath in the entire Viliuisk ulus; by the 1970s, 23 public baths were registered, all of them open only in the winter and then operating only two or three times a week. In the 1990s, the number of baths increased several times and 40% of them were private. By 2007, 42% of patients with VEM versus 92% of control families lived in dwellings with private baths (p <0.025).

Over the past eight decades, the Republic of Sakha (Yakutia) has made significant progress in its healthcare sector. Smallpox was eradicated in 1937, typhoid fever in 1938, malaria and trachoma by the late 1950s, poliomyelitis in the early 1960s, and leprosy in 1969. The incidence of diphtheria has become insignificant, and the incidence of measles has decreased by 7.5 times. The availability of health care in settlements of all sizes has grown.

Conclusion. Although the etiology of VEM, its origin and mechanisms of transmission remain poorly understood, available data characterize VEM as a transmissible disease that broke through the confines of a geographically isolated indigenous population living around *Lake Mastakh* to cause an epidemic of a severe neurological disease involving hundreds of victims. The aggregation of cases in households and small villages suggests that VEM was likely to have been transmitted through prolonged close intra-household contact, the same mechanism involved in transmission of other chronic infectious diseases such as tuberculosis and leprosy. There is a suspicion that the causative agent of VEM probably circulated in nature before it acquired the ability to infect people and pass from person to person [9]. Persistent efforts to reduce close contacts between patients with acute and subacute VEM and the surrounding population was followed by a marked decrease in incidence of VEM, which slowly declined in the 1980s and 1990s and finally reached zero in the 2010s. No new cases of VEM have been reported since 2012. Socio-demographic changes in the Republic of Sakha (Yakutia) increased the standard of living, modernized traditional lifestyle, and improved nutrition, probably also contributing to the elimination of VEM. Taken together, these efforts might well have prevented yet another emerging infectious disease from spreading globally. Since there is no certainty that the epidemic will not resume under some unknown conditions, it would be prudent to continue researching the collected materials and to maintain the qualifications of the doctors responsible for diagnosing VEM.

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MAIN TRENDS OF HIV MORBIDITY, DISABILITY AND MORTALITY IN THE IRKUTSK REGION

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Purpose of the study: assessment of the trend of changes in the incidence of HIV infection, mortality from HIV infection, as well as disability of the adult population from HIV infection in a region with a high prevalence of the population.

Materials and methods of research. A retrospective descriptive-epidemiological study was carried out using continuous samples of statistical data for the Irkutsk region for 2010-2019. The pairwise Spearman correlation coefficient was used to assess the degree of relationship between mortality and HIV incidence over the years. Statistical processing of the results was carried out using the MSExcel 2010 package. Spearman's correlation coefficient, regression equations and χ^2 were calculated using the EpiInfo program.

Results and discussion. In the Irkutsk region, since 2018, there has been a decrease in the incidence of HIV infection in the total population. In recent years, the main risk group is represented by the age group of 30-39 years; the role of women and the heterosexual transmission pathway has significantly increased. Against the background of a decrease in the total mortality of the population from 1544.2 per 100 thousand in 2000 to 1317.7 in 2019 ($p<0.01$), the mortality rate for the class "Some infectious and parasitic diseases" for the analyzed period, on the contrary, increased; the increase was 155.3%. The dynamics of the death rate from HIV infection had a steady upward trend, the Tpr. amounted to 6.31%. During the follow-up period, the rate of primary disability of the adult population due to HIV infection increased by 2.2 times from 1.3 to 2.9 per 10,000 population. Among the subjects of the Russian Federation, the Irkutsk Region ranked first in terms of primary disability of the adult population due to HIV infection in 2010 and second in 2019. Among the adult population recognized as disabled for the first time due to HIV infection, people of working age predominate – 98.6% in 2019.

Conclusion: Despite the decrease in the incidence of HIV infection, the stabilization of the mortality rate from HIV infection and disability of the population due to HIV infection in the Irkutsk region, these indicators significantly exceed the national ones, which indicates an unfavorable situation in general and requires additional measures to optimize preventive programs and epidemiological supervision.

Keywords: HIV infection, morbidity, mortality, disability.

Introduction. The HIV pandemic remains the world's leading global problem. The social and economic significance of HIV infection is due to a number of indicators: a high incidence and prevalence rate, a long course of the disease, damage to a predominantly working-age population, and, despite the success of antiretroviral therapy, a high mortality rate [2, 8]. In the Russian Federation, the decline in the incidence of HIV infection continued in 2019. Against the background of increasing testing of the population, the incidence rate was 66.2 per 100,000 population. HIV is actively spreading in the general population, more than half of the patients diagnosed for the first time in 2019 were infected during sexual intercourse (63.9%). In recent years, age groups at risk are represented by persons 30–35 years of age and older [11]. Against the background of an increase in the total number of HIV-infected patients, the number of deaths from this disease is also increasing. Accordingly, the share of HIV infection in the mortality structure of the population of the Russian Federation is increasing. It is HIV infection that today is the leading cause of death of young people from infectious and parasitic diseases. Recently, HIV infection is increasingly the cause of primary disability in the adult population [5, 6].

For a number of years, the Irkutsk region has been one of the regions, unfavorable in terms of the incidence and prevalence of HIV-infected population. Mortality rates from HIV infection and disability from HIV infection in the region exceed those in the Russian Federation [1, 3, 4].

Purpose of the study: assessment of the trend of changes in the incidence of HIV infection, mortality from HIV infection, as well as disability of the adult population from HIV infection in a region with a high prevalence of the population.

Materials and methods of research. A retrospective epidemiological analysis was carried out according to the data of the state statistical reporting forms: No. 2, 7-social security and C52 for 2010-2019, statistical collections "The main indicators of primary disability in the adult population in the Russian Federation" Center for the Fight against AIDS FBUN "Central Research Institute of Epidemiology" Rospotrebnadzor RF (http://www.hivrussia.info).

Assessment of the relationship of indicators is calculated using the pairwise Spearman correlation coefficient. Statistical processing of the results was carried out using the MSExcel 2010 package. Spearman's correlation coefficient, regression equations and χ^2 were calculated using the EpiInfo program.

Results and discussion. For a long time, the Russian Federation was one of the territories where the increase in the incidence of HIV continued. Since 2018, the number of newly diagnosed cases of diseases has stabilized [6, 8].

In the Irkutsk region, HIV infection began to be registered in the early 1990s. In 1999, an epidemic rise in the incidence was registered; in 2000, the number of registered new cases was the maximum [10]. Until 2017, the incidence of HIV infection tends to grow, the average annual growth rate was 2.16%. Since 2018, there has been a decrease in the incidence rate, as well as in the Russian

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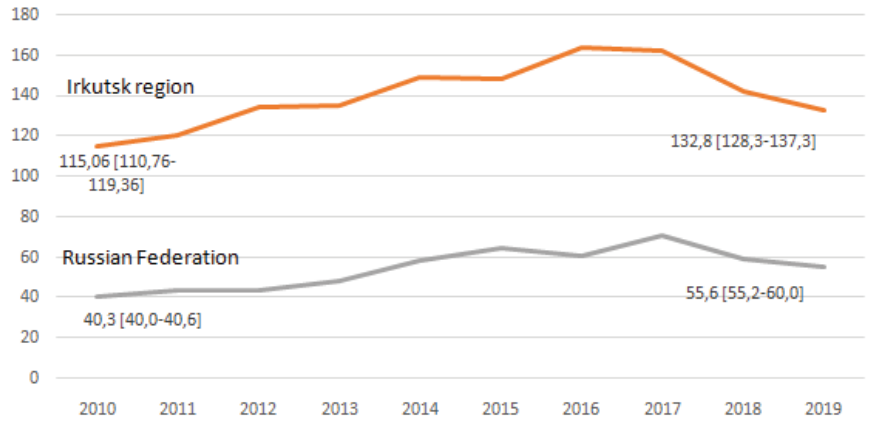

Fig. 1. The incidence of HIV infection among the population of the Irkutsk region and the Russian Federation in dynamics for 2010-2019. (per 100 thousand population)

Federation as a whole. For the analyzed period, the incidence rates in the Irkutsk region are significantly higher than the average for Russia (Fig. 1).

The structure of the incidence of HIV infection on the scale of the epidemic has undergone significant changes: an increase in the proportion of women, the proportion of older age groups and heterosexual transmission.

Infectious diseases are still one of the main causes of death, and to the extent this is due to HIV infection. The spread of HIV infection has a significant impact on the level and nature of mortality in the population. In the Russian Federation, the mortality rate is assessed by a number of authors as unfavorable [6, 8, 9].

In the Irkutsk region, since the beginning of registration of HIV infection, the mortality rate in the class "Some infectious and parasitic diseases" has increased, the increase was 155.3%. These changes took place against the background of a decrease in overall mortality. There was a marked positive correlation with the prevalence of HIV infection, r = 0.83, p <0.01.

When comparing the mortality rates of the population from infectious and parasitic diseases with mortality from HIV infection, a relationship between these indicators was noted, r = 0.81, p <0.01.

In total, more than 30 thousand people died in the region in 2019, 995 of them from HIV infection, which amounted to 3.1% of the total number of deaths and 60.0% of the number of deaths from infectious and parasitic infections (Table 1).

During the analyzed period, the dynamics of the mortality rate from HIV infection in the Irkutsk region, as well as in the Russian Federation in relation to growth trends [12], an increase of 6.31% and 5.29%, respectively. Over the past three years (2017-2019), the mortality rate in the Russian Federation has stabilized. In the Irkutsk region in 2019, there was a slight decrease in the mortality rate, the average annual rate of decline was -1.83%. The indicators of the HIV epidemic in the region are higher than the average for Russia (Fig. 2). In 2019, the region in terms of mortality from HIV infection was ranked II among 85 subjects with an indicator of 41.6 (per 100 thousand population), exceeding the national level by 3 times.

By 2024, the Government has set the task of reducing mortality rates [7], however, in the structure of mortality, the share of HIV infection is increasing and is the leading cause of death in people of working age [6, 8, 12, 13].

Among the constituent entities of the Russian Federation, the Irkutsk Region took 1st place in terms of the level of primary disability of the adult population due to HIV infection in 2010 and II - in 2019 (after the Sverdlovsk Region). During the observation period, the indicator of primary disability of the adult population due to HIV infection in the Irkutsk region increased 2.2 times from 1.3 to 2.9 per 10,000 population, and since 2016 this indicator has stabilized for the period 2016-2018. the maximum level was recorded - 3.2 per 10,000 population with a subsequent decrease in 2019. In the Russian Federation, the level of primary disability continues to grow, the average annual growth rate was 6.23%. The level of primary disability of the adult population in the region was higher than the national average (Fig. 3).

Among the adult population, for the first time recognized as disabled due to "HIV infection", persons of working age prevail - 98.6% in 2019 (of which 18-

Table 1

Causes of death of the population of the Irkutsk region in 2000 and 2019 (according to Irkutskstat data)

Cause of death	2000 г.			2019 г.			χ^2	p
	absolute number	%	per 100 thousand	absolute number	%	absolute number		
All causes	40829	100	1544.2	31553	100	1317.7	449.9	<0.001
Infectious and parasitic diseases	1112	2.7	42.1	1657	5.2	65.4	285.5	<0.001
HIV infection	1	0.002	0.036	995	3.1	41.5	1262.1	<0.001

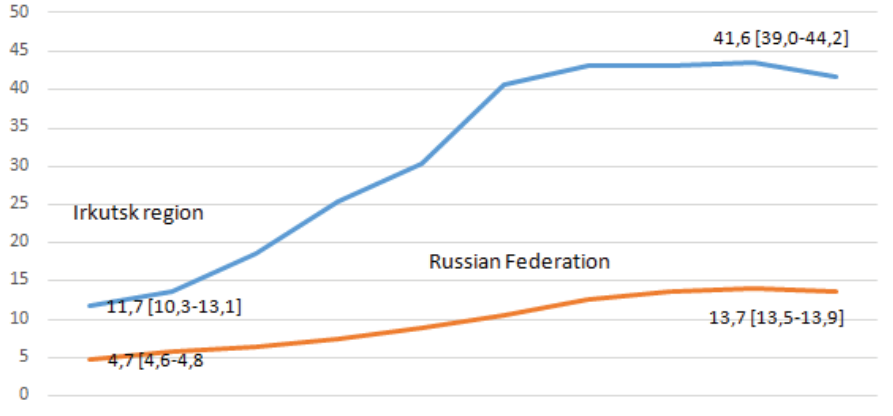

Fig. 2. Mortality from HIV infection of the entire population of the Irkutsk region and the Russian Federation in dynamics for 2010-2019. (per 100 thousand population)

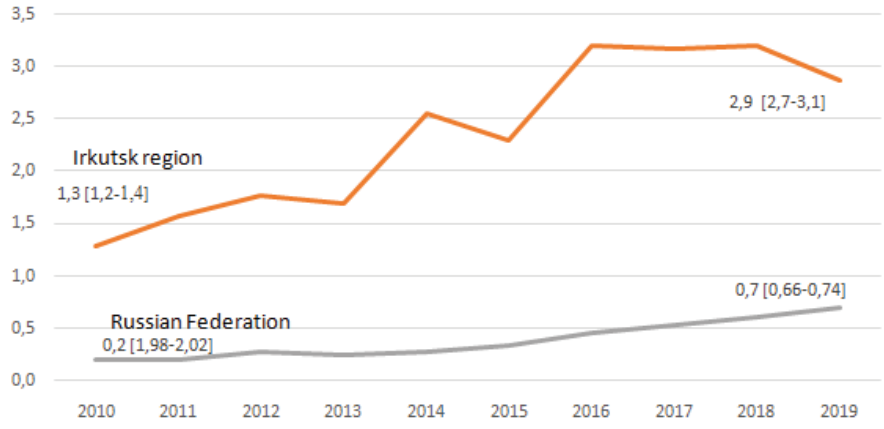

Fig. 3. The level of primary disability due to HIV infection among the adult population of the Irkutsk region and the Russian Federation in dynamics for 2010-2019. (per 10 thousand adults)

44 years - 87.6%). During the analyzed period, the indicators of primary disability among the population aged 18-44 increased from 2.2 to 4.7 per 10,000 people, among women 45-54 years old and men 45-59 years old - from 0.4 to 2.5 per 10,000. 10,000 people. (Table 2). The registered age indicators of primary disability due to HIV infection in the region exceed those in the Russian Federation.

In the structure of the contingent, for the first time recognized as disabled due to HIV infection during the observation period, disabled people of group II prevail (Table 3). the share of disabled persons of group I in the Irkutsk region practically did not change, and in the Russian Federation increased from 9.9 to 14.8%.

Conclusion: Despite the decrease in the incidence of HIV infection, the stabilization of the mortality rate from HIV infection and disability of the population due to HIV infection in the Irkutsk region, these indicators significantly exceed the national ones, which indicates an unfavorable situation in general and requires additional measures to optimize preventive programs and epidemiological supervision:

1. Increase in population screening for antibodies to HIV, including those not belonging to key groups.
2. Timely identification and treatment of secondary and concomitant diseases that are the causes of death and early disability.
3. Increase in funding for prevention programs (including information and educational activities) among different age groups of the population.

Table 2

Primary disability of the adult population of the Irkutsk region and the Russian Federation from diseases caused by HIV, taking into account age (absolute number, level per 10 thousand people of the corresponding age)

Years	Territory	Indicators by age group					
		absolute number			indicator for 10 thousand		
			45-54(f) и 45-59(m)	ст.55(f) и ст.60(m)	18-44	45-54(f) и 45-59(m)	ст.55(f) и ст.60(m)
2010	Irkutsk region	228	17	0	2.2	0.4	0.0
	Russian Federation	1835	231	29	0.3	0.1	0.0
2019	Irkutsk region	426	90	6	4.7	2.5	0.1
	Russian Federation	6211	1564	154	1.1	0.7	0.0

Table 3

Primary disability of the population of the Irkutsk region and the Russian Federation due to diseases caused by HIV, taking into account the disability group (absolute number,%)

Years	Territory	Indicators by disability group					
		absolute number			%		
		I group	II group	III group	I group	II group	III group
2010	Irkutsk region	24	158	63	9.8	64.5	25.7
	Russian Federation	207	1050	838	9.9	50.1	40.0
2019	Irkutsk region	50	310	162	9.6	59.4	31.0
	Russian Federation	1176	4079	2674	14.8	51.4	33.7

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EVALUATION OF PHYSICAL DEVELOPMENT OF CHILDREN AND ADOLESCENTS OF DIFFERENT ETHICAL GROUPS, LIVING IN THE REPUBLIC OF SAKHA (YAKUTIA)

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To justify the use of regional standards for the physical development of children living in the Republic of Sakha (Yakutia) on the basis of their ethnic predisposition the data were analysed obtained during preventive examinations in 2019-2020 in the territory of the republic. The evaluation of the physical development of children aged 3 months up to 17 years was conducted as well as the comparative analysis of the compliance of centile tables CDC, WHO and regional normative scales of physical development. It was found that the regional compliance standards most adequately reflect indicators of the physical development of children in the studied populations.

Keywords: physical development, centile corridor, anthropometry, Sakha, indigenous peoples of the north (ipotn), Russians.

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Introduction. As it is known, the physical development of a child is understood as the dynamic process of growth (increase in body length and weight, development of organs and body systems) and biological maturation of the child as he grows up. The physical development of children is tightly connected with cognitive and social development, and is one of the indications of social wellness of the population [1,5,6].

Negative factors that affect the prenatal period and early childhood can disrupt the sequence of growth and development of the body, in some cases causing irreversible changes [4].

External factors, such as nutritional conditions, upbringing, the presence of diseases, social and other factors during the period of intensive growth and development of a child, can have a great influence on the characteristics of physical development [5,7]. The share of physical development, inherited and acquired in programming, has been the subject of discussion over the past several decades. [8,9,10,11,12]. The variability of the share of inherited factors in the determinants of the final growth of an individual is 45 - 85%, and this share is largely determined by the assessment method and is associated, in particular, with race and ethnicity [11].

In recent years, a tendency towards disharmonious development of children has been observed everywhere, mainly due to the widespread prevalence of both overweight and underweight [3,5].

The known polygenicity of the peoples inhabiting the Republic of Sakha (Yakutia), and the lifestyle of the indigenous population formed in the course of biocultural adaptation to harsh climatic conditions, suggest the presence of a peculiarity of the anthropometric characteristics of the population, which are

the result of adaptive processes [2].

The purpose of this work is to study the features of the physical development of children and adolescents in the Republic of Sakha (Yakutia).

Materials and methods. The physical development of 1099 children was assessed, including 611 girls and 488 boys aged from 3 months to 17 years (Slavs - mostly Russians - 241, Sakha - 498, Evens - 155, Evenks - 111, Yukaghirs - 21, Dolgans - 73) surveyed in Churapchinsky, Allaikhovsky, Anabarsky, Bulunsky and Verkhnekolymsky regions of the Republic of Sakha (Yakutia).

The procedure for assessing the dynamics of the physical development of children included:

1. Measurement of body length (height) and body weight using current recommendations.

2. Calculation of body weight by body length/height, or BMI, according to well-known formulas.

3. Assessment of each recorded value for body weight, length/height, bodyweight for height, body mass index, taking into account age and sex:

A) according to Centers for Disease Control and Prevention standards for children 2 years and older living in the United States (CDC, Centers for Disease Control and Prevention) [13];

B) according to the standards of the World Health Organization (WHO) for children under 2 years of age [14];

C) according to regional standards developed in 2017 to assess the age dynamics of the physical development of children aged from birth to 17 years old living in the Republic of Sakha (Yakutia) with additional consideration of ethnicity [2].

To select adequate standards for assessing the dynamics of the physical development of children, living in the Republic of Sakha (Yakutia), we formed a comparative analysis of the correspondence of the use of the CDC and WHO centile scales, as well as regional normative scales of physical development, taking into account the ethnicity of children.

Additionally, demographic indicators and peculiarities of the organization of medical care in the surveyed areas were analyzed according to the official reporting data.

Database maintenance and statistical analysis were carried out using the software packages "Excel", "Statistica for Windows".

Results. The results are presented in the form of tables describing the proportion of the surveyed children (% of their total number and % of the number of children of the corresponding ethnic group) falling into the normative corridors of the standard. Full compliance with the standard implies the correspondence of the calculated numbers in the cells of the table with the values of the centile cut-off values for the corresponding age and gender.

Table 1 shows the proportion (%) of the examined children who fall into the CDC centile corridors of age body weight.

As can be seen from the data presented in Table 1, the assessment of the conformity of the bodyweight of the children examined by us with the CDC standards leads to the conclusion that there is a tendency towards bimodal distribution for some ethnic groups and that, in general, the cut-off curves are shifted to the region of small values. With the exception of the Yukaghirs, Dolgan and, to a lesser extent, Sakha, small body weights prevail in the studied subpopulations. Some correspondence can be noted for values corresponding to 75 centile and above.

Table 2 shows the proportion (%) of the surveyed children who fall into the CDC centile corridors of height/body length according to age.

Analysis of the data presented in Table 2 also leads to the conclusion that the cut-off values are shifted to the area of low values for all ethnic groups, with the exception of the Yukaghirs.

Table 3 shows the centile corridors for body weight by height / length according to the CDC standards and the proportion of children examined who fall into the corresponding centile corridors.

As can be seen from the data presented in Table 4, when assessing the dynamics of the physical development of a child under the age of 2 years using WHO standards, small values of height / body length in the studied subpopulations prevail (extreme values are 4 times more common).

A more detailed interpretation of the description of compliance with WHO standards and the possibility of their use is difficult.

Table 5 presents the data of the examined children (%) used in the corresponding centile body mass corridors in terms of regional analytical indicators of the development of the Republic of Sakha (Yakutia).

Analysis of the data presented in Table

Table 1

Proportion of examined children meeting CDC weight standards

Proportion (%) of children falling into the corridor	Ethnic group	Centiles, %						
		5	**10**	**25**	**50**	**75**	**90**	**95**
	by body weight							
	In total	9.2	15.4	33.0	58.9	78.8	91.5	95.0
	sakha	7.7	14.7	32.3	59.8	81.2	94.1	96.0
	evens	15.5	21.9	40.6	61.9	80.0	89.7	92.3
	evenki	12.6	19.8	35.1	60.4	83.8	88.3	95.5
	yukaghirs	0	4.8	33.3	42.9	66.7	81.0	95.2
	dolgans	5.5	12.7	28.2	57.5	83.6	93.2	97.3
	russians	10.1	13.0	29.2	57.9	72.2	88.9	93.5
	by height/body length							
	In total	12.0	19.4	40.1	66.2	84.4	94.0	96.4
	sakha	10.3	18.2	41.4	68.9	87.3	95.6	96.8
	evens	15.5	26.5	41.9	68.4	82.6	92.3	92.9
	evenki	20.7	30.6	51.4	77.5	89.2	99.1	100
	yukaghirs	0	5.8	23.8	47.6	66.7	90.5	95.2
	dolgans	13.7	19.2	45.2	71.2	84.9	94.5	98.6
	russians	10.2	14.4	30.6	54.6	78.7	89.4	95.0
	by body weight by height/body length							
	In total	9.5	12.6	23.8	40.0	61.1	78.7	85.2
	sakha	6.8	10.2	22.6	37.9	62.1	78.5	85.3
	indigenous peoples of the north	14.8	16.5	23.5	40.0	62.4	81.2	84.7
	russians	9.1	13.6	26.1	44.3	59.1	77.3	85.2

Table 2

Proportion of surveyed children meeting the WHO standards in terms of body weight, height/length, body weight by height/length

	Parameter	Centiles, %						
		5	10	25	50	75	90	95
Proportion (%) of children falling into the corridor	Body mass	4.3	5.8	10.1	23.2	47.8	68.1	78.3
	Length/height	20.0	22.9	35.7	44.3	67.1	81.4	87.1
	Weight by height/length	5.7	7.1	11.4	20.0	37.1	51.4	60.0

Table 3

Proportion of examined children meeting regional weight standards

	Ethnic group	Centiles, %						
		5	10	25	50	75	90	95
	по массе тела							
Proportion (%) of children falling into the corridor	In total	6.9	15.7	35.9	59.6	77.4	88.3	92.4
	sakha	6.6	16.0	38.9	61.3	80.7	90.8	94.1
	indigenous peoples of the north	6.4	16.8	30.7	55.0	72.1	84.1	88.5
	russians	8.5	13.7	37.6	63.2	78.6	89.7	94.9
	по росту/длине тела							
	In total	6.0	13.5	30.9	58.5	80.4	89.6	94.5
	sakha	5.5	12.3	30.1	59.0	83.0	91.6	95.5
	indigenous peoples of the north	5.6	13.7	29.3	55	74.3	84.9	91.6
	russians	7.7	15.8	35.0	62.4	84.1	92.7	97.0

5 indicates that regional standards better reflect the distribution of body weight values in the studied population. It should be noted that, while the proportion of children falling into a diagnostically significant corridor (5%) is quite satisfactory, there is a tendency for the curves corresponding to 10 and 25 centiles to shift to the region of small values.

Table 6 shows the centile corridors for height/body length in terms of regional standards for assessing the physical development of children in the Republic of Sakha (Yakutia) and the proportion of the examined children falling into the corresponding centile corridors.

Analysis of the data presented in Table 6 indicates that regional standards quite accurately reflect the distribution of height/body length values in the studied population, which indicates that, in general, the dynamics of the physical development of children in the studied areas is average for the corresponding ethnic group of children, living in the Republic of Sakha (Yakutia).

Thus, the use of regional standards of physical development of children that takes ethnicity into the account can evidently be recommended for practical use in mathematical modelling in the course of describing the impact of industrialization on the child population of the Republic of Sakha (Yakutia).

A preliminary analysis of the age dynamics of the height and weight of children in populations of different social composition, ethnicity and occupation confirms the need to take these factors into account when modelling the effects of industrialization. It is also evident that it is necessary to compare the dynamics of anthropometric indicators with the standards developed for individual ethnic groups living in the Republic of Sakha (Yakutia) since the lifestyle and diet in the villages located in the circumpolar and polar regions are largely determined by the ethnic characteristics of the subpopulations.

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PREVALENCE OF THE EAST ASIAN CAGA AND WESTERN CAGA GENES OF HELICOBACTER PYLORY IN YAKUTIA

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There are several alleles of the *cagA* gene, in which a change in the carboxyl end of its protein is the main distinguishing feature between different alleles. Polymorphisms at the C-terminus occur in the so-called EPIYA region and usually involve changes in amino acid sequences flanking repetitive five amino acid sequences – Glu-Pro-Ile-Tyr-Ala. In Yakutia, the estimation of prevalence of the East Asian *cagA* and Western *cagA* genes in *Helicobacter pylori* strains has not been studied. The aim of this work is to analysis of the prevalence of the Western *cagA* and East Asian *cagA* genes among *Helicobacter pylori* strains in Yakutia. The study sample consisted of 30 *Helicobacter pylori* DNA samples isolated from biopsy specimens of patients with gastroduodenal diseases. As a result of the endoscopic and histological examination, the presence of *Helicobacter pylori* infection was confirmed in 30 patients. The Western *cagA* was identified in 12 of 30 samples (40%). In 18 samples (60%) the *cagA* gene variant was not identified, since it did not belong to either the Western *cagA* or the East Asian *cagA*. The analysis of the prevalence of East Asian and Western *cagA* among *Helicobacter pylori* strains in Yakutia revealed that the prevailing variant of the *cagA* gene is the Western *cagA* and the East Asian *cagA* was not found in none of the samples. In 60% of cases, the samples did not belong to either the Western or the East Asian *cagA* gene, which may indicate the presence of their own *cagA* gene sequence among *Helicobacter pylori* strains in Yakutia.

Key words: *Helicobacter pylori,* East Asian *cagA,* Western *cagA*, EPIYA motif, Yakutia

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Introduction. *Helicobacter pylori* (*H. pylori*) – is a gram-negative, spiral-shaped bacterium that colonizes the gastric mucosa of the human stomach and duodenum, causing various gastroduodenal diseases (chronic gastritis, gastric and duodenal ulcers, MALT lymphoma, and stomach cancer) [5, 20]. The family is the basic unit of transmission for *H. pylori*. Children often become infected with a strain that is genetically identical to one of the parent's strain [23]. Consequently, transmission is likely to occur more frequently in the family or among infants. Once the infection has entered the human body, subsequent infection with other strains of *H. pylori* becomes unlikely [11]. According to some estimates, more than half of the world's population is infected with *H. pylori. H. pylori* infection is often not clinically apparent [19]. Only a certain part of the infected (20%) over time develop clinically significant symptoms of the disease: chronic gastritis, gastric and duodenal ulcer, stomach cancer [1, 24].

H. pylori is genetically more diverse than other types of bacteria. The characteristics of DNA samples and the sequence of any different DNA fragments almost always differ between independent pairs of isolates, and a comparison of the genomes of two strains in one study showed that 7% of genes are specific for each strain [23]. *H. pylori* DNA sequences can be used to distinguish between closely related human populations and are superior in this respect to classical human genetic markers [13, 28].

There are several alleles of the *cagA* gene, in which a change in the carboxyl end of its protein is the main distinguishing feature between different alleles. Polymorphisms at the C-terminus occur in the so-called EPIYA region and usually involve changes in amino acid sequences flanking repeated amino acid sequences – Glu-Pro-Ile-Tyr-Ala. In 2006, based on the deep sequence of the *cagA* gene was built a phylogenetic tree, and it was noted that the most spread motifs are EPIYA -A, -B, -C and -D and are in two different combinations by geographic location [4, 6, 12, 17]. The combination of EPIYA-A, -B and -C motifs (identified up to five motifs EPIYA-C) belongs to the Western *cagA*, while the combination of EPIYA-A, -B and -D motifs belongs to the East Asian *cagA* [6, 8, 9, 22]. The EPIYA-C motif is found everywhere (Iran, India, Kazakhstan, Greece, Italy, Sweden, Ireland, USA, Costa Rica, and Colombia) [3], as well as from 8 to 40% in samples of Southeast Asian countries (Japan, China, Korea, Thailand and Malaysia) [4]. The EPIYA-D motifs were clustered separately from strains isolated in Europe and found only in the countries of Southeast Asia (Malaysia, Vietnam, Thailand, Korea, China, and southern Japan) [14, 25, 29]. Subsequently, it was noted that both East Asian *cagA* and Western *cagA* circulate in Southeast Asia [26, 27, 30]. Interesting, that Truong et al., in 2009 among the inhabitants of the Okinawa island, Japan, there were found *H. pylori* strains with the motif of the *cagA* gene is very similar to the "Western *cagA*" [14], but it forms separate isolated cluster, that locate between two branches on the phylogenetic tree – Western CagA и East Asian CagA [14]. Thus, authors

conclude, that there is Japanese subtype of the Western CagA from an enclave of populations from the Okinawa island (J-Western *cagA* subtype) [14, 19].

In Yakutia, the estimation of the frequency of the East Asian *cagA* and Western *cagA* genes in *Helicobacter pylori* strains has not been performed. The aim of this work is to analysis of the frequencies of the Western *cagA* and East Asian *cagA* gene strains of *Helicobacter pylori* in Yakutia.

Materials and methods. The study sample consisted of 30 *H. pylori* DNA samples isolated from biopsy specimens of patients with gastroduodenal diseases. The diagnosis was confirmed by histological and cytological methods in the endoscopy department of the Republican Hospital No. 1 - National Center of Medicine of the Ministry of Health of the Republic of Sakha (Yakutia). Among the studied patients 12 were women (40%), 18 were men (60%). Distribution by age – 21 patients were children and adolescents (from 8 to 17), 9 were adults (21-57 years old), the median age was 19.16 years. Among 30 patients 26 were Yakuts (86.6%), 2 were Russians (6.6%), 1 were Yukaghir (3.3%) and in one patient (3.3%) nationality was not established.

Genomic DNA of *H. pylori* was isolated from frozen gastrobiopsies of the examined patients by using phenol-chloroform extraction [16]. Fibrogastroduodenoscopy was performed in the morning on an empty stomach. Biopsy specimens were taken from the antrum in amount of 2-3 pieces during endoscopic examination using a GIF-P3 fiberscope ("Olympus", Japan). Obtained biopsies of the gastric mucosa were fixed in 10% formalin solution. Dewaxing of sections and staining with hematoxylin and eosin were carried out according to the standard technique. For targeted bacterioscopy, sections are stained according to the Romanovsky-Giemsa method. The study was performed under magnification x100, x400 and x1000 on microscope "Axioskop" ("Opton", Germany). The morphological criteria of chronic gastritis were assessed in accordance with the visual analogue scale according to the modified Sydney system (Houston, USA, 1996).

The genomic DNA of *H. pylori* was isolated from frozen gastrobiopsy specimens from patients with confirmed histological diagnosis of chronic gastritis and chronic gastritis with erosions and ulcers using phenol-chloroform extraction. To perform genotyping of the DNA fragments of the *H. pylori* East Asian *cagA* and Western *cagA*, oligonucleotide primer sequences were used that flanking required marker regions of this gene (Table 1). Visualization of PCR products was carried out using a gel-video documentation device ("Bio-Rad") using Image Lab ™ Software.

Sequences of oligonucleotide primers for two variants of the cagA gene: East Asian cagA and Western cagA of H. pylori

Gene	Sequences of oligonucleotide primers	Size (b.p.)
East Asian *cagA*	F: 5'- AAAGGAGTGGGCGGTTTCA-3' R: 5'- CCTGCTTGATTTGCCTCATCA-3'	91
Western *cagA*	F:5'- AGGCATGATAAAGTTGATGAT-3' R:5'- AAAGGTCCGCCGAGATCA-3'	88

The surveys, provided by the framework of research work, were carried out strictly after the informed consent of participants, parents or legal representatives of minor patients. This study was approved by the local committee on biomedical ethics of the Yakutsk Scientific Center for Complex Medical Problems. Protocol No. 41 of November 12, 2015. Decision No. 5

Results and discussion. In the course of the endoscopic and histological examination, the presence of *H. pylori* infection was confirmed in 30 patients. Further, a molecular genetic analysis of the prevalence of *H. pylori* East Asian and Western *cagA* gene circulating in Yakutia was carried out. The East Asian *cagA* variant was not detected among 30 *H. pylori* DNA samples. The Western *cagA* was identified in 12 out of 30 samples (40%) (Fig. 1). 18 samples (60%) did not belong to either the Western *cagA* or the East Asian *cagA*.

It is known that on the basis of the amino acid sequence of the repeated fragments of the EPIYA motif in the *cagA* gene, it is possible to estimate the geographical origin of the *H. pylori* studied strain. Repeated EPIYA-D motifs in the *cagA* gene were not detected in our samples, that indicates the absence of the East Asian *cagA* in studied sample. In our study, it was noted that *H. pylori* strains with Western *cagA* dominate in Yakutia, since have repeating EPIYA-C motifs.

It is known from previously published works that in almost all countries, except the countries of Southeast Asia, the Western *cagA* gene variant is the predominant with EPIYA-C motifs (Canada – 95%, USA – 98%, Mexico – 73.8%, Colombia – 83, 7%, France – 95%, Italy – 100%, Greece – 74.8%, Iran – 88.1%, Mongolia – 79.6%) [7, 10, 26, 31, 32, 34]. The East Asian *cagA* with EPIYA-D motifs dominates in the Japanese Islands (98.4%), the Korean Peninsula (86.6%), China (42%), as well as in some countries washed by the South China Sea (Vietnam – 56.7% and Malaysia – 37.2%) (Fig. 2) [4, 12, 26, 27, 30]. In other regions of Asia, the East Asian *cagA* with EPIYA-D motifs occurs with lower frequencies (Thailand – 14% and Mongolia

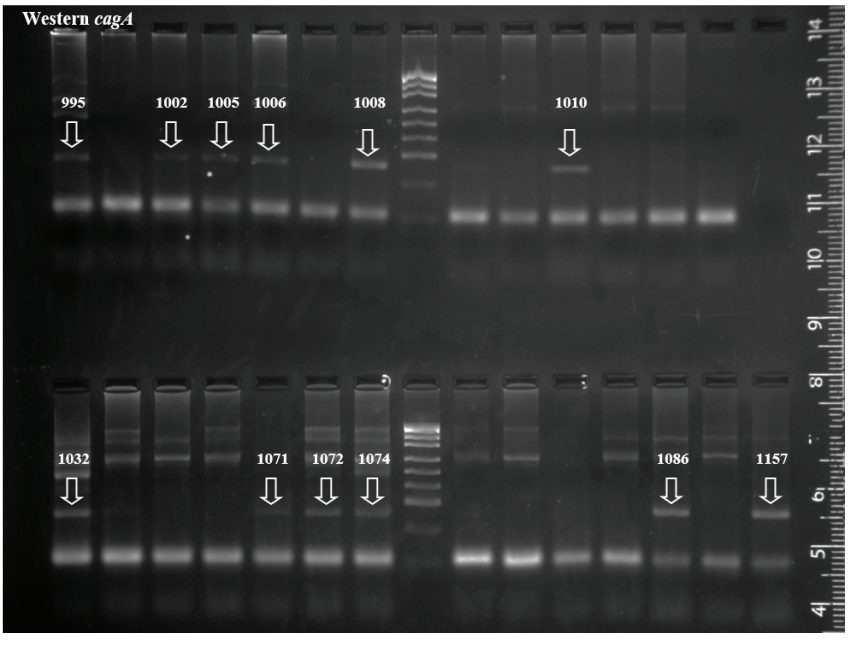
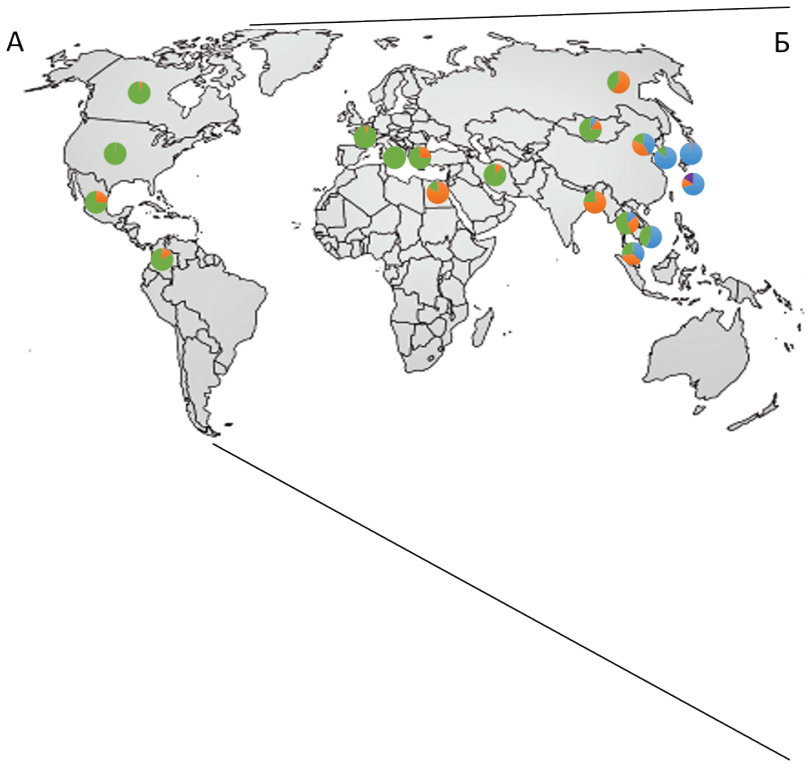


Fig 1. Electrophoregram of DNA samples with Western cagA gene (only positive samples are numbered).



Страна	Количество образцов	Western *cagA*	East Asian *cagA*	Неопределенный вариант *cagA*	Ссылка
Япония (Окинава)	337	54 16,1%	237 70,2%	46 13,6%	[5]
Япония (Фукуи)	65	0 0%	64 98,4%	1 1,5%	[4]
Китай (Далянь)	141	27 19%	59 42%	55 39%	[27]
Корея	45	6 13,3%	39 86,6%	0 0%	[12]
Таиланд	50	28 56%	7 14%	15 30%	
Вьетнам	171	73 42,6%	97 56,7%	1 0,6%	[26]
Малайзия	578	160 27,6%	215 37,2%	203 35,1%	[30]
Иран	159	140 88,1%	0 0%	19 11,8%	[10]
Египет	60	10 16,6%	0 0%	50 83,3%	[23]
Бангладеш	78	19 24,3%	0 0%	59 75,6%	[17]
Россия (Якутия)	30	12 40%	0 0%	18 60%	Данная работа
Монголия	368	293 79,6%	12 3,2%	63 17,1%	[34]
Греция	135	101 74,8%	0 0%	34 25,1%	[31]
Италия	20	20 100%	0 0%	0 0%	[26]
Франция	100	95 95%	0 0%	5 5%	
США	100	98 98%	0 0%	2 2%	
Канада	20	19 95%	0 0%	1 5%	
Колумбия	80	67 83,7%	0 0%	13 16,2%	[7]
Мексика	287	212 73,8%	0 0%	75 26,1%	[32]

Fig 2. The prevalence of two cagA gene variants among H. pylori strains in the world: A – The distribution of East Asian cagA and Western cagA gene variants in the world; B – initial data; Note: blue color – East Asian cagA, green color – Western cagA, violet color – J-Western cagA, orange color – undefined cagA.

3.2%), giving way to the Western *cagA* with EPIYA-C motifs (Thailand – 56% and Mongolia 79.6%) [12, 34]. A rather high percentage of detection of the Western *cagA* in Yakutia (40%) is consistent with the available information about the European origin of most *H. pylori* lines (hpEurope haplotype – 89.3%) circulating in Yakutia, according to the three housekeeping genes *atpA*, *mutY*, *ppa* [2].

The presence of undefined variants of the *cagA* gene (60%) in Yakutia is most likely explained by the fact that the *cagA* gene may have a sequence that differs from the sequences of East Asian and Western *cagA*. Undefined variants of the *cagA* gene were also found in Mexico (26.1%), Colombia (16.2%), Greece (25.1%), Egypt (83.3%), Iran (11.8%), Bangladesh (75.6%), Thailand (30%), Malaysia (35.1%), China (39%), Mongolia (17.1%) and Japan (13.6%), which may also indicate about local variants that differ from the previously identified East Asian and Western *cagA*.

Conclusion. Analysis of the prevalence of East Asian and Western *cagA* among *Helicobacter pylori* strains in Yakutia revealed that the prevailing variant of the *cagA* gene is the Western *cagA* and the East Asian *cagA* was not found in none of the samples. In 60% of cases, the samples did not belong to either the Western or the East Asian *cagA* gene, which may indicate about the presence of their own *cagA* gene sequence among *Helicobacter pylori* strains circulating in Yakutia.

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TOPICAL ISSUE

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PERINATAL OUTCOMES OF CORONAVIRUS COVID-19 PNEUMONIA IN PREGNANT WOMEN IN 2020 ON THE BASIS OF SBI RS (Y) POLYCLINIC NO. 1

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Since the end of 2019, the problem of the coronavirus pandemic has been the most urgent, including in pregnant women. Physiological changes in the immune system, susceptibility to respiratory viral infections, including COVID-19, can lead to unfavorable perinatal outcomes, but still many questions remain controversial.

The aim of the study was to identify the relationship between adverse perinatal outcomes and coronavirus pneumonia. The material was individual cards of pregnant women and women in childbirth aged 18 - 49 years with a confirmed new COVID - 19 infection. Statistical significance was assessed using the Pearson chi-square test. Differences were considered statistically significant at $p < 0.05$.

This article presents an analysis of perinatal outcomes in 82 patients with confirmed new COVID-19 infection, reveals an association of coronavirus pneumonia with an increased frequency of abdominal delivery ($p = 0.034$) and preterm birth ($p = 0.006$).

Keywords: pregnancy, coronavirus infection, coronavirus pneumonia, premature birth, abdominal delivery.

Introduction. Investigating the influence of the new COVID-19 infection on pregnancy and its outcomes are the most important tasks which attract big interest. It's known that clinical manifestations of the new coronavirus infection are not specific [10], the risk of severe course of acute respiratory virus infections is increased, the range of drugs is limited and there is no approved vaccine.

By the years of the investigations, it's noted that physiological changes that are happening in a pregnant woman in the form of immunological tolerance which is aimed at preserving the alloantigenic fetus lead to susceptibility to respiratory virus infections [1].

It's known that during pregnancy physiological changes are occurring in the respiratory and circulatory system. For example, the job of the respiratory system is complicated after the second half of gestational age due to high standing of the diaphragm occured by the result of growing uterus and necessity in strengthening lungs functions. COVID-19 pneumonia is progressing rapidly from focal to diffusive bilateral form which predisposes to fast development of respiratory distress [4].

There are few data in the literature that confirm the influence of the new COVID - 19 infection on the unfortunate perinatal outcomes [8,11,13.17], thereafter further study of this topic is logical.

Objective: to identify the relationship between adverse perinatal outcomes and coronavirus pneumonia.

To achieve this goal, the following research objectives were identified:

1. To assess the structure of extragenital morbidity, complications in childbirth, delivery routes, term of delivery and weight indicators of the newborn.
2. To identify the relationship between CT scan-signs of coronavirus pneumonia and preterm birth.
3. To identify the relationship between CT scan - signs of coronavirus pneumonia and the frequency of abdominal delivery by cesarean section.
4. Determine the relationship between CT scan - signs of coronavirus pneumonia and low birth weight.
5. To identify the presence of a link between adverse perinatal outcomes in coronavirus pneumonia and the period in which it was recorded.

Materials and methods: The study was carried out on the basis of the GBU RS (Y) "Polyclinic No. 1" women's consultation. A retrospective study of 82 individual cards of pregnant and postpartum women aged 18 - 49 years with a confirmed new COVID - 19 infection was carried out.

Identification of the SARS-CoV-2 virus was carried out by polymerase chain reaction (PCR) in nasopharyngeal material.

The criteria for the inclusion of patients in the study groups were: pregnant women with a confirmed new COVID-19 infection, the absence of severe concomitant somatic diseases (except for pneumonia associated with SARS-CoV-2), autoimmune and genetic diseases, and multiple pregnancies.

Statistical methods were used to process the data. The assessment of the statistical significance of the differences in outcomes depending on the impact of the risk factor was performed using the Pearson chi-square test. Differences were considered statistically significant at $p < 0.05$.

Results:

The average age of patients was 27 years. All subjects were divided into two groups depending on the presence of CT - signs of lung damage: group - CT 0 (no signs of viral pneumonia) n = 61, CT group 1-4 (CT 1 - pneumonia less than 25 percent, CT 2 - pneumonia 25 -50%, CT 3 - pneumonia 50-75%, CT 4 - pneumonia more than 75%) n = 21.

In the structure of extragenital diseases, the following diseases were identified: anemia - 57.3% of all studied, gestational diabetes mellitus - 12.1%, pathology of

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the hepatobiliary system - 1.2%, pathology of the genitourinary tract - 13.4%, gastrointestinal tract - 3.6%, cardiac - vascular system - 7.3%, respiratory system - 8.5%, respectively.

53.6% of pregnant women received outpatient treatment, 46.3% received inpatient treatment, respectively. Of the 82 infected with the new COVID-19 infection, three had a spontaneous miscarriage in the 1st trimester. Due to the severe course of the new coronavirus infection, in one case, a connection to a ventilator took place.

Picture 1. The structure of operative labor depending on the presence of CT signs of coronavirus pneumonia.

As shown in Picture 1, the total number of abdominal deliveries (AD) was 29 out of 79 deliveries, in the "CT 0" n - 17 ARs, n - 41 spontaneous deliveries (SD), "CT 1-4" n - 12 ARs, n - 9 SD, respectively.

The analysis of the structure showed that complications in childbirth, depending on the presence of CT signs of coronavirus pneumonia, as shown in Table 1, adverse perinatal outcomes were more common in the CT 1-4 group, but with a small amount of data, it is not possible to draw such unambiguous conclusions.

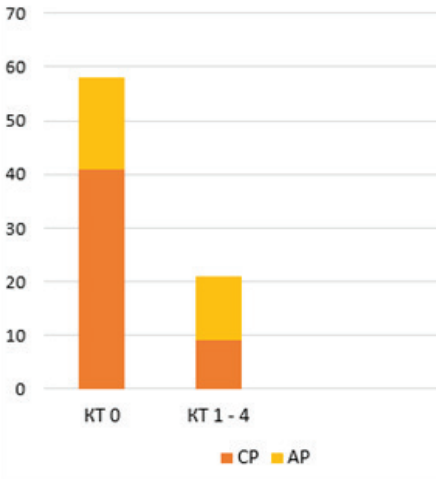
The total number of premature births (PB) was n - 11, very early PB n - 0, early PB n - 4, PB n - 9. In groups "CT 0" n - 4, "CT 1-4" n - 7, respectively.

As can be seen from Table 2, the authors assessed the statistical significance of the effect of coronavirus pneumonia on the term of delivery and the weight of the child using the calculation of the Pearson chi-square test using four-field contingency tables.

Then, as presented in Table 3, when comparing the two groups (CT 0 and CT 1-4), depending on the presence of CT - signs of lung damage and the type of labor (spontaneous and operative), we found a statistically significant relationship between these groups.

When assessing a statistically significant relationship between the perinatal outcomes presented (Table 4) and the trimester in which coronavirus pneumonia was recorded, using the Pearson chi-square index, it was not possible to identify a significant relationship (p> 0.05).

Discussion: Despite the accumulated experience, many aspects related to the management of pregnancy and the tactics of delivery of pregnant women with the new COVID - 19 infection are controversial, and each country has its own characteristics in the adopted algorithms for the management of pregnancy, childbirth and the postpartum period [7].



Structure of operative labor depending on the presence of CT signs of coronavirus pneumonia

According to different authors, the course of pregnancy in patients with COVID-19 varies. For example, in the conclusion of studies in Italy, China and the United States, it was shown that the course of COVID-19 in pregnant women does not differ from that in the general population [12]. A systematic review in 2020 by Liu D, which included 108 pregnant women, found an increased risk of severe disease in this contingent with COVID-19 [15].

It is known that the characteristic complications of pregnancy for patients with COVID-19 are: premature birth (21.3–39%), fetal distress (10.7%), fetal growth retardation (10%) and miscarriage (2%) [3] A meta-analysis by Mascio D.D., which included 79 pregnant women, also showed that the new coronavirus infection was associated with relatively

Table 1

The structure of complications in childbirth depending on the presence of CT signs of coronavirus pneumonia.

index	Premature rupture of amniotic fluid	Severe preeclampsia	Antenatal fetal death	Bleeding	Threat of fetal asphyxiation
CT 0	3	2	0	0	0
CT 1-4	4	1	1	2	1

Table 2

Criteria for assessing the significance of differences in outcomes depending on the impact of a risk factor (CT picture of coronavirus pneumonia)

index	Delivery on time	Premature birth	Birth weight (> 2500g)	Birth weight (<2500g)
Group CT 0	54	4	56	2
CT group 1-4	14	7	5	16
Fisher's exact test (two-sided)	0.00621 p<0.05		0.000 p<0.05	
X2 test	8.99		46.37	
Odds ratio	6.7 ± 0.7 (95% ДИ 1.7 – 26.3)		0.01 ± 0.88 (95% ДИ 0.002 – 0.06)	

Table 3

Criteria for assessing the significance of differences in outcomes depending on the impact of a risk factor (CT picture of coronavirus pneumonia)

Index	Spontaneous childbirth	Childbirth by caesarean section
Group CT 0	41	17
CT group 1-4	9	12
Fisher's exact test (two-sided)	0.03428 p<0.05	
X^2 test "chi-square"	5.14	
Odds ratio	3.2 ± 0.5 (95% ДИ 1.1 – 9.0)	

Table 4

Criteria for assessing the significance of differences in outcomes depending on the impact of a risk factor (trimester in which coronavirus pneumonia was recorded)

Index	Spontaneous childbirth	Abdominal labor	Premature birth	Delivery on time	Birth weight (> 2500g)	Birth weight (> 2500g)
1 trimester	4	1	1	5	5	2
2 trimester	16	13	4	25	25	3
3 trimester	30	15	6	38	38	6
Chi-square	1.644		0.041		1.497	
p	0.440		0.980		0.474	

higher rates of preterm birth, caesarean section and perinatal death [13].

The results obtained in the course of our study demonstrated a significant incidence of preterm birth and, as a consequence, low birth weight, which is consistent with the works of Gao Y.J., Abourida Y., 2020 [5, 9] in pregnant women with COVID-19 pneumonia. The most likely cause of these results was severe respiratory failure of the mother with hypoxemia, which can disrupt the uteroplacental blood flow and cause premature birth [16].

In some cases, obstetricians-gynecologists are forced to carry out induction of labor according to indications from the mother.

The presence of COVID-19 pneumonia showed a significant association with an increase in the frequency of abdominal delivery, which is also consistent with the authors Liu D., Li L., Wu X [12], in a study of which 15 pregnant women with COVID-19 and pneumonia have an increase in the frequency of operative deliveries. due to the development of fetal distress syndrome. According to the authors Chen D., Yang H., Cao Y 2020 [6], this may be due to the need to avoid prolonged labor, which can aggravate the course of COVID-19 in pregnant women.

Along with the above, it should be noted that we failed to identify a significant relationship between confirmed coronavirus pneumonia in groups divided by trimesters and an increase in the frequency of caesarean section operations, and there was no connection for premature birth and low birth weight. A small sample did not allow us to identify a significant relationship.

Of 6 pregnant women who underwent COVID-19 infection in the 1st trimester, 3 pregnancies ended in spontaneous miscarriage, but given the small sample size, the data cannot be considered representative.

Conclusion: The results of our study show a significant role of the influence of the new COVID-19 infection on perinatal pregnancy outcomes, which confirm an increase in the frequency of delivery by caesarean section, preterm birth and, accordingly, low birth weight.

To date, it can be concluded that there are risks of unfavorable outcomes in pregnant women, which undoubtedly requires their more dynamic monitoring.

It is important to take into account that patient management should be individualized, and questions about labor management tactics should be considered and accepted collectively, taking into account concomitant diseases, clinical picture, and fetal condition.

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INTERREGIONAL ANALYSIS OF CERVICAL CANCER INCIDENCE AND MORTALITY IN THE SIBERIAN PART OF RUSSIA (2008-2019)

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The study presents the differences in cervical cancer incidence and mortality in the period from 2008 to 2019 among the state entities, including national state entities, located in Siberia and Russia as a whole. Taking into account the study results, the entities, which are characterized by a multiethnic population – Kemerovskaya oblast, Buryatia and Zabaikalski Krai are acutely, but Tyva is in critical need of cervical cancer preventive measures, including HPV vaccination.

Keywords: human papillomavirus, vaccination, health, ethnic, minorities, disparities.

Introduction. Cervical cancer (CC) is the fourth most common cancer among women worldwide, and currently more than 300,000 women die from it every year. The highest burden of CC is in low- and middle-income countries where access to public health services is limited [1].

In the Russian Federation, which belongs to countries with a middle income level [2], in the structure of the cancer incidence and mortality in 2020, the indicators of CC took the 4th place of women's cancers [3]. For example, in countries with a high income level, such as the United States and Canada, in 2020, CC incidence took the 13th place, CC mortality - the 10th and the 12th respectively [3].

Along with routine screenings HPV vaccination is important to prevent CC. The vaccination targets the types of HPV that are the most important CC risk factors [1].

In the United States the HPV vaccination are covered by health insurance plans [4]. In Canada all jurisdictions offer the HPV vaccination through government-funded programs [5].

In Russia the HPV vaccination has not yet been included in the national routine immunization schedule, named "The Russian national preventive vaccinations and the vaccinations by epidemic indications calendar" approved by the Order of the Ministry of Health of the Russian Federation, March 21, 2014, N 125n, and therefore is not free for the population of Russia's state entities.

Meanwhile, the HPV vaccination has been highlighted in the WHO strategy for the period 2020-2030 [1]. Therefore, there is a hope that HPV vaccines will be included in the Russian national vaccinations calendar and will become available to all Russia's citizens in the foreseeable future. When these vaccines will be free, their supplies at the start of vaccination would be limited. In the period between the inclusion of vaccines in the Russian national vaccinations calendar and their widespread distribution, it is necessary to develop a plan that figures out which people, including the territories of their residence, should get vaccines first. Accordingly, the studies highlighted the disparities in the CC incidence and mortality depending on the territories of residence, ethnicity / race, gender, etc., are of undoubted relevance [6,7].

Our previous study was devoted to the analysis of CC incidence and mortality in the national-state entities located in Siberia, in which the indigenous population and national minorities live - the republics Altai, Buryatia, Tyva, Khakassia and Sakha (Yakutia) in the period from 2007 to 2019. Taking into account its results we concluded that in Tyva and Buryatia the urgent need for HPV vaccination is.

To confirm the results of our previous study and compare them with foreign studies, which also noted an unfavorable CC situation in multiethnic territories [8,9,10], we have expanded the number of state entities in the comparison group and have included in it other state entities located in the Siberian part of Russia. The retrospective year at the beginning of this study is 2008 since the Zabaikalski Krai was formed in 2008 by the unification of the Chitinskaya Oblast and the Agin-Buryat Autonomous Okrug [11], and when its data on the CC incidence and mortality appeared. The retrospective final year of the study remained 2019, its data on cancer were marginal for access on the portal for medical and pharmaceutical workers "ONCOLOGY.ru". In this study we have also introduced analysis for other indicators - the annual changes of the CC incidence and mortality for every year of the period 2009- 2019 from 2008, which was chosen as baseline. Accordingly, the purpose of this study was to compare the CC incidence and mortality in the period from 2008 to 2019 among state entities, located in Siberia, as well as Russia as a whole.

The following tasks were set: to compare the CC incidence and mortality among these state entities, and also compare them to the all-Russian analogous indicators; to determine state entities with maximum and minimum rates of

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CC incidence and mortality; to establish the disparities among state entities in the CC incidence and mortality annual baseline changes; to highlight state entities with the highest baseline increase in the CC incidence and mortality; to identify state entities with the correlation between CC incidence and mortality baseline changes.

Materials and methods. To establish the disparities in CC incidence (CCI) and mortality (CCM), the data of state medical statistics on cancer from 2008 to 2019 were used. The data that published annually in the books of the Moscow Oncological Research Institute named after P.A. Herzen - a branch of the Federal State Budgetary Institution "National Medical Research Center of Radiology" of the Ministry of Health of Russia on the portal for medical and pharmaceutical workers "ONCOLOGY.ru", from 2008 to 2020 were extracted [12].

The CCI and CCM were estimated by the age-standardized CCI rates (ASIR) and CCM rates (ASMR) per 100 thousand populations. To standardize by the age in the data sources, mentioned above, the world standard for the population distribution by the age was used. The time period was from 2008 to 2019. State entities (SEs) – Altaiski Krai (AK), Krasnoyarski Krai (KK), Irkutskaya Oblast (IO), Kemerovsraya Oblast (KO), Novosibirskaya Oblast (NO), Omskaya Oblast (OO), Tomskaya Oblast (TO), Zabaikalski Krai (ZK), and the republics – Buryatia (RB), Altai (RA), Tyva (RT), Khakassia (RKh) and Sakha (Yakutia) (RSY) were included in the comparison group. There were 13 SEs in total and Russia as a whole (RF). The retrospective time period is from 2008 to 2019. The cancer site is the cervix (C53).

Because the annual values of CC ASIR and ASMR did not have a normal distribution the Friedman's test was used for finding differences across multiple attempts. To identify paired differences the Wilcoxon's test was used.

The values of the CCI and CCM annual baseline changes (ABC) were calculated as the ratio of the difference between the current annual value of ASIRi and ASMRi (where i - [2009, 2019]) and the value of the CCI and CCM in 2008 (ASIRo, ASMRo) to the value of ASIRo, ASMRo.

CCI ABC = (ASIRi – ASIRo) / ASIRo;

CCM ABC = (ASMRi – ASMRo) / ASMRo.

To identify the correlation between the CCI ABC and CCM ABC, the Spearman correlation coefficient (r) was calculated, using the formula for small sample sizes. Chan's scale was used to assess the connection strength [13].

Differences and r were considered significant at $p \leq 0.05$.

Results and discussion. *Research on the cervical cancer incidence.*

The annual values of the CC ASIR from 2008 to 2019 in the SEs located in Siberia and in Russia as a whole are presented in Table 1. The null hypothesis of Friedman's test is rejected, so Friedman's test revealed their unevenness ($p = 0.000$).

The average ranks of the annual values of the CC ASIR from 2008 to 2019 in the SEs and in Russia as a whole are shown graphically in Figure 1. Based on them, the lowest CCI in this period of time was in the NO. The paired differences in the NO's average rank with the all-Russian and with the rest of the SEs' average ranks reached the level of the chosen significance ($p < 0.05$). The CCI average ranks in the KO and in the OO did not differ from the all-Russian ones ($p = 0.979$). In the remaining ten SEs, the CCI average rank significantly exceeded the all-Russian ($p < 0.05$) while ZK and Tyva had the highest CCI average ranks, which exceeded the all-Russian by more than 4 times. There were no differences between the ZK's and RT's CCI average ranks ($p = 0.638$), and they both significantly exceeded the ranks of all other eleven SEs ($p < 0.05$).

Our previous study on the CCI differences in the period 2007-2019 among the republics located in Siberia also showed that the highest CCI rates was observed in Tyva [14].

The all-Russian CCI baseline change rates increased steadily from year to year in the period 2009-2019 and have the character of a steady trend towards the further increase. The rates of the all-Russian CCI ABCs, as well as the CCI ABCs in SEs located in Siberia, are graphically presented in Figure 2. An increase of CCI ABCs that are close to the above-described characteristics of the all-Russian one and confirmed by the strong correlation on the Chan scale [6], was observed in the KK ($r = 0.945$, $p = 0.000$), in the IO ($r = 0.855$, $p = 0.001$), in the KO ($r = 0.873$, $p = 0.000$), in Buryatia ($r = 0.845$, $p = 0.001$) and in Tyva ($r = 0.882$, $p = 0.000$).

The increase in the CCI ABCs close to the all-Russian, but confirmed by a moderately strong correlation, was observed in the RSY ($r = 0.755$, $p = 0.007$). The CCI ABCs in the OO, the NO, the TO, the ZK and the RKh in 2009-2019 varied and did not correlate with the all-Russian, but at the same time always showed an increase or no change in rare years (1-2 years out of the selected 12 years). The absence of the CCI ABCs in these SE is shown in Figure 2 as a decrease in the CCI ABCs in certain years. The absence because when we calculated the differences between the CC ASIR values in these SEs in 2008 and in the years in which, according to our calculations, the CCI ABCs had negative values, the significance did not reach the chosen level ($p > 0.05$).

The CCI ABCs in the AK as well as in the RA were in opposite directions, and have the fair and poor negative correlation coefficient with the all-Russian ones, but the p values did not reach the required level ($r = -0.205$, $p = 0.545$; $r = -0.336$, $p = 0.312$, respectively). In the AK the CCI ABCs' increase was noted in 2012 and 2013, in other years the baseline CCI decreased or did not change. In the RA the CCI ABCs' increase was observed in 2009, 2013 and 2015, in other years the baseline CCI decreased or did not change. In our opinion in the AK and the RA there is a tendency towards a decrease of the CCI baseline changes but so far without the formation of a stable trend.

In addition our previous study showed that despite the fact that in the RA, with a 1.55-fold decrease of the CC ASIR value in 2019 compared to 2007, the difference between them still did not reach the required level of significance ($p=0.108$) [14].

When we carried out the multiple rank analysis of the CCI ABCs in the period 2009-2019, we found that their distribution is also heterogeneous ($p = 0.000$). These rank rates are graphically presented in Figure 3. Despite the fact that the CCI ABCs average ranks in the KK, the IO, the KO, the NO, the TO, Buryatia, ZK, Tyva and RKh graphically exceed the all-Russian ones, the average ranks were distributed unevenly ($p = 0.049$). Therefore, we had begun to form comparison groups among these SEs by the descending order of their average ranks, to identify a group of SEs or a single SE, which had the maximum rates of CCI ABCs. We had formed such a group, that included the KK, the KO, the ZK, and the RKh, among which the distribution of the CCI ABCs was the same ($p = 0.664$) and the median of the CCI ABCs' differences between in these SEs and the all-Russian reached the significance ($p < 0.02$).

Research on cervical cancer mortality. The annual values of the CC ASMR from 2008 to 2019 in SEs located in Siberia and in Russia as a whole are presented in Table 1. Analysis of their distribution

Table 1

Annual values of CC ASIRs and CC ASMRs in the SEs located in Siberia and RF

	2008				2009				2010				2011			
	ASIR		ASMR		ASIR		ASMR		ASIR		ASMR		ASIR		ASMR	
	M	m	M	m	M	m	M	m	M	m	M	m	M	m	M	m
RF	12.84	0.11	4.99	0.07	13.40	0.12	5.13	0.07	13.71	0.12	5.12	0.07	13.70	0.12	5.27	0.07
AK	16.27	0.99	5.40	0.54	15.04	0.94	4.99	0.52	15.81	0.97	5.67	0.56	16.25	1.01	4.81	0.54
KK	14.25	0.85	6.60	0.56	13.76	0.83	6.73	0.56	15.13	0.87	7.06	0.59	14.94	0.88	6.93	0.58
IO	19.00	1.06	8.36	0.70	17.68	1.02	8.26	0.69	19.72	1.09	7.45	0.66	21.18	1.16	7.61	0.68
KO	10.78	0.73	3.96	0.43	12.77	0.81	4.18	0.45	12.06	0.78	4.36	0.45	12.90	0.83	6.18	0.56
HO	11.40	0.78	4.99	0.50	13.08	0.85	4.27	0.46	12.24	0.82	4.23	0.46	12.15	0.84	4.48	0.49
OO	12.84	0.96	5.55	0.61	14.63	1.05	5.93	0.64	12.61	0.95	5.57	0.61	14.13	1.02	4.71	0.57
TO	16.61	1.56	7.09	1.01	19.13	1.68	6.41	0.92	20.40	1.73	6.44	0.91	15.85	1.49	6.43	0.92
ZK	24.92	1.93	5.43	0.87	30.74	2.12	9.08	1.13	28.06	2.04	7.90	1.04	30.38	2.11	8.39	1.11
RB	22.90	1.98	11.69	1.42	18.66	1.75	11.60	1.38	15.57	1.62	8.71	1.17	21.09	1.86	11.13	1.33
RA	18.60	3.77	10.28	2.96	27.03	4.83	12.91	3.28	14.15	3.45	10.98	3.00	18.56	3.88	6.18	2.21
RT	27.54	3.98	6.24	1.90	17.45	3.12	9.20	2.33	20.66	3.54	10.44	2.48	27.94	4.11	13.46	2.85
RKh	14.04	1.99	8.39	1.45	16.36	2.09	6.60	1.27	16.97	2.11	4.31	1.02	22.00	2.47	5.77	1.23
PSY	17.21	1.74	6.77	1.09	17.44	1.72	5.29	0.96	13.36	1.52	3.82	0.79	16.68	1.70	4.91	0.93
	2012				2013				2014				2015			
	СПЗ		СПСм		СПЗ		СПСм		СПЗ		СПСм		СПЗ		СПСм	
	M	m	M	m	M	m	M	m	M	m	M	m	M	m	M	m
RF	13.90	0.12	5.23	0.07	14.17	0.12	5.35	0.07	14.47	0.12	5.18	0.07	15.01	0.12	5.39	0.07
AK	17.78	1.05	5.25	0.55	17.19	1.01	5.92	0.58	15.56	0.96	5.66	0.56	16.38	0.99	6.32	0.59
KK	16.10	0.91	6.09	0.54	17.29	0.94	6.12	0.56	19.73	1.00	6.70	0.56	20.62	1.03	7.26	0.60
IO	22.39	1.18	8.82	0.72	22.21	1.17	8.51	0.72	22.63	1.19	7.93	0.68	22.45	1.19	9.31	0.76
KO	14.51	0.88	6.77	0.59	14.29	0.86	7.12	0.60	14.72	0.89	5.80	0.54	16.53	0.94	7.30	0.61
HO	14.23	0.89	5.28	0.54	15.13	0.90	5.14	0.51	12.62	0.82	5.09	0.51	15.19	0.89	4.74	0.49
OO	12.61	0.97	5.26	0.59	14.20	1.02	5.91	0.62	13.54	1.00	5.58	0.61	16.39	1.09	5.79	0.64
TO	19.81	1.66	7.26	1.00	19.56	1.64	7.06	0.97	26.86	1.92	7.84	0.98	25.74	1.90	7.66	0.99
ZK	25.15	1.96	8.95	1.12	29.23	2.10	8.57	1.13	32.77	2.23	8.73	1.13	35.58	2.36	10.69	1.24
RB	18.86	1.76	10.57	1.31	31.27	2.27	12.47	1.42	27.83	2.10	12.48	1.39	29.91	2.20	11.46	1.36
RA	18.97	3.87	6.93	2.21	22.43	4.16	11.19	2.82	16.46	3.35	8.95	2.54	21.86	4.04	8.21	2.37
RT	20.20	3.43	9.04	2.29	24.07	3.66	12.00	2.59	30.97	4.15	9.38	2.22	35.40	4.48	12.21	2.64
RKh	20.15	2.34	8.08	1.43	20.55	2.37	10.55	1.72	26.57	2.69	7.46	1.42	17.20	2.12	8.50	1.50
PSY	16.58	1.69	5.58	0.99	20.30	1.84	6.95	1.06	19.59	1.83	6.47	1.03	19.20	1.79	6.21	0.97
	2016				2017				2018				2019			
	ASIR		ASMR		ASIR		ASMR		ASIR		ASMR		ASIR		ASMR	
	M	m	M	m	M	m	M	m	M	m	M	m	M	m	M	m
RF	15.45	0.12	5.26	0.07	15.76	0.13	5.18	0.07	15.80	0.12	5.07	0.07	15.38	0.12	5.01	0.07
AK	14.92	0.97	4.73	0.51	16.38	1.02	5.83	0.60	14.96	0.96	4.28	0.50	16.24	1.02	5.01	0.54
KK	18.90	0.99	6.61	0.56	21.06	1.03	6.80	0.57	21.49	1.03	6.40	0.55	21.03	1.04	6.21	0.54
IO	26.43	1.31	9.24	0.75	23.56	1.21	8.34	0.71	25.18	1.26	8.87	0.74	21.83	1.17	7.94	0.69
KO	16.22	0.94	6.38	0.57	16.97	0.97	7.27	0.61	15.65	0.92	6.67	0.57	15.24	0.91	6.94	0.59
HO	13.75	0.84	4.01	0.44	14.28	0.86	4.37	0.45	13.05	0.81	5.89	0.54	12.60	0.80	4.99	0.48
OO	13.80	1.01	5.49	0.62	15.61	1.08	5.07	0.6ц0	17.33	1.14	5.83	0.65	16.73	1.11	4.91	0.57
TO	23.44	1.82	6.92	0.95	19.20	1.62	7.11	0.97	17.26	1.52	5.74	0.85	19.49	1.58	5.74	0.83
ZK	44.75	2.64	8.85	1.12	41.54	2.59	7.17	0.99	40.64	2.54	9.55	1.15	22.85	1.86	7.30	1.00
RB	30.03	2.25	11.39	1.36	42.55	2.67	13.09	1.47	35.64	2.43	11.41	1.30	39.92	2.56	14.32	1.53
RA	16.75	3.71	9.18	2.68	17.08	3.60	7.61	2.12	18.19	3.59	2.14	1.07	14.50	3.14	5.12	1.86
RT	43.88	4.96	9.11	2.23	44.98	4.92	9.41	2.17	49.01	5.09	12.62	2.62	51.37	5.33	13.60	2.81
RKh	17.26	2.10	8.78	1.53	20.29	2.30	8.55	1.45	19.48	2.33	6.21	1.18	22.10	2.44	7.93	1.40
PSY	22.85	1.94	6.84	1.07	20.20	1.82	6.11	1.01	21.57	1.89	4.61	0.84	18.38	1.75	5.49	0.93

Note: See the explanation of abbreviations in Tables 1-3 and Fig.1-4 in the text

revealed the heterogeneity (p = 0.000) also as the ASIR and the CCI ABCs distributions.

The average ranks of the annual CC ASMR values from 2008 to 2019 in the selected SEs and in Russia as a whole are shown graphically in Figure 1. Based on the average ranks, the lowest CCM was, also as the CCI, in the NO (p <0.05 in comparison with the all-Russian rank and with the remaining SEs' ranks). The CC mortality average rank not different from the all-Russian one was observed only in the AK (p = 0.328). In the remaining eleven SEs, the CC mortality average ranks significantly exceeded the all-Russian level (p <0.05). The highest CCM average ranks during this period were in Buryatia and Tyva, which exceeded all-Russian by more than 4 times. There were no differences between the values of these ranks (p = 0.158) and they both significantly exceeded the ones of all other eleven SEs (p <0.05).

Our previous study also showed that the highest CCM in the period 2007-2019 among the republics located in Siberia was observed in Buryatia and Tyva [14].

In contrast of the all-Russian CCI ABCs, its CCM ABCs in the period 2009-2019 did not have the stable increase. The CCM ABCs in SEs located in Siberia and in Russia as a whole are shown in Figure 4. A steadily increase in the CCM ABCs can be seen in the KO; but in Tyva and in the ZK the increase is accompanied by peaks. Such increasing rates of CCM ABCs can even be called critical in comparison with all-Russian and with the rates in the remaining ten SEs, in which their CCM ABCs had a downward and upward direction. The largest number of years in which a decrease of the CCM ABCs was observed was in the RSY; there were 9 years of them in total (2009-2012, 2014-2015, 2017-2019).

When we carried out the multiple rank analysis of the CCM ABCs rates among all SEs and Russia as a whole, it was found that their distribution is also heterogeneous (p = 0.000). The rates of the average ranks of the CCM ABCs in SEs located in Siberia as well as all-Russian ones are graphically presented in Figure 3. Based on the rates, the KO, the ZK and Tyva have the most increase in the CCM ABCs, which is about 1.5 times higher than the all-Russian ones (the paired differences between each of them and all-Russian have p = 0.002). The rates of the CCM ABCs average ranks in these SEs had no difference (p> 0.05). The CCM ABCs average ranks in the AK, the KK, the IO and Buryatia did not differ from the all-Russian ones (p> 0.05).

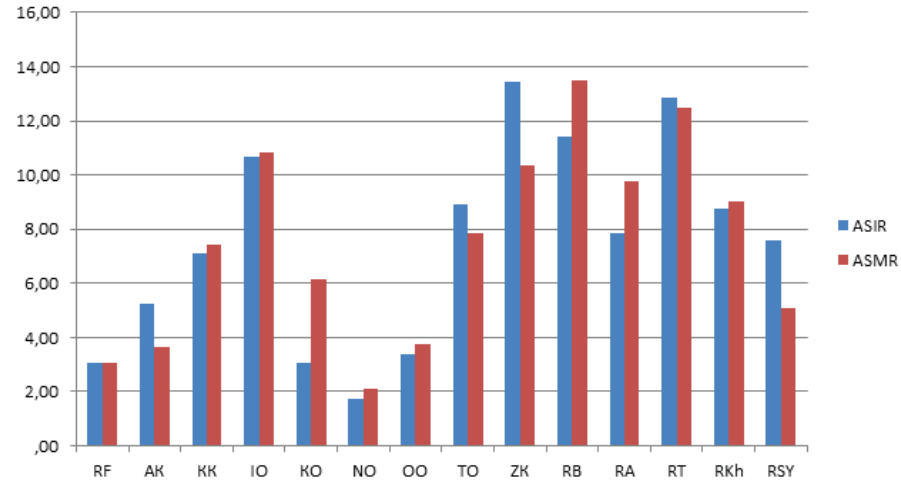

Fig 1. Average ranks of the CC ASIRs and ASMRs in the SEs (2008-2019)

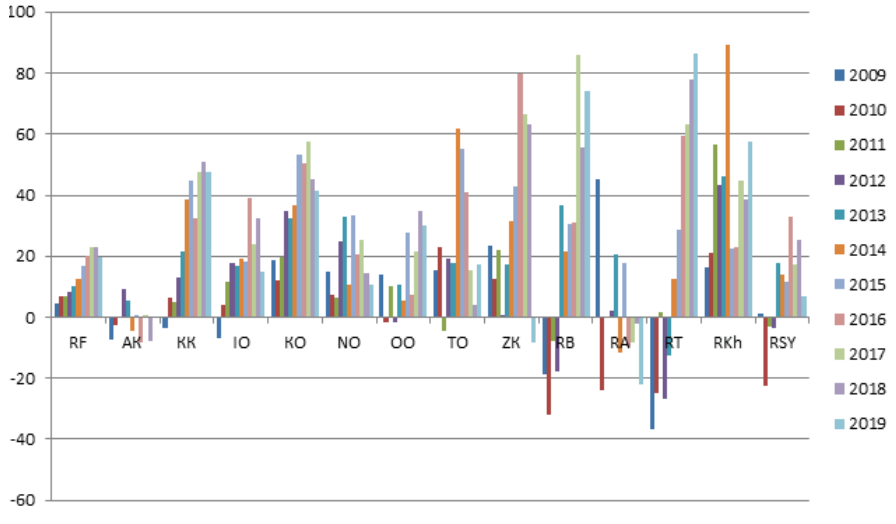

Fig 2. The rates of the CCI ABCs in the SEs

Fig 3. The average ranks of the CCI ABCs and the CCM ABCs in the SEs (2008 – 2019)

Significantly lower ranks than of all-Russian were in the NO (paired differences p = 0.034), the OO (p = 0.034), the TO (p = 0.015), the RA (p = 0.0340), the RKh (p = 0.028) and in the RSY (p = 0.003); in the RSY, as we wrote above, a decrease in the CCM baseline changes was revealed in most annual periods. When we were performing the rank analysis of the CCM ABCs of these six

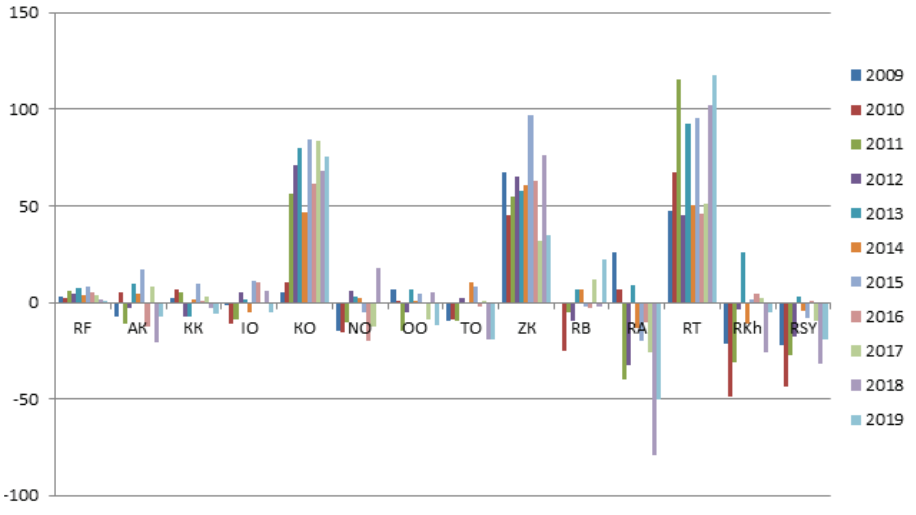

Fig 4. The rates of the CCM ABCs in the SEs

SEs it was found that their distribution is uniform, that is, no significant differences were found between them (p = 0.075).

In our opinion, only in relation to the RA we can talk about the beginning of the stable decreasing CCI baseline changes trend predominantly at the period 2014-2019.

Presented in our previous study the analysis of differences in CCM in 2019-2007 in the republics located in Siberia, also showed that Tyva had the most significant increase and the RA had most significant decrease [14].

Analysis of the correlation between cervical cancer incidence and mortality.

Analysis of the correlation between CCI ABCs and CCM ABCs, the results of which are presented in Table 2, showed the presence of a moderately strong correlation in the AK, the IO, the KO, the TO and Buryatia. Of these SEs, only in the AK there was a relatively favorable picture in relation to CC.

In Global Strategy the WHO recommends to achieve the CCI rate less than 4 cases per 100,000 women per year for accelerating the CC elimination as a public health problem [1]. According to the 2019 data in all SEs included in this study, there is a significant excess of this rate (see Table 1).

Nevertheless, according to the results of our study, it is important to choose SEs that are in highly need of CC prophylaxis including the HPV vaccination. So, finally, we assigned ranks to the indicators calculated by us. The ranking results are presented in Table 3, in which 1 point is the presence of the indicator, 0 is its absence. The indicator "Maximum values of ASMR" was assigned 2 points, since currently a maximum reduction of CCM is the first goal.

As a result, the maximum final rank was assigned to Tyva, which characterizes the burden of CC in this SE as critical that is also shown in our previous study [1]. Further, in descending order the KO, and having a common border Buryatia and the ZK followed. Their ranks indicate the need for urgent CC prevention.

Tyva, the KO, Butyatia and the ZK are the SEs characterized by a significant share of the indigenous population and ethnic minorities. These data consist with other third-party international research, which established an unfavorable situation in relation to CC in multiethnic areas [8,9,10].

Conclusion. Our research shows that the HPV vaccination among the population of the SEs located in Siberia is a very urgent task.

In all SEs selected for the study, as well as in Russia as a whole, the CCI in 2019 exceeded the WHO-established 4 cases per 100,000 women per year.

The lowest CCI and CCM in 2008-2019 detected in the NO, where the rates were lower than the all-Russian ones. In most of the selected SEs, the CCI (10 SEs) and the CCM (11 SEs) significantly exceeded the all-Russian ones. The

Table 2

Spearman correlation coefficient (r) between the annual values of CC ASIRs and ASMRs (2008-2019)

	RF	AK	KK	IO	KO	NO	OO	TO	ZK	RB	RA	RT	RKh	RSY
r	-0.182	0.683*	-0.145	0.645*	0.709*	0.164	0.155	0.724*	0.291	0.682*	0.264	0.518	0.136	0.573
p	0.574	0.020	0.670	0.032	0.015	0.631	0.649	0.012	0.385	0.021	0.433	0.102	0.689	0.066

Note: * - the r has the required significant p-value

Table 3

The sum of the calculated CCI and the CCM indicator ranks

	AK	KK	IO	KO	NO	OO	TO	ZK	RB	RA	RT	RKh	RSY
Max ASIR								1			1		
The increase of CCI ABCs		1	1	1					1		1		
Max increase of CCI ABCs		1		1				1				1	
Max ASMR									2		2		
The increase of CCM ABCs				1				1			1		
Max increase of CCM ABCs				1				1			1		
Correlation between CCI ABCs and CCM ABCs			1	1			1		1				
The sum		**2**	**2**	**5**			**1**	**4**	**4**		**6**	**1**	

highest CCI was observed in the ZK and Tyva, the highest CCM was in Buryatia and Tyva.

Based on the changes in the CCI from baseline it have showed an increase of the CCI in Russia as a whole and in all SE selected for the study, except for the AK and the RA. But in Russia as a whole, in the KK, the IO, the KO, Buryatia and in Tyva the CCI increased steadily. The maximum increase was noted in the KK, the KO, the ZK and the RKh.

The changes in the CCM from baseline in most SEs (10 SEs) had two direction - downward and upward. A critically increase of CCM based on the changes in the CCM from baseline was found in the KO, the ZK and Tyva. The correlation between the CCI ABCs and the CCM ABCs reflecting an unfavorable CC situation found in the IO, the KO, the TO and Buryatia.

Thus, taking into account the results of our study in total, among SEs located in Siberia the most acute CCI and CCM situation has developed in the SEs of Russia, which have significant proportion of the indigenous population and ethnic minorities, such as Tyva, Kemerovskaya Oblast, Buryatia and Zabaikalski Krai. These SEs, especially Tyva, highly need to carry out CC preventive measures, including HPV vaccination.

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COMPARATIVE ANALYSIS OF BIOLOGICAL PROPERTIES OF THE MAIN FAMILIES OF *M.TUBERCULOSIS* GENOTYPES IN NEWLY DIAGNOSED PATIENTS WITH PULMONARY TUBERCULOSIS

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Using advanced molecular genetic methods for identification of *M.tuberculosis* isolates, we were able to compile detailed description of *M.tuberculosis* (MTB) population circulating in one of the underexplored northern territories of Russia. Isolates circulating in the Sakha Republic (Yakutia) were presented by 9 key heterogeneous clusters: Beijing, T, S, Ural, Lam, Haarlem, Orphan, Uganda, and X. Analysis of growth rates, colony counts, drug sensitivity of the key genotypes of MTB isolated from newly diagnosed patients with PTB showed lack of uniformity in MTB isolates, in terms of their biological properties. Viability of the causative agent can reliably be linked to emergence of primary drug resistance in MTB population; this can be clearly seen in the case of S family. Consequently, excessive MTB colony count implies that patients isolating such strains pose epidemiological risk, especially in cases where fast growth is coupled with multidrug-resistance.

Keywords: Mycobacterium tuberculosis, genotypes, biological properties, molecular genetic techniques.

Introduction. Current situation with tuberculosis (TB) is shaped in large part by the changing biological properties of the causative agent, and particularly, by *M.tuberculosis* (MTB) population composition with increasing absolute and relative proportion of drug-resistant organisms [1].

Considerably less attention is paid to exploring another changing biological property of MTB, namely its viability (in terms of growth rate and colony count), while viability has an important predictive value [3, 5]. MTB viability has been shown to be a factor determining disease severity, characteristics of disease progression, and treatment success [8, 9, 10, 13]. Also there is a correlation between such biological properties as viability, primary/secondary drug-resistance (DR) and basic epidemiological parameters (incidence, mortality, treatment effectiveness, etc.) [2, 4, 6].

Beyond that, changes in biological properties of the causative agent are defined by recently discovered genetic heterogeneity of MTB species, implying large dependence of the virulence of causative agent on its membership within specific genetic family [10].

Based on the above said, it seemed relevant to employ advanced techniques for the purpose of characterizing biological properties of MTB in newly diagnosed pulmonary tuberculosis (TB).

Aim. Define key MTB genotype families circulating in the Sakha Republic (Yakutia), and comparatively analyze their biological properties in newly diagnosed pulmonary TB.

Material and methods. The study was performed at the Bacteriological laboratory of the Phthisiatry Research-Practice Center, and at the laboratory of Epidemiology and Microbiology Institute of the Scientific Center for Family Health and Human Reproduction Problems SB RAMS (Irkutsk).

315 MTB strains were selected for the study, which were isolated from 315 newly diagnosed culture-positive patients with pulmonary tuberculosis (PTB) who underwent treatment in the in-patient clinic of the Phthisiatry Center and in rural central district hospitals.

110 (34.9%) strains were isolated from rural residents, and 205 (65.1 %) from urban residents (Yakutsk). Additionally, we used classification of rural districts to 4 socio-geographical zones first proposed by M.A. Tyrylgin [14].

Presence of MTB in culture specimens was established using Lowenstein-Jensen and Finn-2 solid egg media, after preliminary digestion and decontamination with BBLMycoPrepNALC-NaOH solution (BD, USA), in compliance with the Russian Federation Health Ministry Order no.109 (21.03.2003) [12].

To assess the growth of MTB cultures in solid media, we registered the following parameters:

1. Growth rate or the first detectable growth (based on the date of first detected growth per tube). MTB colony detection in less than 30 days was considered fast growth; growth in 30 or more days was a slow growth.

2. Load or level of growth: colony count per tube. If simultaneous growth was observed in all tubes inoculated with the same material, a total number of colony-forming units (CFU) in all of those tubes was counted. Colony count under 20 was assessed as scanty (1+); between 21 and 100 colonies – as moderate (2+); above 100 colonies – as excessive (3+).

3. Presence of contaminant flora («overgrowth»).

4. Absence of growth. This was established at week 10 since inoculation.

Viability of MTB cultures isolated from specimens before therapy was assessed based on combined assessment of growth rate and colony count, using conventional methods described elsewhere [11]: colony count less than 20 with a growth rate of longer than 30 days was considered a low viability; more than 100 colonies with a growth rate of less than 30 days was considered a high viability. Any other combinations of colony count

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and growth rate were labelled moderate viability.

Drug sensitivity was determined based on absolute concentration method, using Lowenstein-Jensen medium, following the procedure described in the Russian Federation Health Ministry Order no. 109 (21.03.2003) [12]. The following resistance types were determined: monoresistance; poly-resistance (resistance to 2 or more drugs, with the exception of simultaneous resistance to isoniazid and rifampicin); multidrug-resistance (MDR, resistance to at least isoniazid and rifampicin); extensive drug resistance (XDR, resistance to rifampicin + isoniazid + fluoroquinolone + capreomycin/kanamycin).

Further, the isolated MTB strains were genotyped, using molecular genetic methods; this was performed at the laboratory of Epidemiology and Microbiology Institute of the Scientific Center for Family Health and Human Reproduction Problems SB RAMS (Irkutsk). DNA was extracted from inactivated cultures. For inactivation, one or several colonies from Lowenstein-Jensen medium were re-suspended in 500 mL of 1% mixture of N-acetyl-N,N,N-trimethyl ammonium bromide (CTAB) with 50% isopropanol, as described in the literature [17]. DNA isolation was performed using DNA-sorb-B extraction kit (Interlabservis, Russia), following the manufacturer's protocol.

To define key genetic families forming MTB population in Yakutia, MIRU-VNTR genotyping was performed using the protocol described at MIRU VNTR plus website [18]. Primary determination of MTB genotypes was done using phylogenetic software available at the web-site. Prevalence of MTB strains was reconfirmed using the publicly available SITVIT database [15]. Beijing family genotypes were additionally sub-typed based on RD105/RD207 genome region, as described earlier [7]. Strain profiles, which were verified as Beijing genotype using the above methods, were then compared with the database published by M.Merker and colleagues, against 24 MIRU-VNTR loci [16].

Statistical data processing was done using conventional software (Microsoft Excel, StatSoft Statistica 6), and calculated based on mean values (M±m) and the significance of differences observed between variables compared (p).

Results and discussion. Based on the results of MIRU-VNTR typing (Table 1), of 315 isolates of MTB, Beijing family was detected in the highest number of strains (152; 48.2%) over entire republic: of them, 95 (30.1%) from Yakutsk, and 57 (18.1%) from rural administrative districts. Proportions of genotypes belonging to Beijing family among urban and rural patients made 46.3% (95/205) and 51.8% (57/110), respectively. Based on distribution by socio-geographical zones, the following variations were observed: 42.8% (9/21) in Arctic districts, 44.0% (11/25) in hybrid districts, 44.4% (4/9) in industrial districts; 60.0% (33/55) in agrarian districts.

The second largest group comprising 37 isolates (11.7%) consisted of genotypes referred to family T, based on their spoligoprofiles. Individual strains were identified as MANU 2, H37Rv, SIT 877, SIT 1562. Family T was detected in 12.2% (25/205) of urban residents (Yakutsk), and in 10.9% (12/110) of rural residents. By socio-geographic zones, T family was absent in industrial zone (including 5 administrative districts); 7.3% (4/55) were detected in agrarian zone, 12.0% (3/25) in hybrid zone, and 23.8% (5/21) in the Arctic zone.

The third most frequent in Yakutia was a clustered S family (32; 10.1%) with a single spoligotype (SIT 1253). The family formed a distinctive cluster on phylogenetic tree, and was presented by similar MIRU-VNTR profiles. Proportions of S family in Yakutsk and in rural districts were 10.2% (21/205) and 6.4% (7/110), respectively. Family S showed an interesting distribution by socio-geographic zones: it was absent in Arctic zone (consisting of 11 districts); proportions in agrarian, hybrid, and industrial zones were respectively, 7.3% (4/55), 8.0% (2/25), 11.1% (1/9).

The fourth by occurrence was Ural family (26; 8.2%). Profiles identified included MIT 197, 756 and some other ones, not registered in SITVIT database, but characterized by typical MIRU-VNTR features distinguishing Ural family. Ural family was found in 8.8% (18/205) of the residents of Yakutsk, and in 7.3% (8/110) of rural residents. Distribution by socio-geographic zones was as follows: 5.4% (3/55) in agrarian zone; 8.0% (2/25) in hybrid zone; 14.8% (3/21) in Arctic zone; no cases in industrial zone.

Even more heterogeneous Haarlem family was established in 21 (6.7%) cases. Haarlem was present in 7.3% (15/205) of Yakutsk cases, and in 5.4% (6/110) of rural cases. By socio-geographic zones, the incidence was as follows: 8.0% (2/25) in hybrid zone; 9.5% (2/21) in Arctic zone; 22.2% (2/9) in industrial zone; no cases in agrarian zone.

MTB belonging to LAM family were detected in 21 (6.7%) cases. Of them, 14 (6.8%) in Yakutsk, and 7 (6.4%) in rural districts. Distribution of LAM family by socio-geographic zones was as follows: 4.0% (1/25) in hybrid zone; 7.3% (4/55) in agrarian zone; 22.2% (2/9) in industrial zone; no cases in Arctic zone. LAM cluster included both registered profiles (MIT 1, 140, 326), and some strains with MIRU-VNTR codes proximal to LAM family. This heterogeneous group comprised different spoligotypes (SIT 42, 254, 1337, new), assigned to different sub-families, based on Spol tool of SITVIT database (LAM 9, LAM 5). Also, strains within single MIT or SIT type had variations in a number of loci (Mtub 4, Mtub 30, Qub 26), which may imply long-time circulation of LAM family in the territory of Sakha Republic (Yakutia).

Next group (15 isolates) was presented by a cluster formed by Orphan family (15; 4.8 %): 8 (2.5%) in Yakutsk, and 7 (2.2%) in rural districts. Orphan family was found in the following socio-geographic zones: agrarian (3.6%; 2/55), Arctic (4.8%; 1/21), hybrid zone (16.0%; 4/25). No cases in industrial zone were detected.

The rest of the strains belonged to families Uganda (7; 2.2%) and X (4; 1.3%). Incidences of these families in Yakutsk and in rural districts were, respectively: 5 (1.6 %) and 2 (0.6 %); 2 (0.6 %) and 2 (0.6 %).

Biological properties, such as growth rate and colony count, were analyzed for key genotypes identified in MTB cultures (Table 2).

As is seen in Table 2, out of 315 cultures, 194 (61.6%) showed scanty colony count, 95 (30.1%) had moderate colony count, and 26 (8.3%) showed excessive colony count. Fast growth rate was observed in 238 (75.5%), and slow growth rate in 77 (24.4%) cases. Mean growth rate was 27.4±1.2 days.

Scanty, moderate and excessive growth rates were observed in the following genotype families, respectively: Beijing: 92 (60.5%), 51 (33.6%), 9 (5.9%); T: 19 (51.4%), 16 (43.2%), 2 (5.4%); S: 21 (65.6%), 3 (9.4%), 8 (25.0%); Ural: 18 (69.2%), 4 (15.4%) 4 (15.4%); Orphan: 11 (73.3%), 3 (20.0%), 1 (6.7%); Uganda: 3 (42.9%), 2 (28.6%), 2 (28.6%). Scanty and moderate growth rates were observed for genotypes Lam (14 (66,7%); 7 (33.3%)) and Haarlem (12 (57.1%); 9 (42.9%)). Genotype X showed scanty growth rate in all 4 (100%) cases.

Higher colony counts occurred statistically more often ($p<0.001$; $p<0.05$) in genotype S (8-25.0%), then in genotypes Beijing (9-5.9%) and T (5-5.4%), based on detections of excessive colony counts in culture media, but compared to Ural, Orphan, Uganda genotypes, S family

Table 1

Prevalence of key genetic families of *M. tuberculosis* population in the entire region of Yakutia, in rural socio-geographical zones, and in Yakutsk

Administrative districts by socio-geographical zones	Genotypes, n (%)								
	Beijing	T	S	Ural	Haarlem	Lam	Orphan	Uganda	X
Arctic zone: 21 (6.7%)									
Abyysky	-	2	-	-	-	-	-	-	-
Allaikhovsky	1	-	-	-	-	-	-	-	-
Anabarsky	1	-	-	-	-	-	-	-	-
Bulunsky	-	-	-	2	1	-	-	-	-
Zhigansky	1	1	-	-	-	-	-	-	-
Momsky	1	-	-	-	1	-	-	-	-
Nizhnekolymsky	2	-	-	-	-	-	-	-	-
Olenyoksky	-	2	-	1	-	-	-	-	-
Srednekolymsky	2	-	-	-	-	-	1	-	-
Ust-Yansky	-	-	-	-	-	-	-	-	-
Eveno-Bytantaysky	1	-	-	-	-	-	-	1	-
Total, Arctic zone:	9 (2.8)	5 (1.6)	-	3 (0.9)	2 (0.6)	-	1 (0.3)	1 (0.3)	-
Industrial zone: 9 (2.8%)									
Aldansky	1	-	1	-	1	-	-	-	-
Lensky	-	-	-	-	-	1	-	-	-
Mirninsky	-	-	-	-	-	-	-	-	-
Neryungrinsky	2	-	-	-	-	-	-	-	-
Oymyakonsky	1	-	-	-	1	1	-	-	-
Total, industrial zone:	4 (1.3)	-	1 (0.3)	-	2 (0.6)	2 (0.6)	-	-	-
Agrarian zone: 55 (17.5%)									
Amginsky	3	-	-	1	-	-	1	-	1
Verkhnevilyuysky	7	-	1	-	-	-	-	-	-
Vilyuysky	3	2	-	-	-	-	-	-	-
Gorny	1	-	-	-	-	-	-	-	-
Megino-Kangalassky	7	-	-	1	-	1	-	-	-
Namsky	1	-	-	-	-	-	-	-	-
Nyurbinsky	4	-	4	-	-	-	1	-	-
Suntarsky	3	-	-	-	-	1	-	-	-
Tattinsky	2	-	-	-	-	1	-	-	-
Ust-Aldansky	-	1	-	1	-	1	-	1	-
Churapchinsky	2	1	1	-	-	-	-	-	1
Total, agrarian zone:	33 (10.5)	4 (1.3)	6 (1.9)	3 (0.9)	-	4 (1.3)	2 (0.6)	1 (0.3)	2 (0.6)
Hybrid zone: 25 (7.9%)									
Verkhnekolymsky	-	-	-	-	-	-	-	-	-
Verkhoyansky	2	1	-	-	1	-	-	-	-
Kobyaysky	3	2	-	-	-	-	-	-	-
Olyokminsky	2	-	-	-	-	-	-	-	-
Tomponsky	-	-	1	-	-	1	-	-	-
Ust-Maysky	-		-	1	1	-	-	-	-
Khangalassky	4	-	1	1	-	-	4	-	-
Total, hybrid zone:	11 (3.5)	3 (0.9)	2 (0.6)	2 (0.6)	2 (0.6)	1 (0.3)	4 (1.3)	-	-
Total, all rural socio-geographical zones n=110 (34.7%)	57 (18.1)	12 (3.8)	9 (2.8)	8 (2.5)	6 (1.9)	7 (2.2)	7 (2.2)	2 (0.6)	2 (0.6)
City of Yakutsk n=205 (65.3%)	95 (30.1)	25 (7.9)	23 (7.3)	18 (5.7)	15 (4.8)	14 (4.4)	8 (2.5)	5 (1.6)	2 (0.6)
Total, Sakha	152 (48.2)	37 (11.7)	32 (10.1)	26 (8.2)	21 (6.7)	21 (6.7)	15 (4.8)	7 (2.2)	4 (1.3)

showed no statistically meaningful differences.

Genotypes Haarlem, Lam, Uganda, S, T, Beijing, and Orphan showed fast growth rates, with mean durations varying between 22.9±1.5 and 28.8±2.0 days. Ural and X genotypes were characterized by slow growth rates (30.9±2.0 and 34.3±2.1 mean number of days, respectively). Predominating Beijing cluster did not differ from other genotypes, in terms of growth rate or colony count.

Table 3 shows comparative analysis of culture viability for key MTB genotypes.

As is seen in Table 3, out of 315 cultures, viability (combined assessment of growth rate and colony count) was high in 25 (7.9%), moderate in 206 (65.4%), and low in 84 (26.7%) cultures, respectively.

Comparison of genotypes by their viability showed high, moderate, and low viability as follows: Beijing: 37 (24.3%), 106 (69.7%), 9 (5.9%); T: 11 (29.7%), 24 (64.9%), 2 (5.4%); S: 6 (18.7%), 18 (56.2%), 8 (25.0%); Ural: 11 (42.3%), 12 (46.1%), 3 (11.5%); Orphan: 11 (73.3%), 3 (20.0%), 1 (6.7%). Low and moderate viability, respectively, was observed in genotypes LAM (2 (9.5%); 19 (90.5%)) and Haarlem (2 (9.5%); 19 (90.5%)). Genotype Uganda showed moderate (5; 85.7%) and high (2; 28.6%) viability. All isolates with X genotype (4; 100%) showed low viability. Interestingly, cultures with S genotype (8-25.0%) demonstrated reliably ($p<0.001$; $p<0.05$) higher viability compared to Beijing (9-5.9%) or T (5-5.4%), but showed no meaningful differences compared to Ural, Orphan, Uganda.

Shown in Fig. 1 is distribution of phenotypic MTB drug resistance types by key genotypes.

As is seen, out of 315 cultures, 207 (65.7%) were drug sensitive. Monoresistance was detected in 10 (3.2%) cases, and poly-resistance in 20 (6.3%) cases. MDR was present in 78 (24.8%) cases, of them, 5 (1.6%) were XDR.

Preserved sensitivity to all drugs was observed in the following genotypes: Beijing (101; 66.4%), T (31; 83.8%), S (3; 9.4%), Ural (14; 53.8%), LAM (17; 80.9%), Haarlem (20; 95.2%), Orphan (12; 80.0%), Uganda (6; 85.7%), X (3; 75.0%). Monoresistance was observed in Beijing (2; 1.3%), T (4; 10.8%), Ural (1; 3.8%), LAM (1; 4.8%), Haarlem (1; 4.8%), Uganda (1; 14.3%) genotypes. Poly-resistance was established in Beijing (2; 1.3%), S (6; 18.6%), Ural (10; 38.5%), LAM (1; 4.8%), X (1; 25.0%) genotypes. MDR was found in Beijing (49; 30.9%), T (2; 5.4%), S (23; 71.9%), Ural (1; 3.8%), LAM (2; 9.5%), Orphan (3; 20.0%). Of

Table 2

Comparative analysis of colony count and growth rate of *M.tuberculosis* strains by genotypes

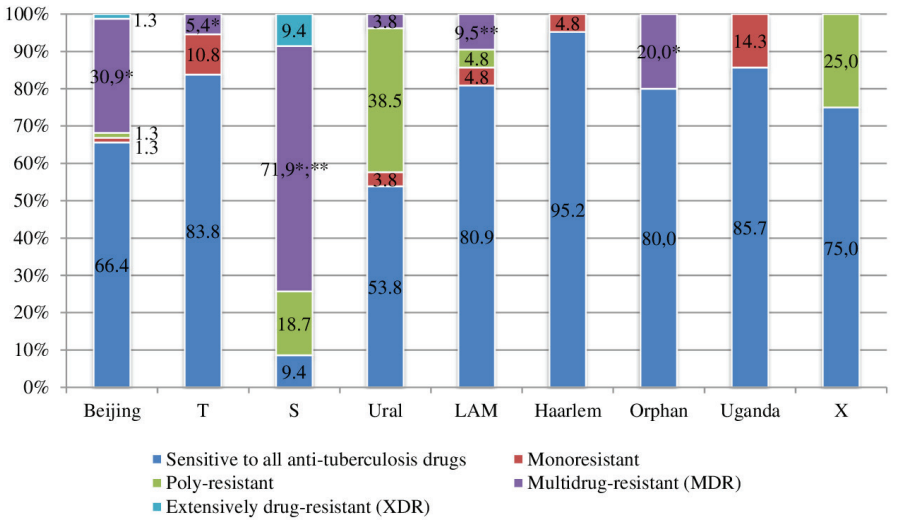
Genotype	Colony count, n (%)			Growth rate, n (%)		Mean growth rate, days (M ± m)
	1+	2+	3+	<30 days	>30 days	
Beijing n=152	92 (60.5)	51 (33.6)	9* (5.9)	116 (76.3)	36 (23.7)	27.6±0.8
T n=37	19 (51.4)	16 (43.2)	2** (5.4)	25 (67.6)	12 (32.4)	27.4±1.5
S n=32	21 (65.6)	3 (9.4)	8*; ** (25.0)	25 (78.1)	7 (21.9)	26.5±1.1
Ural n=26	18 (69.2)	4 (15.4)	4 (15.4)	14 (53.8)	12 (46.2)	30.9±2.0
Lam n=21	14 (66.7)	7 (33.3)	-	19 (90.5)	2 (9.5)	23.6±1.2
Haarlem n=21	12 (57.1)	9 (42.9)	-	19 (90.5)	2 (9.5)	22.9±1.5
Orphan n=15	11 (73.3)	3 (20.0)	1 (6.7)	12 (80.0)	3 (20.0)	28.8±2.0
Uganda n=7	3 (42.9)	2 (28.6)	2 (28.6)	7 (100.0)	-	25.0±1.9
X n=4	4 (100.0)	-	-	1 (25.0)	3 (75.0)	34.3±2.1
Total n= 315	194 (61.6)	95 (30.1)	26 (8.3)	238 (75.5)	77 (24.4)	27.4±1.2

Note: Colony count per tube was scored using 3-grade scale:
(1+): 1-20 CFU – scanty bacterial load;
(2+): 21-100 CFU – moderate bacterial load;
(3+): >100 CFU – excessive bacterial load;
*statistically significant difference between variables compared, $p<0.001$;
**statistically significant difference between variables compared, $p<0.05$.

Table 3

Comparative analysis of *M.tuberculosis* strains by genotypes and viability

Genotypes	Viability level					
	Low		Moderate		High	
	Абс. ч.	%	Абс. ч.	%	Абс. ч.	%
Beijing (n=152)	37	24,3	106	69,7	9*	5,9
T (n=37)	11	29,7	24	64,9	2**	5,4
S (n=32)	6	18,7	18	56,2	8*; **	25,0
Ural (n=26)	11	42,3	12	46,1	3	11,5
Lam (n=21)	2	9,5	19	90,5	-	-
Haarlem (n=21)	2	9,5	19	90,5	-	-
Orphan (n=15)	11	73,3	3	20,0	1	6,7
Uganda (n=7)	-	-	5	85,7	2	28,6
X (n=4)	4	100,0	-	-	-	-
Total: n=315	84	26,7	206	65,4	25	7,9



Comparative characteristics of drug resistance types between *M. tuberculosis* genotypes

them, XDR was determined in Beijing (2; 1.3%) and S (3; 9.4%) genotypes.

In terms of drug-resistance, genotypes Haarlem, Uganda, and X were regarded as favorable, due to absence of MDR cases. Genotypes Ural, Orphan, LAM, and T were deemed less favorable, as 53.8% to 83.8% cases with theses genotypes proved drug-sensitive or else showed minimal spectrum of DR. Genotypes Beijing and S were considered unfavorable, based on the incidence of MDR (47 (30.9%) for Beijing; 23 (71.9%) for S), including cases with XDR (1.3% and 9.4%, respectively). Moreover, genotype S showed statistically higher incidence of MDR ($p<0.001$; $p<0.05$) among all the rest.

Conclusion. Using advanced molecular genetic methods for identification of MTB isolates, we were able to compile detailed description of MTB population circulating in one of the underexplored northern territories of Russia. Isolates circulating in the Sakha Republic (Yakutia) were presented by 9 key heterogeneous clusters: Beijing, T, S, Ural, Lam, Haarlem, Orphan, Uganda, and X. Beijing genotype was the most prevalent in the MTB population (152; 48.2%).

Analysis of growth rates, colony counts, drug sensitivity of the key genotypes of MTB isolated from newly diagnosed patients with PTB showed lack of uniformity in MTB isolates, in terms of their biological properties. Assessment of colony counts showed the following variations: scanty colony count in 43.9% to 100% cases; moderate colony count in 9.4% to 43.2% cases; excessive colony count ranging from 5.4% to 28.6% cases. Assessment of growth rates showed fast growth in 25.0% to 100%, and slow growth in 9.5% to 75.0% of cases. Mean growth rate was 27.4±1.2 days. Viability, based on combined assessment of growth rate and colony count, was assessed as high in 25 (7.9%) cultures, moderate in 206 (65.4%), and low in 84 (26.7%) cultures. By drug sensitivity, 9.4% to 95.2% were sensitive, and 5.4% to 71.9% were multidrug-resistant. Beijing genotype, along with S family, are the two epidemiologically significant families in a region of Yakutia, based on the incidence of MDR.

Viability of the causative agent can reliably be linked to emergence of primary drug resistance in MTB population; this can be clearly seen in the case of S family. Consequently, excessive MTB colony count implies that patients isolating such strains pose epidemiological risk, especially in cases where fast growth is coupled with multidrug-resistance.

Further in-depth studies are necessary to keep looking into the prevailing MTB genotypes circulating in Yakutia, for the purposes of epidemiological surveillance and monitoring of TB infection.

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FIBROBLAST PROLIFERATION AND ADHESION *IN VITRO* ON POLYLACTIDE FILMS

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Abstract: Fibroblasts are a versatile biological model for the *in vitro* study of dynamic molecular regulatory processes underlying cell growth and proliferation, metabolism and transduction of intracellular signals. **The aim of the research** was to evaluate the proliferative activity and adhesive capacity of cultured *in vitro* fibroblasts on polylactide films of different thicknesses depending on the presence of the adhesive factor.

Materials and methods. Laboratory samples of polylactide films were made of L-PLA 4043D NatureWorks (USA) by extrusion and films with a thickness of 100. 125 and 150 μn with a smooth surface were obtained. The subject of the study was cultured fibroblasts (2nd passage) isolated from loose fibrous connective tissue of the peritoneum of a practically healthy person with an appendectomy with pre-signed informed consent. Small pieces of fabric under sterile conditions were ground into pieces from 1 mm to 2 mm and 2-3 pieces each were placed in petri dishes. Cell culture was carried out using DMEM/F-12 (1:1) (1X), FBS 20%, anti-anti (1x100), sodium pyruvate (1x100). Incubated at 37 C in an atmosphere enriched with 5% CO2 for 5 days.

Results. Thus, the addition of adhesive factor AF comprising gelatin to the nutrient medium activates the proliferation of cultured cells almost 1.98 times (p = 0.000) than without it; the largest number of attached fibroblasts is observed on the 3rd day with substrates of 100 μn and 150 μn in wells with AF, while in substrates with a thickness of 125 μn - on the 1st day and the 3rd day of cultivation. Based on supravitally stained samples, it was found that the adhesion of fibroblasts, depending on the thickness of the polylactide film, showed statistically significant differences (p = 0.022) between films with a thickness of 125 microns (37.67 ± 7.63) and 100 microns (20.75 ± 8.51), as well as an upward trend (p = 0.068) between 125 μN (37.67 ± 7.63) and 150 μN (25.67 ± 10.67). Only at a polylactide thickness of 125 microns is the number of fibroblasts on the surface of the film higher than in the well, and at other thicknesses, the number of cells in the well is greater than on the polylactide.

Keywords: proliferation, adhesion, polylactide, *in vitro*, fibroblasts.

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Introduction. Fibroblasts represent a universal biological model for studying in vitro dynamic molecular regulatory processes underlying cell growth and proliferation, metabolism and transduction of intracellular signals [4].

Most tissue engineering substitutes for living skin are created by culturing skin cells under laboratory conditions and combining them with a substrate. Application of skin equivalents accelerates wound healing, reduces pain syndrome, inflammation, as well as prevents scarring, contracture or pigment defects [13].

Polylactic acid, its copolymers and composites are part of the modern class of biodegradable materials and are widely used for the manufacture of various implants [1]. Polymer matrices made of polylactic acid are biocompatible products possessing bioactive properties in relation to regenerative processes and reactions of blood system during subcutaneous implantation in laboratory rats [3]. Biodegradable polymers such as polylactide and poly (e-caprolactone) are widely used in biomedicine as polymer scaffolds to promote tissue and cell growth during bone regeneration, as well as for drug delivery when drugs are mixed with a polymer. matrix and are gradually released as the biopolymer decomposes in the human body [10, 11]. However, due to its hydrophobicity, the interaction of cells with this material is far from optimal [9]. Biofunctionalization of the surface of many biodegradable polymers is one of the strategies used to improve the biological activity of such materials [6]. The high molecular weight polylactide is a colorless, glossy, rigid thermoplastic polymer which can be semi-crystalline and completely amorphous depending on the purity of the polymer backbone. Both lactic acid and polylactide exhibit optical activity, that is, they exist as two L- and D- stereoisomers. Lactic acid is very hygroscopic, so lactides are used instead [2].

The development of scaffolds for use in tissue engineering requires careful selection of properties such as mechanical characteristics, porosity and biodegradation. Scaffold surface properties are an important criterion because they affect cell adhesion, proliferation, and differentiation. It is known that the topography of the surface affects the cellular response, but the mechanisms governing this remain unclear [7].

The aim of the research was to assess the proliferative activity and adhesive capacity of cultured *in vitro* fibroblasts on polylactide films of different thicknesses depending on the presence of adhesive factor.

Materials and methods. Laboratory samples of polylactide films were made of L-PLA 4043D NatureWorks (USA) based on the UNTL "Polymer Nanocomposite Technologies" NEFU named after M.K. Ammosov by extrusion at the

following parameters: melt temperature - 175-180 °C; supply area - 170 °C; sealing zone (plasticisation zone) - 174 °C; injection zone (dosing zone) - 175 °C; die (extrusion head) - 180 °C. As a result, films with a thickness of 100, 125 and 150 nm with a smooth surface were obtained. These films can be easily cut with scissors, which corresponds to the requirement for the matrices.

An in vitro experimental study was carried out in the research laboratory "Cellular Technologies and Regenerative Medicine" of the Medical Institute of NEFU named after M.K. Ammosov. The subject of the study was cultured fibroblasts (2nd passage) isolated from loose fibrous connective tissue of the peritoneum of a practically healthy person with an appendectomy with pre-signed informed consent. Small pieces of fabric under sterile conditions were ground into pieces from 1 mm to 2 mm and 2-3 pieces each were placed in petri dishes. Cell culture was carried out using DMEM/F-12 (1:1) (1X), FBS 20%, anti-anti (1x100), sodium pyruvate (1x100). Incubated at 37 C in an atmosphere enriched with 5% CO2 for 5 days.

After achieving monolayer growth in polystyrene petri dishes (diameter 60x15 mm) on day 2 of culture, a passage was made into bottoms with a bottom size of 75 cm2. Two days later, by trypsinization, the cell suspension (1.0-1.3x106/ml) after 2 times washing with PBS and centrifuging was transferred to a bottom of 15 µl in 24-well standard culture plates with an area of 2 cm2. For everyone their three thickness of polylactide (100, 125 and 150 мкн) about 12 holes from which 6 holes were processed within 5 min. by an adhesive factor - AF (Attachment Factor 1X, Cascade Biologist TM) containing gelatin as an attachment factor were estimated. The polylactide supports cut along the diameter of the wells (1.75 cm) were placed on the bottom of the wells. The culture plates were incubated at 37 C in an atmosphere enriched with 5% CO2 for 5 days.

Daily cell counting was carried out from inverted microscope images (LOMO, Russia) with an increase of 60 times (eyepiece x15, lens x 4) and visualization of 5 fields of vision on each well. A total of 180 wells were analyzed, of which 90 wells with an adhesive factor (30 wells for thicknesses of 100 µn, 125 µn, 150 µn) and 90 wells (30 wells for thicknesses of 100 µn, 125 µn, 150 µn) - without it. The number of rounded (not attached) and elongated (attached) fibroblasts was calculated, as well as their sum - the total number of cells (Fig. 1).

Statistical analysis performed with IBM SPSS Statistics version 19. To compare related groups (in dynamics), the Wilcoxon test was used; to compare independent samples, the nonparametric Mann-Whitney and Kruskal-Wallace tests were used. The critical value of the significance level (p) was taken equal to 0.05.

Results and Discussion. Comparative analysis of the total number of cells (round and elongated cells) in the test wells depending on AF treatment revealed statistically significant differences (p = 0.000) on all follow-up days (1st to 4th days). In 90 wells with adhesive factor, the average value of the total number of cells turned out to be statistically significantly higher (on average 1.98 times) than without it (Fig. 2).

Statistical analysis performed with IBM SPSS Statistics version 19. To compare related groups (in dynamics), the Wilcoxon test was used; to compare independent samples, the nonparametric Mann-Whitney and Kruskal-Wallace tests were used. The critical value of the significance level (p) was taken equal to 0.05.

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It should be noted that on polylactide films, fibroblasts attach unevenly on the surface, significantly more cells are observed closer to the edge of the films than in the center. [7] observed an increase in cellular response with an in-

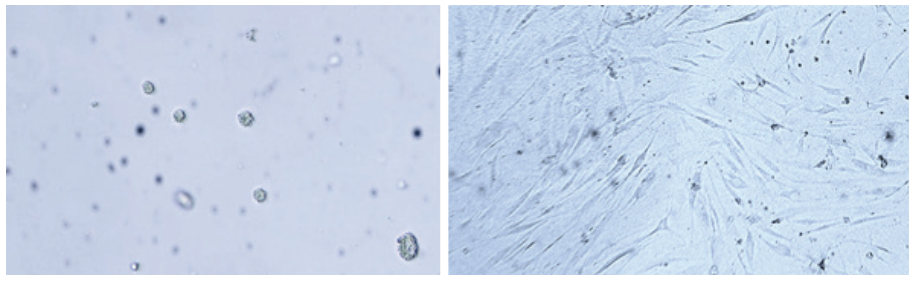

Fig. 1. Rounded unattached fibroblasts on the day of planting (left) and on the 4th day of cultivation (right) - spindle-shaped attached cells

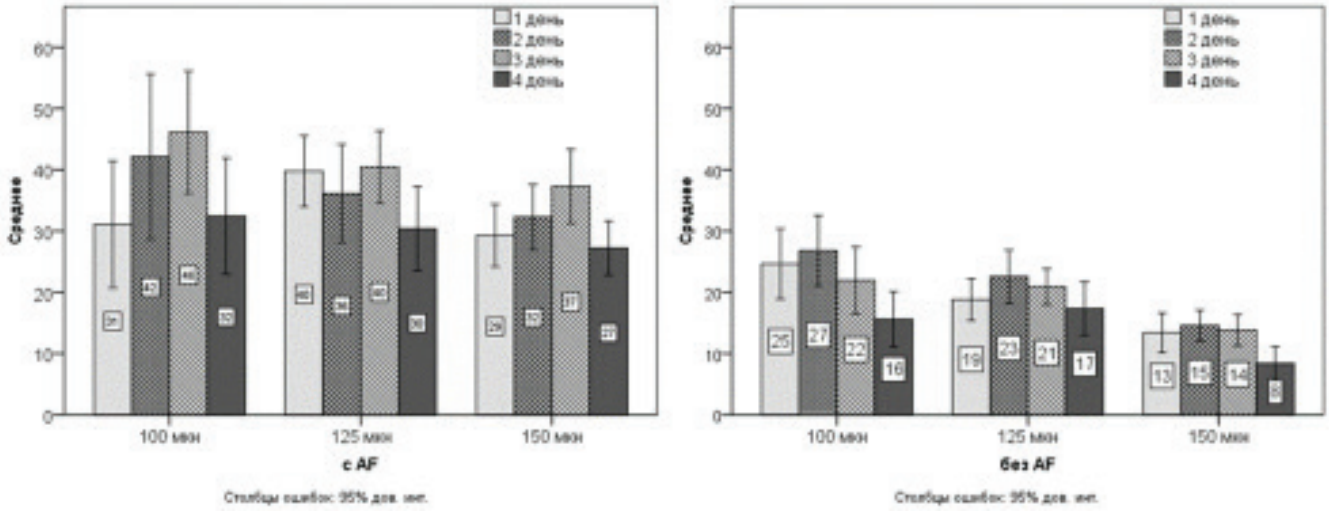

Fig. 2. The total number of cells in wells with AF and without AF in dynamics.

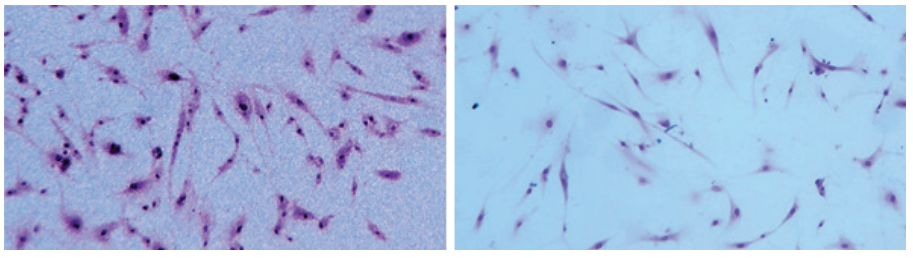

Fig. 3. Stained fibroblasts on day 4 of culture: (left - cells on the surface of the polylactide 125 μn thick, right - on the bottom of the well)

crease in edge density. This may possibly be due to preferred protein adsorption or conformational changes occurring on surface irregularities. Protein adsorption or conformational changes preferably occur on surface irregularities. This has been proposed as a mechanism of the so-called contact alignment phenomenon experienced by cells seeded on parallel ridges, where it has been found that focal adhesions are predominantly formed on the edges and lateral walls of such features [12].

Conclusion. Thus, the following conclusions can be drawn from the results obtained:

- addition in nutrient medium of an adhesive factor of AF including gelatin in the structure activates proliferation of the cultivated cages almost by 1.98 times (p =0.000), than without her;

- the largest number of attached fibroblasts is observed on the 3rd day with substrates 100 μn and 150 μn in wells with AF, while in substrates with thickness 125 μn - on the 1st day and the 3rd day of cultivation;

- according to supravitally colored samples, it was found that the adhesion of fibroblasts depending on the thickness of the polylactide film showed statistically significant differences (p = 0,022) between films with a thickness of 125 μn (37.67 ± 7.63) and 100 μn (20.75 ± 8.51), as well as a tendency to increase (p = 0,068) between 125 mkn (37.67 ± 7.63) and 150 μn (25.67 ± 10.67). Only with a polylactide thickness of 125 μn the number of fibroblasts on the film surface is higher than in the well, and with other thicknesses the number of cells in the well is higher than on the polylactide;

- on polylactide films, fibroblasts attach unevenly on the surface, significantly more cells are observed closer to the edge of the films than in the center.

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MORTALITY AND DISABILITY OF THE POPULAITON IN YAKUTIA FROM EXPOSURE TO LOW AMBIENT TEMPERATURES

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The official statistics was used to study the consequences of cold temperature exposure for the human organism in the Sakha Republic (Yakutia), a region with the subarctic climate, where the average winter temperature is – 35-40°C. The article reviews the mortality rate in the region due to cold temperature exposure in 2011-2019 and the disability level in 2014-2019. It was revealed that cold temperature exposure (cold injury) ranks third among the external causes of death in Yakutia, following suicides and homicides. The mortality rate due to cold injuries exceeds the traffic fatality rate in traffic accidents (TA). In the period 2011-2019, cold injuries killed 1,339 people (mortality rate 15.6 per 100,000 persons), while TA – 1,116 (12.9 per 100,000 persons). The percentages of disability due to severe frostbites were relatively low: in 2017 – 0.06 per 1,000 persons; in 2018 – 0.05; and in 2019 – 0.05.

Keywords. High North, low natural temperature, mortality, disability.

Introduction. The loss of health (disability, premature mortality) due to exposure to cold natural temperatures is one of the poorly understood health problems. The data of scientific publications indicate the global relevance of this issue. For instance, in the period 2003-2013, the USA registered 13,419 hypothermia-related deaths, which makes 0.3-0.5 cases per 100,000 population. Males accounted for 67%; there is also an age-specific feature: for males and females over 65 years of age, the rate was 1.8 and 1.1 cases per 100,000 population, respectively [5]. In Sweden, mortality due to hypothermia, frostbite and cold-water drowning is 3.4, 1.5 and 0.8 cases per 100,000 population, respectively [4]. According to foreign literature, the mortality rate in severe hypothermia ranges from 12 to 80% and depends on age, predisposing factors, causes and how prompt the treatment is [5]. However, the problem is especially acute in the Russian Federation, whose vast territories lie in the northern latitudes. In Amur region, patients with cold injuries account for 12-19% (60-90 people annually) of the patients in the Thermal Injuries Department. In Chita region, the annual regional rate of hospitalization for patients with cold injury is 1.9 cases per 10,000 population [2].

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Among the constituent entities of the Russian Federation, Yakutia is the largest with the area of 3,103.2 square kilometers; yet, in terms of the population density, it ranks 83 out of 85 regions (0.3 people per 1 square kilometer); 90% of the area does not have year-round transport access. The cold season in the region lasts for 7 months a year with the average winter temperature of - 35-40°C. In such conditions, the impact of cold natural temperatures on the human body is significant in the structure of deaths from external causes, and is an urgent medical and social problem.

It should be noted that the medical and demographic situation in the Sakha Republic (Yakutia) in 1990-2014 was characterized by a high rate of premature mortality from preventable causes, primarily accidents, injuries, and poisoning. In the structure of the main causes of deaths, external causes consistently ranked second after circulatory system diseases [1]. Since 2015, external causes have moved to third place (circulatory system diseases – neoplasms – external causes). The decrease in mortality from all types of external causes in 2011-2019 amounted to 39.8% (from 181.8 to 109.4 per 100,000 population). As a rule, the official statistics distinguishes the following external causes of mortality: transport accidents, including traffic accidents, violent deaths (homicides, suicides), accidental alcohol poisoning, accidental drowning, accidental falls, etc. Cold injury falls under the category of "Other causes"; therefore, the real situation with the loss of health by the population due to exposure to cold natural temperatures remains hidden.

The aim of the current study was to assess the morality and disability of the population in the Sakha Republic (Yakutia) due to cold temperature exposure in the period 2011-2019.

Material and Methods. The analysis of mortality rates of the population from exposure of cold natural temperatures in the Sakha Republic (Yakutia) was carried out by a selective statistical method basing on the official data from the Regional Office of the Federal State Statistics Service in the Sakha Republic (Yakutia) for the period 2011-2019. On the disability, the data of the Federal State Institution "Main Bureau of Medical and Social Expertise in the Sakha Republic (Yakutia)" for the period 2014-2019 were studied. The selection criteria under ICD-10 were Code 302 (Exposure to excessive natural cold) to study the mortality; Codes T34 (Frostbite with tissue necrosis), T35 (Frostbite involving multiple body regions and unspecified frostbite), and T69.8 (Other specified effects of reduced temperature) – to study the disability.

Results. The study period witnessed a positive trend of the decreased mortality rate from external causes in the republic: from all types of external causes – by 39.8% (from 181.8 to 109.4 per 100,000 persons); from suicides – by 42.3 % (from 39.7 to 22.9 per 100,000 persons); from homicides – by 52.0% (from 27.9 to 13.4 per 100,000 persons); from cold injuries – by 53.0%; from all types of transport accidents – by 54% (from 18.9 to 8.7 per 100,000 persons), including traffic-related deaths – by 47.0% (from 16.4 to 8.7 per 100,000 persons) (Tab. 1).

A retrospective analysis of the statistical data revealed that more people die annually of exposure to cold natural temperatures than in traffic accidents in the Sakha Republic (Yakutia). According to Rosstat, traffic accidents killed 1,116 people there in the period 2011-2019, whereas exposure to extremely cold natural temperatures – 1,339, which was

Table 1

The mortality in the Sakha Republic (Yakutia) from some types of external causes in 2011-2019 (absolute number, rate per 100,000 persons)

Indicators	2011		2015		2017		2018		2019	
	abs.n	per 100,000 persons	abs.n	per 100,000 persons	abs.n	per 100,000 persons	abs.n	per 100,000 persons	abs.n	per 100,000 persons
Total deaths of external causes	1740	181.8	1392	145.5	1287	133.6	1184	122.6	1061	109.4
Suicides	380	39.7	333	35.1	267	27.7	231	23.9	222	22.9
Homicides	267	27.9	198	20.7	175	18.2	138	14.3	130	13.4
Cold temperature exposure	195	20.4	145	15.1	147	15.3	128	13.3	93	9.6
Transport accidents	181	18.9	125	13.1	119	12.3	117	12.1	84	8.7
- *including traffic accidents*	157	16.4	119	12.4	112	11.6	114	11.8	84	8.7

Table 2

The number of deceased due to traffic accidents and low temperature exposure in 2011-2019

Cause of death	2011		2012		2013		2014		2015		2016		2017		2018		2019		Всего	
	abs.n	per 100,000 persons	abs.n	per 100,000 persons	abs.n	per 100,000 persons	abs.n	per 100,000 persons	abs.n	per 100,000 persons	abs.n	per 100,000 persons	abs.n	per 100,000 persons	abs.n	per 100,000 persons	abs.n	per 100,000 persons	abs.n	per 100,000 persons
traffic accidents	157	16.4	134	14.0	156	16.3	140	14.6	119	12.4	100	10.4	112	11.6	114	11.8	84	8.7	1116	12.9
Cold temperature exposure	195	20.4	164	17.6	150	15.7	158	16.5	145	15.1	159	16.5	147	15.3	128	13.3	93	9.6	1339	15.6

Table 3

The number of deceased due to low temperature exposure in 2011-2019 by main age groups *

Age group	2011		2012		2013		2014		2015		2016		2017		2018		2019		total	share (%)
	male	female	male	female	male	female	male	female	male	female	male	female	male	female	male	female	male	female		
under working age	4	1	0	1	2	0	2	0	0	0	3	-	1	-	-	-	2	-	16	1,2
working-age	108	34	113	24	85	28	91	25	80	35	97	20	92	19	79	19	54	9	1012	78,0
over working age	21	14	12	8	17	11	20	12	18	10	20	13	16	19	19	11	16	12	269	20,7
total	133	49	125	33	104	39	113	37	98	45	120	33	109	38	98	30	72	21	1297	-
age unspecified	13	-	5	1	6	1	6	2	2	-	4	2	-	-	-	-	-	-	42	3,1
TOTAL	146	49	130	34	110	40	119	39	100	45	124	35	109	38	98	30	72	21	1339	-

* under working age – 0-15 years, working age – males 16-59 years, females – 16-54 years, over working age – males 60 years and older, females – 55 years and older.

16.7% more; the mortality rates were 12.9 and 15.6 per 100,000 population, respectively (Tab. 2).

Of all the deceased due to exposure to cold natural temperatures, 75.3% were males (1,008 out of 1,339). In terms of the age structure (in 1,297 cases of reliably established age of the deceased), working-age people accounted for 78.0%, under working age – 1.2%, over working age – 20.7%, with the prevailing share of able-bodied males – 61.6% (799 of 1,297 deceased) (Tab. 3). The presented data confirm the consistent "supermortality" of the male population, despite the significant positive dynamics in the recent years.

The disability due to frostbites in extremities is another serious medical and social problem. Indeed, the amputation of a limb entails a serious injury, leading to limited physical capabilities and deteriorated quality of life. In one of our previous publications, we pointed at a discrepancy between the number of persons who have undergone limb amputation and the number of persons qualified as disabled for this reason. For instance, in 2014-2016, in a specialized ward for patients with thermal injuries, 40 patients underwent lower leg amputation and 9 patients – forearm and hand amputation (excluding the cases of fingers amputation); yet, only 14 were qualified as disabled. According to the data from the Main Bureau of Medical and Social Expertise in the Sakha Republic (Yakutia), 27 people qualified as disabled due to exposure to extremely cold natural temperatures in

the period 2014-2016. The most common cause of disability was amputation of frostbitten limbs (55.6%), including 66.7% arm amputations and 33.3% of leg amputations [3].

According to the official data from the Main Bureau of Medical and Social Expertise in the Sakha Republic (Yakutia), in the period 2017-2019, 154 patients qualified as disabled due to frostbites (hypothermia), including 4 (2.6%) persons under 18 years of age (Tab. 4).

Table 4

The number of people qualified as disabled due to frostbites (hypothermia) in 2017-2019

Years	Total disabled children under 18	qualified at:		Total disabled persons ≥ 18	qualified at:	
		first examination	second examination		first examination	second examination
2017	1	0	1	59	28	31
2018	1	1	0	47	29	18
2019	2	0	2	44	25	19

According to experts, official statistics reflects only about a half, at most, of the people with disabilities who actually exist in society, which is due to several reasons. Firstly, it does not include the disabled who have been examined and recognized as disabled by expert commissions, but have not applied for a pension from the social security authorities. Secondly, since the disability accountability is based on the source of pension provision, people with disabilities receiving other types of pensions (by age, loss of breadwinner, etc.) are not included in the general statistics. Thirdly, some of the disabled who receive pensions in other departments (for example, the Ministry of Defense, the Ministry of Internal Affairs, the Federal Security Service, etc.) are also not included in the general statistics.

Conclusion. Thus, the case of the Sakha Republic of (Yakutia) demonstrates how critical is the impact of excessively cold natural temperatures on the human body in the High North due to its significant frequency, disability of patients and high mortality in cases of severe hypothermia. The problem under discussion, as well as other external causes of mortality, is not only medical, but also social in nature, since it goes far beyond the scope of the health care system responsibilities. There are two main negative factors contributing to the problem: the lack of self-preservation (non-compliance with safety rules) and the spread of alcoholism, like in the situation with traffic-related deaths. In other words, “cold injury” is indeed a preventable factor of premature mortality and disability in people of predominantly young age groups. An unfavorable background is the vast territory of the region, great remoteness of settlements, impassable roads, lack of infrastructure and stable satellite communications for a timely call for professional medical services. In this regard, the problem is especially acute in the Arctic districts of the republic; it can be solved only by a specific set of measures taken by the state authorities.

In this situation, medical workers have to work with something that has already happened, that is to say, to deal with the treatment (elimination) of the consequences of the traumatic factor. It is obvious that the clinical course and outcome of the injury will depend on the timely diagnosis, scope and adequacy of first aid and subsequent pathogen-based treatment. At the same time, many issues related to diagnosis and treatment tactics remain insufficiently studied or are controversial. There are no uniform recommendations, or treatment standards for patients with general hypothermia or frostbites; numerous issues in the patient management tactics remain debatable, including the preference for passive or active warming in cases of hypothermia, use of extracorporeal methods, thrombolytic therapy, as well as the timing of surgical treatment [2]. These facts indicate the need for developing clinical guidelines for the diagnosis and principles of treating the consequences of exposure to excessively cold temperatures (cold injury), based on the latest scientific advances and accumulated practical experience.

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CYTOLOGICAL EXAMINATION IN THE DIAGNOSIS OF PATHOLOGY OF THE CERVIX

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A cytological study of the cervix was conducted in women aged 18 to 44 years. It was found that the highest rate of background and precancerous diseases of the cervix falls on women aged 30-44 years (62.1%).

Early detection and treatment of background and precancerous cervical diseases will help reduce cervical cancer incidence and mortality.

Keywords: diagnostics, dysplasia, cervix.

Relevance. Pathology of the cervix remains an urgent problem, regardless of recent achievements in the diagnosis and treatment of this disease. The pathology of the cervix in women of reproductive age is of particular importance. Recently, cervical diseases have shown an increasing rate among women under the age of 40 [5, 10]. Women in the active reproductive phase are vulnerable to the development of various oncological processes because of the rejuvenation of the disease [11]. The high prevalence of neoplastic pathology of the cervix is caused by the defeat of papillomavirus infection [4,7,12]. Infection with the human papillomavirus (HPV) and other sexually transmitted diseases increases the risk of dysplasia by 10 times [1,2,3,4,14]. In modern practice, the pathology of the cervix is divided into the background, precancerous, and tumor processes. Background diseases include dyskeratosis, glandular hyperplasia, and erosion. Precancerous conditions include moderate and severe dysplasia. The peculiarities of the hormonal balance in women of reproductive age, which depends on exogenous hormones, determine the predominance of background diseases in them [6,8]. Timely diagnosis and treatment of background and precancerous diseases of the cervix contribute to the early prevention of cervical cancer, which ranks fourth among malignant neoplasms in Russia [6,15].

The objective of this article is to study the frequency of background and precancerous diseases of the cervix in women of reproductive age in Yakutia.

Materials and methods. The analysis of smears taken from the mucous membrane of the cervix and cervical canal in 2280 women aged 18 to 44 years was carried out on the basis of the NEFU Clinic named after M. K. Ammosov. The materials were delivered from medical and preventive institutions of the Republic of Sakha (Yakutia) with the informed consent of the respondents to the study according to the protocol of the ethics committee of the YSC CMP

The cytological study included the preparation of exfoliative cervical smears in accordance with the generally accepted method, which was characterized by air drying and staining according to the Romanowsky-Giemsa method. The results of the study corresponded with the clinical and morphological classification of Ya. V. Bokhman (1976)

Results and discussion. Background diseases and dysplasia of various grades were found in 417 examined women (18.28%). Background diseases were diagnosed in 310 women, which was 74.4% of the number of women with cervical pathology. For our study, 2 comparison groups were formed by age characteristics: group I - women from 18 to 29 years old and group II - from 30 to 44 years old. A high frequency of background diseases was found in group II - 193 women , which accounted for 62.3% of all women with background diseases, and in group I - 117 cases (37.7%). The structure of background diseases is shown in picture 1. Squamous metaplasia and proliferation of the glandular epithelium were more often detected in the cervix with background diseases, each of which was detected in 72 cases (23.2% each). Squamous metaplasia occurred in both groups almost equally – in 41 (13.2%) and 31 (10%) cases. The proliferation of the glandular epithelium occurred more frequently in women aged 30-44 years – 47 (15.2%) cases. 35 women were diagnosed with hyperplasia by the type of dysplasia. The maximum value of its frequency was noted in the age group of 30-44 years and amounted to 8.4%. Hyperkeratosis was much less common - in 21 women (in 6.7% of the total number of women with background diseases). The maximum frequency of this pathology was also found in the age group of 30-44 years (5.5%).

110 cases with remote causes of the virus infection were detected, which accounted for 35.5% of the total number of women with background diseases. The highest number of this pathology was detected in women of group II – 62 cases (20%) against 48 cases (15.5%) in group I.

Cervical dysplasia of varying severity was detected in 107 women. Analyzing the frequency of cervical dysplasia, it has been shown that CIN 1was the most frequently registered - 71 cases (66.3%). The highest number was observed in the age group II -1.5 times more (39.2% vs. 27.1%). (Pic. 2). The highest number was observed in the age group II -1.5 times more (39.2% vs. 27.1%). (Pic. 2). CIN 2 was also most common in women aged 30-44 years, which accounted for 16.8%, in group I - 10.3%. A more severe lesion (grade 3 dysplasia) was registered 6 times more in group II (5.6%) than in group I - 0.9%.

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Women aged from 29 to 44 years, who were corresponding to fertility age were examined in the NEFU Clinic. The data obtained showed that the maximum frequency of background and precancerous diseases of the cervix falls on women in mature fertile age – from 30 to 44 years.

Conclusion. Thus, women of reproductive age living in the Republic of Sakha (Yakutia), and who applied for medical and preventive care were examined. The data showed that background diseases are the most commonly registered among women with background and precancerous diseases. Early diagnosis of cervical pathology showed a relatively low incidence of detected precancerous diseases. However, this incidence remains high, as 25 % of women have a precancerous condition. It has been shown that women aged 30-44 years are more often diagnosed with background diseases of the cervix, the remote causes of papillomavirus infection, CIN 1and CIN 2, and, significantly more often, severe epithelial lesions - CIN 3.

This included the need for screening of women for early diagnosis of this pathology, prevention, and timely treatment. It will reduce the number of advanced cancer types, improve the life quality of women, and increase their fertility.

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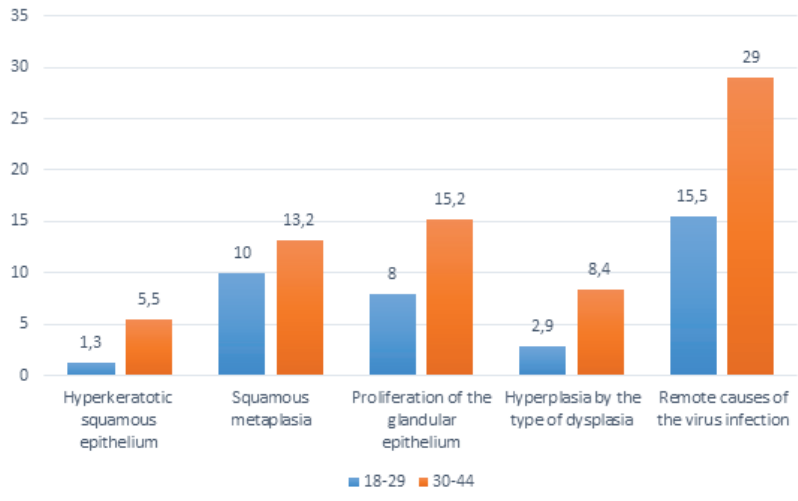
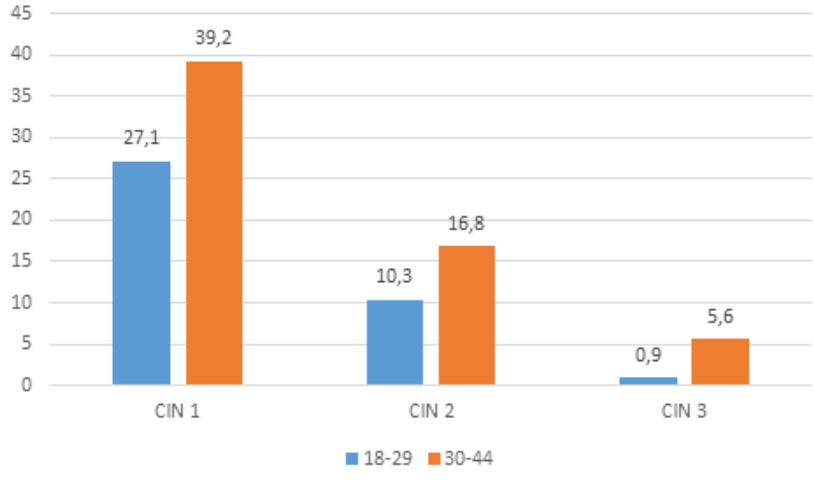


Pic.1. The frequency of background and precancerous diseases of the cervix in women of reproductive age (%)



Pic. 2. Dynamics of the occurrence of dysplasia in women of reproductive age (%)

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ARCTIC MEDICINE

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SEASONAL CHANGES INTHE PITUITARY-THYROID SYSTEM IN CHILDREN OF THE ARCTIC REGIONS OF YAKUTIA

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In recent years, work on the study of adaptive mechanisms and reserves of the body in the Arctic has become increasingly relevant. One of the areas of research is the study of the role of the endocrine system in the adaptation process. The article presents the data of the study of seasonal fluctuations of pituitary-thyroid hormones in 362 children of the Arctic regions of Yakutia. Higher TSH levels were found in winter, which should be considered as physiological shifts reflecting adaptation to the changing season of the year.

Keywords: hormones, pituitary gland, thyroid gland, thyroid-stimulating hormone, thyroid profile, Yakutia, seasons, Arctic.

Introduction. The impact of a complex of extreme climatic and geographical factors of the North is accompanied by dynamic changes of the parameters of the functional systems of human body. In regulation of this adaptive restructuring the leading role is played by the endocrine system. Thyroid gland is considered as one of the main components, providing effective adaptation to conditions of cold climate [3, 9]. In Arctic conditions activity of thyroid gland is often limited by low content of iodine, which is necessary for its normal functioning [1, 4, 7].

In childhood and adolescence even a slight change of adaptive processes can lead to the disease development and, in the absence of correction, to its chronization [5, 7, 8]. Thyroid hormones affect on metabolic processes occurring in the body, height and development of the child. The analysis of the literature showed that despite the presence of researches on age and gender characteristics [2, 5, 7, 8], there is not enough information about seasonal changes of the pituitary-thyroid system of children.

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In this regard the objective of the research was to explore the seasonal dynamics of the pituitary-thyroid system in children of the Arctic regions of the Republic of Sakha (Yakutia) as one of the components of adaptation to extreme environmental conditions.

Materials and methods. Panel researches were conducted among children and adolescents of the Arctic regions of Yakutia during field medical expeditions in 2018-2020. A total of 362 children living in Bulunsky, Anabarsky, Allaikhovsky, Nizhnekolymsky, Verkhnekolymsky districts were examined. The distribution of the examined children and adolescents by gender, age, and season of biological material collection is shown in Table 1.

Laboratory researches were conducted at clinical and diagnostic laboratory of Clinic of Medical Institute of SVFU named after M. K. Ammosov. Thyroid-stimulating hormone (TSH) and free thyroxine were determined by enzyme immunoassay (FT4).

The research was approved by local ethics committee on biomedical ethics by FGBNU « Yakut Scientific Center for Complex Medical Problems» (Yakutsk city, protocol №4, March 1, 2018).

Statistical calculations were complited using software IBM SPSS Statistics 17 (IBM®, USA). For comparison of groups was used Mann-Whitney criteria. Critical value of significance level for statistical hypotheses checking was accepted equal 5%.

Results. Distribution of levels of TSH and FT4, divided by children gender and age is shown in Table 2. In all groups values corresponding to the upper quartile of the distribution of indicators were in the range of the laboratory standarts. Comparison of values of age subgroups between boys and girls in 2 seasons of year revealed no statistically significant differences in the content of the researched parameters in the blood serum (p>0,005). There are also no differences in each age group depending on gender (p>0,005). So, levels of TSH and FT4 in research groups aren't depend on age and gender of children. In the further analysis, the indicators were compared between 2 seasons (winter and spring). The limitation of this research towards the estimation of the seasonal dynamics of indicators is a cross-sectional design, in which independent groups were formed in each season. However, this research is extremely relevant in view of the fact that it determines promising areas of research.

Comparison of each group by the level of the analyzed indicators in different seasons of the year show that more stable differences are observed in the content of TSH (Table 2). Moreover, in all groups they have the same orientation towards higher values in the winter period. In case

Table 1

An amount of examined children by gender and age

Gender and age of examinees	The season of taking blood tests for ELISA	
	Winter	Spring
Girls 10-14 years	59	53
Girls 15-18 years	54	34
Boys 10-14 years	53	40
Boys 15-18 years	33	36
Всего	199	163

Table 2

Comparison of levels of TSH and FT4 between seasons of year*

Indicator	Winter	Spring	
Girls 10-14 years			
TSH, mlU/ml	2.1 (1.4; 3.1)	1.8 (1.2; 2.5)	0.055
FT4, pmol/l	14.0 (9.6; 16.9)	12.7 (11.5; 14.4)	0.857
Girls 15-18 years			
TSH, mlU/ml	1.9 (1.3; 2.6)	1.3 (1.0; 1.8)	0.003
FT4, pmol/l	14.2 (10.5; 16.1)	12.8 (11.0; 14.8)	0.318
Girls 10-18 years			
TSH, mlU/ml	2.0 (1.4; 2.8)	1.6 (1.1; 2.1)	0.002
FT4, pmol/l	14.2 (9.8; 16.7)	12.8 (11.4; 14.5)	0.373
Boys 10-14 years			
TSH, mlU/ml	2.1 (1.7; 3.2)	1.6 (1.1; 2.3)	0.001
FT4, pmol/l	12.8 (9.3; 16.1)	12.9 (11.4; 14.5)	0.411
Boys 15-18 years			
TSH, mlU/ml	2.1 (1.5; 3.3)	1.6 (1.3; 2.4)	0.028
FT4, pmol/l	11.6 (9.4; 15.5)	14.1 (12.6; 15.02	0.015
Boys 10-18 years			
TSH, mlU/ml	2.1 (1.7; 3.2)	1.6 (1.1; 2.3)	<0.001
FT4, pmol/l	12.3 (9.3; 1.0)	13.6 (12.0; 14.8)	0.034

Note: * — data showed as median and interquartile span in ME format (Q_1; Q_3): p – reached level of significance of groups comparison (Mann-Whitney criteria).

of FT4 statistically significant differences are observed only among young men 15-18 years.

Earlier season difference of indicators of в показателях pituitary-thyroid system was described only описаны in the adult population of central Yakutia. The presence of seasonal dynamics of thyroid hormones was shown and the signs of "polar T3 syndrome" were also established [6, 10, 11].

The evidence for seasonal variations of TSH in the literature is several contradictory. During the examination 206 486 patients of Pekin's medical college bigger TSH indicators were observed in winter, a negative correlation was established with the ambient temperature (r = -0,66, p <0,001) [9]. Analysis with using of «big» data that includes 1,5 millons of observations showed that TSH levels were highest at summer and winter regardless of level of peripheral hormones, age, gender and ambient temperature [12].

Results of our research evidence about season changes of regulation of the pituitary-thyroid system in children and adolescents of the Arctic regions of Yakutia. More high levels of TSH at winter should be considered as physiological shifts, reflecting adaptation to the changing season of the year. Tracking of changes in the pituitary-thyroid system more possible in the format of a longitudinal research. Of course, one of the most promising areas of research is determination of the physiological standards for regions with a wide seasonal range and temperature range. In future researches it's necessary to rate influence of photoperiodism and the temperature factor on the degree of these fluctuations. This data can expand our perception of the physiology of adaptation to environmental conditions, as well as to influence the management strategy of patients with subclinical hypothyroidism in the Arctic.

The work was carried out within the framework of the research topic "Monitoring the health status of children of the Republic of Sakha (Yakutia)" (State registration No.: 0120-128-07-98), the basic part of the state task of the Ministry of Science and Education of the Russian Federation (FSRG-2020-0016) and with the financial support of the RFBR grant (no. 18-05-60035_Arctic).

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MORPHOLOGICAL AND FUNCTIONAL FEATURES OF THE MICROCIRCULATION STATE IN CAPILLARY BED AND THERMAL IMAGING OF THE BODY OF YOUNG MALE NORTHERNERS RESIDING DIFFERENT CLIMATIC AND GEOGRAPHICAL ZONES OF MAGADAN REGION

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The research involved 364 young men aged 17-21, permanently residing in the coastal (the city of Magadan, n = 217) and continental (the settlement of Susuman, n = 47) climatic zones of Magadan region. Morphofunctional parameters of the microcirculatory bed vessels, as well as thermographic characteristics of different parts of the body were studied. It was found that the residents of Susuman were characterized by smaller arterial diameter and bigger diameters of the venous and transitional sections of the capillaries as compared with those from Magadan. At the same time, significantly higher average temperatures were observed in Magadan subjects through all the analyzed areas of the thermographic picture than it could be seen with examinees from Susuman.

Keywords: young men, North-East, microcirculation, thermography.

Capillaroscopy is a method of non-invasive investigation of the microcirculatory blood flow [1]. It is created to diagnose functioning of human cardiovascular system in its peripheral parts, the skin and mucous membranes. Capillaroscopy provides the most useful way to promptly visualize the skin capillary blood circulation, and assess density and blood flow rate in capillaries, which gives reliable information about the structural and functional parameters of the capillaries in real time and under real physiological conditions [16]. The most important link in the bloodstream is the capillary system, which provides organs and tissues with all vital substances. Large vessels deliver these substances. They come into tissues through capillaries with simultaneous extraction of metabolic products from the tissues and transferring them to the bloodstream [1]. Microcirculation blood flow maintains health of tissues and organs by delivering oxygen and nutrients [17]. In addition, it regulates blood pressure and tissue perfusion, as well as the body thermal state [18]. Microcirculatory vessels also support functioning and homeostasis in cells. It should be noted that average values for some microcirculation parameters, in particular those characterizing adaptation to the North extremes, remain unclear, which suggests the need for more detailed study of the microcirculatory bed.

Another interesting and easy-to-use method for assessing peripheral blood circulation is thermography [6]. It helps to study skin temperatures, which describe intensity of metabolic processes, since any changes in metabolism or blood circulation affect skin temperatures, and therefore can be seen in the thermogram [2]. Maintaining skin temperature and, consequently, heat balance is represented there as heat production equal to heat loss. Based on this, infrared imaging is of great importance when studying physiological adaptation of the body. This method has proven to be useful for thermal imaging of the body (skin) surface, as well as for monitoring of the body thermoregulation response under different conditions [18].

The body microcirculation system and thermal imaging map formation under the chronic influence of the cold factor is undoubtedly a problem for the modern science that studies adaptation processes. In this regard, visualization and objective assessment of capillary blood flow with both video capillaroscopy and thermography may be useful for the North residents in prognostic and diagnostic terms.

Based on the above, the objects of this work was studying functional state of microvessels and thermal images of the body in the North inhabitants under different environmental extremes of coastal or continental climatic zones in order to identify and justify the use of these assessment methods in providing mechanisms for adaptive changes.

Materials and methods. A random sample was made up of 364 males aged 17-21 permanently residing in coastal (Magadan, n=217) and continental (Susuman, n=47) climatic zones of Magadan region. The structure of capillaries and microcirculation was studied in the epon-

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ychium of the nail bed since this area is easily accessible for research, and the main axis of the capillaries there is parallel to the skin surface while in other areas it is visualized as perpendicular [14]. The study was conducted using a drop of immersion oil to maximize the transparency of the keratin layer on the fourth and fifth fingers of the left hand due to the high light transmission of the skin in these areas [19]. A video capillaroscope of Capillaroscan-1 (New Energy Technologies, Skolkovo) equipped with an optical probe was used within the study course. A 15 minute rest session preceded the recordings which were made at a comfortable ambient temperature of 22-25°C, before afternoon, with the hands at the heart level position [19]. None of the participants had frostbites, hand injuries or vascular pathologies that could affect their microcirculation. In addition, the criterion for inclusion in the research was medical examination as an admission to PE classes within the framework of the educational program.

Microcirculation morphometric characteristics were registered, and vascular density was evaluated in the mode of constructing a panoramic static image of the first-line capillary net with an optical 200-fold magnification. It was necessary that characteristic (contrasting) capillaries could be seen throughout the field of vision. A more detailed analysis was performed using a 10-second video recording with a 400 optical magnification of a specific skin area followed by automatic calculation of the observed capillaries. That made it possible to assess all visually observed processes and anatomical structures, and obtain an average value of morphometric indicators and the speed of red blood cell movement in the specifically studied capillaries [20].

The paper analyzes the following morphofunctional parameters of microcirculatory vessels: the diameter of the arterial, venous, and transitional sections of the capillary (mcm), which refers to the area of the vessel filled with visible red blood cells (the vessel walls are hardly distinguishable by light microscopy); capillary length (mcm); capillary net density (arb. units) indicating the number of observed capillary loops of the first echelon; the amount of the perivascular zone (mcm) namely its linear size from the most remote point of this zone to the nearest point of the transition section of the capillary; the speed in the arterial, venous and transitional sections (mcm\s), which suggests the speed of the red blood cell movement in the capillary; and the deformation coefficient (arb. units), which shows the number of strongly wound and damaged capillaries [12].

Thermography was performed in a sitting position, using a thermal imaging camera (FLIR SC620 Thermal Imager, FLIR Systems, Sweden), which provides long-wave (7.5–13 mcm) imaging with a thermal sensitivity of 0.1 °C. The spatial resolution was 640 x 480 pixels. The study was conducted in accordance with the standards set by the European Thermographic Association [13]. Quantitative analysis of thermal images was performed for 7 front and back zones of the body, in the sitting position (Fig. 1): C_1 – average temperature of the left subclavian region (°C), C_2 – average temperature of the right subclavian region (°C), C_3 – average temperature of the forehead (°C), C_4 – average temperature of the chest (°C), C_5 – average temperature of the abdominal surface (°C), C_6 – average temperature of the upper back (°C), C_7 – average temperature in the area of the shoulder blades (°C). For each selected area, the average surface temperature was obtained, which was more representative of this area than the minimum or maximum values. The advantage of infrared systems in comparison with other methods of temperature measurement is the possibility of simultaneous analyzing a large number of image elements (pixels) in a short period of time followed by real time image processing [15].

Prior to the examination, all participants acclimated for more than 10 minutes, which is the optimal time to get used to the ambient temperature [8]. It was necessary that the subjects reported no chronic diseases in the acute stage or complaints about the health state. The study was carried out in accordance with the principles of the Helsinki Declaration. The study Protocol was approved By the Commission on Bioethics of the Federal State Institution of IBPN, FEB RAS (Protocol No. 1 dated March 25, 2019). Prior to the study, all volunteers gave their written informed consent. The obtained material was processed using the Statistica 7.0 application package. The average values of indicators (M) and errors of averages (m) were calculated. The statistical significance of the differences was ascertained using the t-test by Student at normal distribution, which was determined using the Kolmogorov-Smirnov test. The significance level criterion (p) was assumed to be ≤ 0.05.

Research results. Table 1 shows the main morphological and functional indicators of capillary microcirculation in young men residing in different climatic zones of Magadan region. Examples of capillaroscopic patterns of young male residents of the coastal and continental zones are presented in Figure 1. The data from Table 1 suggest significantly smaller diameter of arterial section typical for young males of the continental area with bigger diameters in venous and transition sections, and longer capillaries. No difference in density of the capillary net as compared to the subjects of the coastal zone was found though. The velocity rate characteristics in the arterial, venous, or transitional sections of the capillary bed did not differ through the examined groups either. A significant growth was observed in size of perivascular area and coefficient of deformation in the group of young men from Susuman. A higher number of slugs in the group of coastal residence subjects could be seen.

Table 2 shows thermal imaging patterns observed with young male northerners residing in different climatic zones of Magadan region. Examples of thermograms recorded in subjects from coastal and continental zones can be seen in Figure 2. The data show significantly higher average temperatures demonstrated by coastal examinees through all analyzed areas of the heat picture. We analyzed average values of the thermal imaging patterns through the two groups. The abdomen (C_5) area proved to have the lowest temperature with significantly higher average temperatures observed in the chest (C_4), forehead (C_3), upper back (C_6), and subclavian areas (C_1, C_2). That is associated with the temperature of internal organs that emit heat as a result of their normal metabolic processes, as well as with a low thickness of subcutaneous fat [22].

Discussion of results. It is known that the capillary diameter is the structural parameter that determines the volume of blood flow passing through the entire length of microcirculatory bed [3]. In this case, the capillary diameter refers to the area of the vessel filled with visible red blood cells, while the capillary walls are hardly distinguishable under light microscopy. [3]. Young men of the continental zone of residence were characterized by significantly lower values of the arterial capillary diameter as compared to the subjects of the coastal area, with this indicator located at the lower limit of the standard range (from 7 to 17 microns; on average 11.91 ± 1.87 microns) [11], which could be better seen in the group of Susuman examinees.

The venous capillary diameter indicator normally ranges from 11 to 20.6 microns (on average 15 ± 2.42 mcm) [11]. In our research, subjects from Susuman

were significantly higher in this parameter with the average value reaching the upper limit of the standard, while the group of Magadan proved to be close to the lower limit. The loop diameter of the capillary tip (the transitional section of the capillary) was bigger in subjects of the continental area of residence (normally it varies from 8 to 21 mcm; on average, 17.17 ± 2.12 mcm) [11]. Examinees from the coastal territory demonstrated this indicator within the presented range, and those from Susuman significantly exceeded it.

When we were comparing the results obtained in our study with those cited in foreign references, (from 92 to 295 mcm; on average 240 ± 38.3 mcm) for the capillary length [20], we found that the examined males from both coastal and continental areas were higher in their values vs. the standards with the longest capillary length observed in young men living in more extreme climatic conditions (continental zone). It is known that longer capillaries may be associated with the arterial hypertension [21], which is fully consistent with the earlier results where higher indicators of both systolic (133±1.6 mmHg) and diastolic (75±1.2 mmHg) blood pressure were observed in young men in Susuman while the group from Magadan demonstrated the following values: 125±0.9 mm Hg and 73±0.7 mm Hg, respectively [5].

The next indicator is the value of the perivascular zone. This indicator is a relatively new parameter used to study microcirculation and reflect the degree of the interstitial hydration, which makes it possible to assess the effectiveness of metabolism in the aspect of its filtration-reabsorption mechanisms [12]. This indicator can be also used to estimate the number of functioning (i.e. open) capillaries at the moment of the study. Of note that, in a normal state, not all micro vessels are open at the same time. In general, the state of the perivascular zone characterizes the barrier function of micro vessels, i.e. the permeability of the vessel wall, and its size proportionally depends on the degree of edema. Normally perivascular zone in healthy individual's nail area is 93.6±9,0 mcm [4], which fully corresponds to indicators observed with subjects from the city of Magadan, and is much lower than shown by the examinees from Susuman who were on average 29% higher in the perivascular zone than the standard rate. We found no intergroup differences regarding the density of the capillary net. With this factor, the rise of the perivascular zone typical for the residents of the continental zone

Table 1

Microhemocirculation indicators in young men of coastal (Magadan) and continental (Susuman) climatic

Indicators	Indicators		Significance Level for Differences
	Continental Climatic Zone (Susuman)	Coastal Climatic Zone (Magadan)	
Diameter of Arterial Section. mcm	7.8±0.2	8.5±0.1	p=0.011
Diameter of Venous Section. mcm	18.3±0.3	12.1±0.2	p=0.008
Diameter of Transition Section. mcm	28.7±0.6	16.8±0.2	p=0.004
Capillary Length. mcm	368.1±8.8	317.35±6.1	p=0.005
Amount of Perivascular Zone. mcm	120.4±2.5	91.28±1.5	p=0.004
Density of Capillary Net. arb. units	0.039±0.001	0.040±0.001	-
Velocity in Arterial Section. mcm/s	209.0±10.2	214.08±8.4	-
Velocity in Venous Section. mcm/s	153.2±5.2	154.39±6.2	-
Velocity in Transitional Section. mcm/s	186.2±12.3	183.82±7.4	-
Slugs. n p s	2.43±0.3	3.27±0.1	p=0.009
Coefficient of Deformation. arb. units	0.37±0.01	0.32±0.01	p=0.008

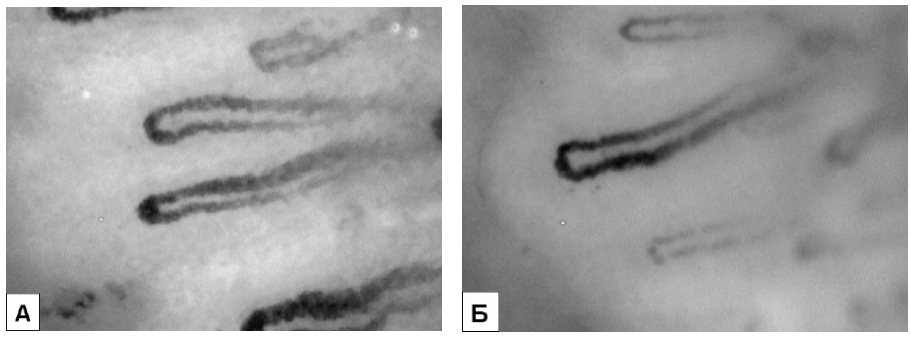

Fig. 1. Examples of microcirculatory bed images in young male residents of the continental (A) and coastal (B) climatic zones of Magadan region

Table 2

Temperature indicators of different zones of the body in male northerners inhabiting coastal (Magadan) and continental (Susuman) climatic zones of Magadan region

Indicators	Examined Cohorts		Significance Level for Differences
	Continental Climatic Zone (Susuman)	Coastal Climatic Zone (Magadan)	
C_1	35.20±0.07	35.57±0.11	p=0.016
C_2	35.37±0.08	35.68±0.12	p=0.038
C_3	33.95±0.08	35.14±0.13	p=0.012
C_4	33.95±0.09	34.62±0.14	p=0.013
C_5	33.42±0.15	33.88±0.16	p=0.005
C_6	34.03±0.11	35.39±0.11	p=0.011
C_7	33.58±0.11	35.03±0.12	p=0.008

Note. See the symbols C1-C7 in the text.

testifies to the growth of the interstitial hydration, apparently, due to the chronic and more pronounced impact of the cold factor. In our opinion, that may be caused by a bigger diameter of the venous section, a larger size of the transition section, and 53% lengthening of the capillary.

After V. I. Kozlov, the velocity rate of erythrocytes in capillaries varies within the range of 400-800 mcm/s, and on average is 617± 0.72 mcm/s in healthy men at rest. That is much higher than we could observe in our studies [4]. It is necessary to specify that such changes in structural and functional characteristics of the capillary bed could be seen on the background of significantly high values of the coefficient of deformation

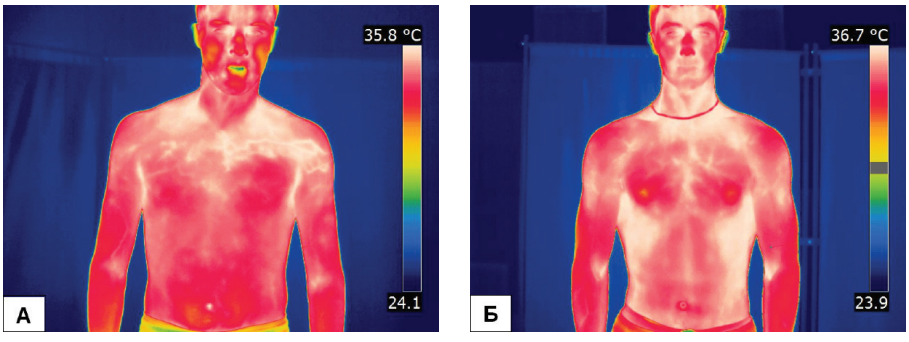

Fig. 2. Examples of thermal images in young male residents of the continental (A) and coastal (B) climatic zones of Magadan region

in male residents of the continental zone.

Changes in skin surface temperature occur primarily due to the changes in peripheral blood flow. The blood acts as a conductor of heat exchange between the internal and external environment. It is also the influence of the tonic state of surface vessels [7]. On the whole, significantly lower values of the temperature pattern were typical for the young men of the continental zone. The lowest temperatures were found in the abdominal area for both groups since this is a poorly vascularized area with adipose tissue [9]. The highest temperatures in examinees of the two groups were recorded in the chest, forehead, upper back, and subclavian zones, which is associated with the temperature of internal organs that emit heat as a result of their normal metabolic processes, as well as with quite thin subcutaneous fatty layer [10].

Conclusion. It should be noted that young male northerners were similar in the microcirculation morphological characteristics: they demonstrated constriction in arterial diameter, lengthening in capillaries, and reduced blood flow rate through all parts of the capillary bed. Along with the above capillary profiles, subjects of the continental zone had such changes in the functionality of the capillary blood flow as more pronounced growth of the perivascular zone, which indicates a higher degree of interstitial hydration due to chronic and more extreme impact of the cold factor, and also due to the bigger diameter of the venous section, significantly larger transition section, and 53% lengthening of the capillary together with higher values of the deformation coefficient. Such changes in the capillary bed are considered to be early signs of microcirculation disorders, which usually begin with a weaker blood refill in the microcirculatory bed and lead to outflow violation accompanied by venous stagnation and further bad disorders of capillary blood flow [3]. In general, the analysis of temperature patterns has revealed significantly lower values through all the studied body areas with representatives of the continental zone of residence. The observed differences could be seen in compensatory and adaptive rearrangements of the microcirculatory bed, and the body thermal image of the young men residing in different natural and climatic territories. We have found the relationship between changes in the architectonics and the capillary bed functionality, as well as between the body temperature picture and the degree of severity of climatic extremes in different areas of residence. Our research has shown the importance of ascertaining the structural and functional parameters of microcirculation and temperature pattern to understand how the environmental extremes affect the body cardiovascular system. In addition, we have revealed that morphofunctional changes in microcirculation and thermal imaging can serve as integral and informative markers, as well as determining criteria of the degree of human adaptation to the North conditions and its severe impact.

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ASSESSMENT OF RENAL FUNCTION IN THE INDIGENOUS PEOPLES OF YAKUTIA WITH ARTERIAL HYPERTENSION

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The aim of the study was to assess the functional state of the kidneys in the group of people with arterial hypertension (n=159), representatives of the indigenous population of the Republic of Sakha (Yakutia). The average age of the subjects was 60.6 years. 78% of the examined individuals have signs of decreased renal function. Most number of participants, along with existing hypertension, have additional risk factors that contribute to the development of renal dysfunction. The surveyed women are characterized by a more unfavorable profile of risk factors for chronic non-communicable diseases, which probably accounts for a higher proportion of women with signs of chronic kidney diseases.

Keywords: arterial hypertension, renal function, glomerular filtration rate, risk factors, indigenous population, Arctic.

Diseases of the circulatory system remain the leading cause of death in the world. In the Russian Federation, according to data from 2019, these diseases attributed to 46.8% of all deaths [2]. Arterial hypertension (AH) and chronic kidney disease (CKD) are considered as independent risk factors for the development of cardiovascular diseases and their complications, simultaneously, each of these conditions can be the cause of the other. Thus, a decrease in renal function leads to an increase in blood pressure, and long-term hypertension affects the glomerular filtration rate, leading to renal failure [9, 10, 12, 14].

Chronic kidney disease is a term that encompasses all degrees of decline in kidney function. Moreover, all its stages are associated with an increased risk of cardiovascular diseases, premature mortality and a decrease in the quality of life [5]. Currently, the glomerular filtration rate (GFR) and the level of albuminuria are used for: determining the stage of CKD, risk stratification, and prognosis [1, 11]. Due to the fact that GFR is difficult to measure directly, various equations are used that take into account serum creatinine level, age, race, sex and body surface area [4, 6, 7].

The aim of the study: assessment of renal function in the indigenous peoples of Yakutia with arterial hypertension.

Materials and methods. A group of people with arterial hypertension was formed during an epidemiological study among the populations of the Tomtor village of Oymyakonskiy district and the city of Srednekolymsk of Srednekolymskiy disctrict in expeditionary conditions. Inclusion criteria were: ages 20 and older, belonging to an indigenous ethnic group by self-identification, presence of an elevated blood pressure when measured (according to ESH/ESC criteria, 2013), taking antihypertensive drugs during the examination period or less than 2 weeks before the examination, regardless of the measured blood pressure level. The Yakuts and representatives of the indigenous small-numbered peoples of the North were attributed to the indigenous peoples of Yakutia. The analysis included data from 159 people, including 58 men and 101 women. The average age of men was 59.3 ± 2.36 years, women 61.4 ± 1.36 years (p = 0.347). All participants were representatives of the indigenous ethnic groups of Yakutia (Yakuts, Evens, Evenks, Chukchi, Yukagirs).

Research program included the following sections: a questionnaire for objective assessment of state; informed consent of the respondent to conduct research and donate blood (according to the protocol of the Ethics committee of YSC CMP); anthropometric examination with measurement of height and weight with calculation of body mass index, waist and hips measurements; blood sampling from the cubital vein in the morning on an empty stomach with 12-hour abstinence from food. After centrifugation, blood serum was stored in a freezer (-70°C) until analysis.

For further analysis, the traditional indicator was used - body mass index (BMI) or Quetelet index, which was calculated by the following formula: BMI (kg / m²) = body weight (kg) / height (m²). Overweight was considered to be a BMI ≥ 25 and <30 kg/m², obesity was determined at a BMI of ≥ 30 kg/m² [according to European recommendations of the III revision, 2003]. 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) according to the instructions for the correct measurement of blood pressure, outlined in the European clinical guidelines for the diagnosis and treatment of

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hypertension. Hypertension is present at the 140/90 mmHg or taking antihypertensive drugs during the study or stopping them less than 2 weeks before the study (2017 ACC/AHA Guideline).

Laboratory methods of the research included biochemical analysis: blood lipids test total cholesterol (TC), triglycerides (TG), HDL Cholesterol, LDL Cholesterol, levels glucose, urea, creatinine.

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 total cholesterol (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. 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.

Kidney function was assessed by the value of the glomerular filtration rate (GFR). The CKD-EPI formula (Chronic Kidney Disease Epidemiology Collaboration) was used to calculate the indicator. The formula includes correction factors based on race and gender. The coefficients for "white and other races" were used in the calculation.

CKD-EPI = a × (blood creatinine (mg/dL)/b) c × (0.993) age,

where a has the following values depending on race and gender: women = 144; men = 141; b: women = 0.7; men = 0.9; variable c has the following values for women: blood creatinine <= 0.7 mg/dl = -0.329; blood creatinine> 0.7 mg/dl = -1.209. Men: blood creatinine <= 0.7 mg/dl = -0.411; blood creatinine > 0.7 mg/dl = -1.209 [4].

In accordance with the Clinical Guidelines for Chronic Kidney Disease of the Association of Nephrologists of Russia in 2019, renal function was assessed depending on the level of GFR in ml/min/1.73m2 as “high or optimal” at GFR values> 90; "Slightly reduced" - at 60-89; "Moderately reduced" - at 45-59; "Significantly reduced" - at 30-44; "Sharply reduced" - at 15-29; end-stage renal failure - at <15 [1].

Table 1

Characteristics of indigenous people with arterial hypertension

Indicator	Men n=58	Women n=101	p
	Me (Q1-Q3)	Me (Q1-Q3)	
Height, sm	163 (158-166)	151 (147.5-156.5)	<0.001
Body mass, kg	66 (60-75)	65 (54-75)	0.193
Body mass index, kg/m^2	25.6 (23-27.5)	27.6 (23.2-31.8)	0.036
Waist measurement, sm	93 (85-99)	93 (85-105.5)	0.346
Systolic blood pressure, mmHg	150 (138.8-160)	160 (140-180)	0.003
Diastolic blood pressure, mmHg	90 (87.5-100)	100 (90-100)	0.001
TC mmol/l	4.9 (4.2-5.3)	5.4 (4.6-6.1)	0.001
HDL mmol/l	1.2 (1-1.5)	1.4 (1.1-1.6)	0.018
LDL mmol/l	3.0 (2.6-3.6)	3.5 (2.9-4.1)	0.002
TG mmol/l	0.9 (0.7-1.3)	1 (0.7-1.2)	0.416
Glucose mmol/l	4.7 (4.2-5.3)	4.4 (4.1-4.8)	0.016
Urea mmol/l	5.7 (4.8-6.9)	5.9 (4.7-7.1)	0.476
Creatinine mg/dl	1 (0.9-1.1)	0.9 (0.8-1.0)	<0.001
CKD-EPI ml/min/1,73m^2	80.4 (73.3-91)	72.2 (59.5-84.2)	0.003

Note: p is the achieved level of significance of differences when comparing groups (Mann-Whitney test).

Table 2

The frequency of metabolic disorders and concomitant diseases in the examined group

Condition / disease	Men n (%)	Women n (%)	p
Obesity at a BMI	5 (9.1)	35 (34.7)	0.002
Abdominal obesity	28 (48.3)	91 (90.1)	<0.001
Hypercholesterolemia	20 (34.5)	58 (57.4)	0.005
The low HDL Cholesterol	14 (24.1)	41 (41.0)	0.047
The high LDL Cholesterol	5 (8.6)	23 (23.0)	0.024
Hypertriglyceridemia	6 (10.3)	9 (8.9)	0.766
A hyperglycemia on an empty stomach	5 (8.6)	6 (5.6)	0.521
Accompanying diseases			
Diabetes 2 type	3 (5.2)	2 (2.0)	0.267
History of cerebral stroke	7 (12.1)	13 (12.9)	0.883
Ischemic heart disease	14 (24.1)	16 (15.8)	0.198

Note: p is the achieved level of significance of differences when comparing groups (Pearson $\chi 2$ test).

Table 3

Distribution of the subjects under study by the level of GFR

CKD stage	Characteristics of global kidney function	Men		Women		Total	
			%	n	%	n	%
C1	High or optimal	15	25.9	20	19.8	35	22.0
C2	Slightly reduced	37	63.8	54	53.5	91	57.2
C3a	Moderately reduced	4	6.9	24	23.8	28	17.6
C3б	Significantly reduced	1	1.7	2	2.0	3	1.9
C4	Sharply reduced	1	1.7	1	1.0	2	1.3

Statistical analysis was performed using the IBM SPSS Statistics software package (version 22.0). Qualitative variables are described by absolute and relative frequencies (percentages), quantitative variables are described using the median (Me) and the interquartile range in the Me format (Q_1; Q_3). Spearman's correlation analysis was used to determine the relationship between the indicators. When comparing groups, the Pearson χ^2 and Mann-Whitney tests were used, depending on the data type. Differences were considered statistically significant at $p < 0.05$.

Results and discussion. The anthropometric and laboratory characteristics of participants of the study are presented in Table 1. Women, in contrast to men, had statistically significant higher body mass index, blood pressure levels, total cholesterol, HDL cholesterol, LDL cholesterol, and lower serum glucose and creatinine levels. The quartile distribution of SBP and DBP levels indicates that the target blood pressure levels have not been achieved in the overwhelming majority of cases. Renal function in the whole group, in accordance with the calculated CKD-EPI, was assessed as "slightly reduced". Patient age correlated positively with serum creatinine values (r=0.17, p=0.033) and negatively with CKD-EPI values (r=-0.61, p <0.001).

Further analysis demonstrated (Table 2) that in the examined group there is a high frequency of such risk factors for chronic noncommunicable diseases as obesity and dyslipidemia. That is, along with existing hypertension, there are additional factors that contribute to the development of renal dysfunction. The high frequency of AO, which is a metabolically more unfavorable type of fat accumulation, should be noted as well. AO was observed in 48% of men and 90% of women with hypertension (p <0.001). In general, the frequency of obesity, metabolic disorders of cholesterol and its fractions was statistically significantly higher in women than in men (p <0.05). 12-13% of the surveyed had a history of cerebral stroke, 16-24% suffered from ischemic heart disease. Thus, in the studied group, there is a high frequency of risk factors for renal dysfunction, more pronounced in women.

Assessment of renal function using GFR showed that 78% of the examined individuals with hypertension have renal dysfunction (Table 3). This has also been observed in numerous studies demonstrating the negative effect of hypertension on renal function. According to the researchers' estimates, each increase in blood pressure by 10 mm Hg. is associated with a decrease in calculated GFR [3, 8, 13, 14].

In the study group, women were more likely to have higher degrees of decline in renal function. Thus, in 26.8% of women and 10.3% of men, the assessment of renal function by stages of CKD ranged from "moderately reduced" to "sharply reduced" (χ^2 = 6.02, p = 0.014). Gender differences in the progression of chronic kidney disease continue to be studied. In a systematic review and meta-analysis of the results of studies of 6 cohorts, with a total of 2382712 people, the effect of hypertension on the risk of developing CKD and end-stage renal failure was assessed. The analysis demonstrated that the relative risk of developing these conditions in women was 23% lower than in men (RR 0.77 [95% CI, 0.63–0.95]) [15]. It is likely that the gender differences identified in this study are largely due to a more unfavorable profile of risk factors in women than in men (Tables 1-2).

Conclusion. The results of the study indicate that 78% of the examined individuals with arterial hypertension have a decrease in kidney function. In general, a significant number of participants have additional risk factors, such as obesity and dyslipidemia, in addition to existing hypertension, which contribute to the development of renal dysfunction. The examined women are characterized by a more unfavorable profile of risk factors for chronic noncommunicable diseases, which probably accounts for a higher proportion of women with signs of CKD.

It should be noted that the examined group of people with arterial hypertension with an average age of 60 years is a typical cross-section of real outpatient practice in the regions of the Republic of Sakha (Yakutia). The revealed high frequency of renal dysfunction in patients with hypertension indicates the need to determine the glomerular filtration rate in the presence of arterial hypertension everywhere in the Republic, including the hard-to-reach places of compact residence of the indigenous population. This will make it possible to adjust the tactics of non-drug and drug therapy in a timely manner, monitor renal function and prevent the early development of cardiovascular complications.

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VARIATIONS OF CARDIAC RHYTHMS IN CHILDREN OF INDIGENOUS AND NEWCOMER POPULATION LIVING IN THE ARCTIC OF THR EUROPEAN PART OF RUSSIA

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The article presents the results of analysis of heart rate variability in preschool children (3-7 years old) living in the Arctic region of the European part of Russia. The study was conducted in three districts of the Murmansk region that differ by ethnic sotav, and also have contrasting climatic conditions. A comparative assessment of the state of the cardiovascular system of pre-school children of the indigenous and non-indigenous population in the Murmansk region showed that there were no pronounced differences between these contrasting groups. The most pronounced differences are observed in representatives of the non-indigenous population in the form of reduced spectral characteristics (TP and HF), which are manifested in children with all four types of vegetative regulation.

Keywords: heart rate variability, preschoolers, polar regions, indigenous and non-indigenous populations.

Introduction. One of the factors that affect the human body in high latitudes is the specific natural conditions that pose a threat to the health of the local population [3]. The impact of high-latitude environmental factors on the human body is reflected at all hierarchical levels of the body [4, 2, 5]. At the same time, the child's body is the most vulnerable to external environmental influences [7, 9]. therefore, early diagnosis and detection of abnormalities in the cardiovascular system in children of the Arctic region will not only identify predictors of adaptation failure, but also help in the development of methods to increase resistance to extreme exposure.

The population of the Murmansk region is a product of complex interethnic hybridization. It can be divided into two conditional groups: the indigenous (Sami and Pomor) and the non-indigenous population. Despite the assimilation processes that have developed in the region over the past decades, the population of Lovozersk and Tersk districts have managed to maintain their ethnic differences. In addition, they are distinguished by contrasting living conditions and critical morbidity of children and adults.

A comparative assessment of the heart rate variability of children of indigenous and foreign populations of these regions will lay the foundation not only for theoretical ideas about the mechanisms of human adaptation in the Arctic, but also for the methodology for creating new health-saving technologies for high-latitude regions.

Objects and methods. The study was conducted in the settlements of the Murmansk region: Lovozero, Umba, and Apatity. In total, we examined 347 preschool children aged 3-7 years. In Lovozero, 122 children were examined, in Umba – 108 children, in Apatity-117 children. The study was approved by the Bioethics Council of the RCHAA KSC RAS. According to the principles of medical ethics approved by the UN General Assembly (1992) and the Council of Europe Convention on Bioethics (1997), all parents of the examined children were informed about the purpose and conditions of the study and gave their written consent to the participation of their child in this study.

The electrocardiogram (ECG) and heart rate variability (HRV) were taken using the complex KFS-01.001 «Cardiometer-MT». HRV analysis was performed according to the standards adopted by the European Society of Cardiology and the North American Society of Electrical Stimulation and Electrophysiology in 1996 [12]. To HRV assess were used temporal (RRNN, SDNN, RMSSD, Amo, MxDMn, pNN50) and spectral (HF, LF, VLF, TP) indicators. In addition, the tension index of the regulatory systems was evaluated (SI).

Statistical analysis was performed using the software "STATISTICA 6.0". To identify the significance of inter – group differences, the Mann-Whitney U test was used. The criterion U represents the median of possible differences between the elements of one and the second sample, and the level of significance of the differences, which in this study corresponded to $p < 0.05$.

Results and discussion. Assessment of cardiohemodynamic parameters was performed using HRV and ECG analysis. According to the ECG results, 30 people were excluded from the HRV analysis. Due to the fact that the assessment of the average HRV indicators of subjects with different types of regulation is not considered reliable and may lead to a false interpretation of the results, we determined the thresholds of the functional norm of heart rate variability (HRV) indicators, taking into account the type of vegetative regulation proposed by I. N. Shlyk [8]. Therefore, at the first stage, all children were preliminarily grouped into 4 groups according to the type of vegetative regulation (VR): type I-moderate predominance of central regulation, type II-pronounced predominance of central regulation; type III-moderate predominance of autonomous regulation; type IV-pronounced predominance of autonomous regulation.

A comparative analysis of heart rate variability (HRV) indicators showed that no significant differences were found between children of the indigenous and non-indigenous population in terms of temporary HRV indicators (RRNN, SDNN, RMSSD, Amo, pNN50, CV) (Table 1). Mainly significant differences are observed only in the spectral characteristics of HRV.

Children with a moderate predominance of the central regulatory circuit

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Table 1

HRV indicators in children 3-6 years old, by type of vegetative regulation, (M±m)

HRV type	I			II			III			IV		
City	Lovozero	Umba	Apatity	Lovozero	Umba	Apatity	Lovozero	Umba	Apatity	Lovozero	Umba	Apatity
Quantity	n=37	n=30	n=30	n=10	n=12	n=15	n=53	n=41	n=36	n=15	n=14	n=24
Heart rate, bpm	98.4 ±1.5	98.6 ±2.5	97.5 ±2.3	105.9 ±3.9	105.9 ±4.3	108.9 ±1.6	89 ±0.6	86.9 ±1.0	89.7 ±1.5	78.7 ±2.9	78.4 ±2.5	81.2 ±4.3
RRNN, ms	610.3 ±9.2	599.6 ±7.8	616.8 ±6.3	559.6 ±16.1	568.7 ±13.5	552.9 ±17.2	674.9 ±21.9	694.4 ±39.7	667.5 ±6.7	761.7 ±60.3	762.3 ±71.6	736.1 ±183.1
Amo	37.2 ±0.9	36.7 ±3.3	38.5 ±0.6	54.6 ±1.3	53.1 ±2.7	57.2 ±4.1	26.9 1±0.6	26.3 ±0.8	28.2 ±0.4	19.2 ±0.3	18.3 ±0.8	20.3 ±0.7
SDNN, ms	42.5 ±0.8	55.5 ±1.2	40.5 ±0.7	33.1 ±29.2	26.8 ±26.4	24.9 ±10.6	60.8 ±0.8	59.4 ±1.4	60.9 ±2.2	94.8 ±0.5	92.7 ±2.6	**90.9 ±0.3** *,**
MxDMn, мс	210.5 ±4.0	210.4 ±5.0	206.4 ±2.7	132.6 ±9.2	142.1 ±12.1	136.6 ±17.8	280.2 ±6.9	270.7 ±3.6	292.5 ±5.3	362.3 ±2.5	389.8 ±3.1	374.8 ±3.0
RMSSD, ms	37.7 ±2	36.9 ±2.7	36.6 ±1.1	21.9 ±6.8	20.5 ±6.0	18.7 ±5.9	61.3 ±4.0	61.8 ±6.5	61.5 ±9.5	101.9 ±3.6	101.7 ±2.8	**98.3 ±6.4** *,**
pNN50, %	16.9 ±2.3	16.4 ±3.2	15.4 ±2.8	3.6 ±6.6	3.0 ±3.1	2.2 ±4.6	38.2 ±5.2	38.4 ±1.1	35.8 ±1.7	61.5 ±5.2	60.6 ±5.3	**57.2 ±8.0** *,**
TP, ms ^{2}	1683.4 ±47.7	1694.9 ±73.3	**1563.7 ±63.9** *,**	666.1 ±67.1	673.3 ±47	**578.7 ±53.3** *,**	3414.70 ±283.5	3444.50 ±205.8	2980.8 ±277.5	8707.7 ±3127.7	8157.7 ±3450.3	**6607.6 ±1203.6** *,**
HF, ms ^{2}	599.9 ±51.2	563.3 ±51.2	**411.7 ±36.8** *,**	235.4 ±28.8	216.7 ±24.1	**124.1 ±34.6** *,**	1403.00 ±129.1	1234.50 ±84.7	1060.50 ±55.2	3952.5 ±479.8	3353.4 ±759.8	**2776.6 ±826.0** *,**
LF, ms ^{2}	543.1 ±23.3	585.9 ±37.9	560.2 ±39.2	216.3 ±9.2	193.6 ±12.1	226.9 ±17.8	1188.20 ±129.1	1158.20 ±84.7	989.3 ±55.2	2629.0 ±271.4	2704.7 ±564.3	2416.9 ±784.4
VLF, ms ^{2}	540.4 ±32.4	545.7 ±44.3	591.8 ±38.7	214.4 ±52.6	263.0 ±51.2	227.7 ±41.7	823.5 ±83.3	1051.8 ±91.0	931 ±121.6	2126.2 ±182.2	2099.6 ±287.4	1414.1 ±196.6
IS, y.e.	158.1	159.3 ±12.8	165.2 ±9.2	399.8 ±35.8	377.2 ±34.8	437.1 ±26.0	76.4 ±4.8	75.0 ±7.7	78.1 ±1.4	33.5 ±1.3	33.4 ±2.0	43.7 ±3.8

Note-Significance of differences (according to the Mann-Whitney test): *1 - between lovozer and Apatity; *2 - between Umba and Apatity;

(type I) from Apatita have lower values of the total power of the spectrum (TR, ms2) compared to children from Lovozero (U=135.0; p<0.022) and Umba (U=4393.5; p<0.024). The same pattern is present in children with a pronounced predominance of the central regulatory circuit (type II) U=239.0 (p<0.038) and U=4359.0 (p<0.019), respectively. The total power (TP, ms2) decreases due to lower values of its high-frequency component (HF, mc2).

As a result, in children from Apatity (non-indigenous population), there is a decrease in the contribution to the total spectrum of the power of respiratory waves (HF, ms2) and an increase in vasomotor (LF, ms2) (Fig. 1), which leads to an increase in sympathetic influence and a decrease in the tone of the vagus nerve.

Thus, it can be assumed that the children of the non-indigenous population with the predominant influence of the sympathetic nervous system (type I and II) have a more pronounced stress

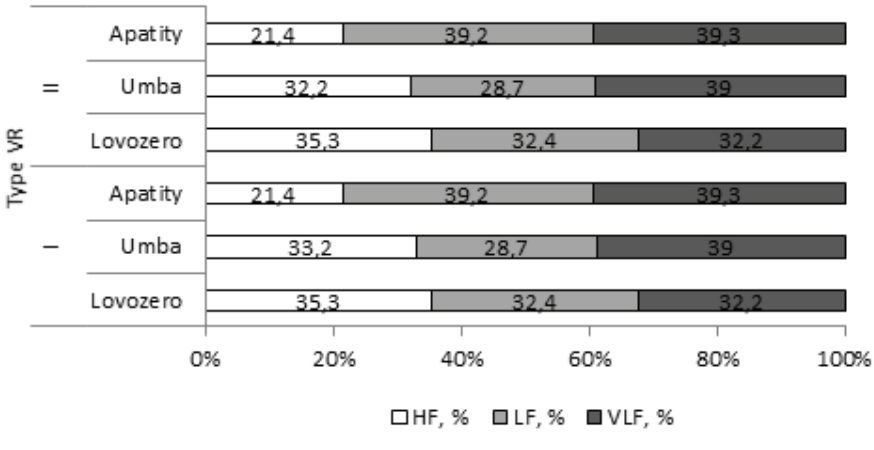

Fig. 1. Contribution of spectral parameters to the total power spectrum (TP, ms^2)

of regulatory mechanisms, manifested in an increase in the SI stress index, which leads to a decrease in the adaptive potential. Detection of type II regulation in children primarily serves as a marker of prenosological conditions, overstrain and overwork. And also requires the need for additional medical examination.

The moderate predominance of the autonomous circuit of regulation (type III) is characterized by the completion of the improvement of cardioregulatory mechanisms, as a result of which HRV indicators of this group are considered to be the physiological norm [1]. In spite of the fact that there were no significant

Table 2

Assessment of the prevalence of ECG syndromes in examined children aged 3-6 years, %

ICD-10-CM	Apatity	Umba	Lovozero
Other conduction disorders (I45)	2.6	1.9	2.5
Atrioventricular and left bundle-branch block (I44)	8.5	4.6	5.7
Atrial fibrillation and flutter (I48)	0.9	-	-
Abnormalities of heart beat (R00)	3.4	0.9	0.8
Other cardiac arrhythmias (I49)	3.4	3.7	1.6
Abnormal results of cardiovascular function studies (R94.3)	13.7	19.4	13.1

Note. The codes are given according to the International Classification of Diseases of the 10th revision (ICD-10) https://mkb-10.com/

differences in HRV indicators between children of the alien population and the indigenous population, children from Apatity have lower values of the total power spectrum (TR, ms2) and its components HF, LF and VLF (ms^2).

A comparative analysis of children with a pronounced predominance of the autonomous circuit of regulation (type IV) showed that in this group the most pronounced differences in HRV indicators. This may be due to the fact that children with type IV regulation are characterized by pronounced heart rate variability, which is determined by a significant predominance of parasympathetic influences on the heart rate and a sharply reduced activity of the sympathetic centers of vascular regulation. This may indicate an imperfect central regulation and autonomic dysfunction. This is especially pronounced in children from Apatity (Table 1). Significant differences are noted in the total power of the spectrum (TR, ms2) compared to children from Lovozero and Umba (U=526.0 p<0.031 and U=182.0 p<0.001, respectively) due to a decrease in its high-frequency component HF, ms2 (U=242.0 p<0.014 and U=453.0 p<0.024, respectively). The decrease in the influence of the parasympathetic system on vegetative regulation is reflected in lower values of temporal indicators SDNN (U=211,0 p<0,003; U=377.0 p<0,001), RMSSD (U=226,0 p<0,007; U=231,0 p<0,001) и pNN50% (U=226,0 p<0,007; U=211,0 p<0,001).

Additionally, 40% of children with type IV of VR have a spread of MxDMn values of more than 530 ms, which indicates not only a pronounced inclusion of autonomous regulation, but also a shift in the rhythm driver. Against the background of normocardia or bradycardia, this can be interpreted as an imperfection (immaturity) or dysfunction in the state of regulatory mechanisms. In addition, in children with excessive parasympathetic activity, frequent arrhythmias are not only the result of dysregulation of the central and autonomic nervous systems. It is this group that accounts for more than 50% of deviations from the norm and pathologies ECG associated with rhythm disorders.

Many studies have shown that various disorders and pathologies of the cardiovascular system are associated with heart rate variability. This allows the analysis of HRV disorders to be used for predictive purposes [11, 10]. Among the main deviations from the norm and pathological changes in the ECG in children were noted: rhythm disturbance, AV block, intraventricular conduction disorders (incomplete and complete blockages), etc. (Table 2).

The analysis of electrocardiograms among the examined children showed that the variant of the ECG norm varies from 47% (Apathy) to 72% of the examined children (Umba). The highest percentage of pathologies (16%) was found in children from Apatity, which is reflected in the incidence of the cardiovascular system in the region.

Thus, the differences in HRV indicators in children of indigenous and non-indigenous populations are manifested in spectral indicators (TP and HF), which is confirmed by previous studies, which show that in the adult population living in high latitudes, compared with the average latitudes, the spectral components of HRV are significantly lower, in particular, the total power spectrum (TP, ms2) and its components (HF and LF, ms2) [6].

Results: The studies conducted to identify the features of HRV in pre-school children of the non- indigenous and indigenous population in the Murmansk region showed that there are no pronounced differences in HRV indicators. The nature of the response of the cardiovascular system to external influences in the comparison territories depends more on the type of regulation of the heart rate than on ethnicity. The greatest differences in HRV indicators in children of indigenous and non- indigenous populations are manifested in the spectral characteristics (TP и HF). In children of the non- indigenous population (Apatity), activation of higher levels of regulation is noted, as a result of which the activity of the lower centers is suppressed. This can lead to energy deficiency, disruption of adaptation processes and disorders of the circulatory system.

Thus, despite the fact that there are no pronounced differences between the indicators of heart rate variability in preschool children of the indigenous and non- indigenous population, the cardiovascular system of children of the alien population is exposed to a stronger influence of climatic and geographical factors, leading to a more pronounced stress of regulatory mechanisms.

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SCIENTIFIC REVIEWS AND LECTURES

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OLFACTORY DYSFUNCTION AS A KEY SYMPTOM OF COVID-19: A REVIEW BASED ON CURRENT RESEARCH

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The 2019 coronavirus pandemic (COVID-19) has caused a large-scale epidemic and a global crisis around the world. The growing scientific evidence suggests that olfactory dysfunction (OD) is present in COVID-19 patients. It can occur on its own or in combination with other symptoms of coronavirus infection. In patients with COVID-19, the main clinical manifestations are fever, cough, accompanied by lymphocytopenia and changes in the lungs with computed tomography of the chest (lesions of the lung tissue in the form of "ground glass"). Also, patients with a severe form of infection may develop neurological disorders, such as acute cerebrovascular diseases, impaired skeletal muscle function, and loss of consciousness. However, the pathogenetic mechanism and clinical characteristics of anosmia in COVID-19 patients remain unclear. Due to the unique anatomical localization of the olfactory system, including the olfactory bulb and olfactory nerve, coronaviruses are able to penetrate and infect the central nervous system through the cribriform (ethmoid) plate. Numerous studies have shown that the incidence of olfactory dysfunction in patients with COVID-19 correlates from 33.9% to 68%, and in women much more often. Anosmia (loss of smell) and dysgeusia (taste disorder) are often associated symptoms in patients with coronavirus infection. Patients with COVID-19 may experience sudden olfactory dysfunction without any other symptoms. Before the onset of anosmia, other symptoms in the form of a dry cough may be present. Otolaryngologists should pay attention to the symptom of anosmia on an outpatient basis in order to diagnose COVID-19 as quickly as possible. To avoid cross-infection, a physician may consider a remote sense of smell assessment for a patient with this diagnosis. In this article, we have reviewed the relevant evidence based on the current literature. Many questions that are somehow related to the loss of smell in coronavirus infection remain unresolved to date.

Keywords: SARS-CoV-2, COVID-19, anosmia, olfaction, dysgeusia.

Introduction. In December 2019, an outbreak of coronavirus infection (COVID-19) occurred in Wuhan, Hubei province (China) and spread very quickly around the world [13,22,39]. On February 12, 2020, WHO named the disease caused by the novel coronavirus as COVID-19 [40]. Clinical studies have shown that SARS-CoV-2 can be transmitted from person to person [13]. Researchers found a high concentration of SARS-CoV-2 RNA in the air in some public areas of 2 Wuhan hospitals during the COVID-19 outbreak [23]. They suggested that the SARS-CoV-2 pathogen may have an aerosol transmission type [23]. The number of COVID-19 cases has skyrocketed worldwide over the last 2020. As of January 27, 2021, WHO reports that since the beginning of the pandemic, 98.2 million cases and 2.1 million deaths have been registered worldwide. [39]. The COVID-19 pandemic has put tremendous pressure on global health systems and the economic stability of all countries in the world.

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In patients with COVID-19, the main clinical manifestations are fever, cough, accompanied by lymphocytopenia and changes in the lungs on computed tomography of the chest (lesions of the lung tissue in the form of “ground glass”) [22]. Also, patients with a severe form of infection may develop neurological manifestations such as acute cerebrovascular diseases, skeletal muscle injury and impaired consciousness [26]. In addition, some patients experience respiratory symptoms such as pharyngolaryngitis, sore throat, rhinorrhea, and changes in the sense of smell [24,25]. Olfactory dysfunction (OD), including anosmia and hyposmia, is very common among all symptoms in patients with COVID-19 [42]. However, the degree of manifestation of OD in COVID-19 remains unclear to date.

To find out the relationship between the development of OD in COVID-19, we conducted a broad search in the literature databases: PubMed, Google Scholar, Web of Science, Wiley Online Library and Nature. The key sample of words included the concepts: anosmia, hyposmia, olfactory dysfunction, COVID- 19. We also looked at the preprint databases (Medrxiv, Biorxiv) to get updated information on research. **This review summarizes the results of studies published on the problem of olfactory dysfunction in patients during the COVID-19 pandemic, as well as examines the mechanisms of its occurrence.**

Etiology of the pathogen

There are 7 types of coronavirus pathogens that can cause disease in humans: SARS-CoV, SARS-CoV-2, MERS-CoV, HCoV-229E, HCoV-NL63, HCoV-OC43, and HCoV-HKU1 [41]. The genome sequence of SARS-CoV-2 is represented by single-stranded unsegmented RNA [4]. SARS-CoV-2 and SARS-CoV belong to the genus Betacoronavirus of the coronavirus family and have 82% similarity in the gene sequence [45].

The SARS-CoV-2 virus uses the S1 spike glycoprotein, which is found in the envelope of the virus, to attach to and infect host target cells. The receptor for coronavirus on the host cell surface is angiotensin-converting enzyme-2 (ACE2) [4]. ACE2 is a functional receptor for SARS-CoV-2, and its expression and localization in the nervous system is very extensive. Therefore, it is assumed that SARS-CoV-2 can cause neurological disorders in both direct and indirect ways [26]. Due to the unique anatomical localization of the olfactory system, including the olfactory bulb and the olfactory nerve, viruses are also able to penetrate and infect the central nervous system through the cribriform (ethmoid) plate [5,20].

Overwhelming evidence has confirmed that the nasal cavity is a vital area tropic to COVID-19. Using a macaque model of coronavirus infection, the researchers compared the pathogenesis and primary localization of SARS-CoV-2, SARS-CoV, and MERS-CoV [31]. The study showed that these pathogens have different areas of localization of the pathological process: SARS-CoV-2 (nose and throat); SARS-CoV (lungs); MERS-CoV

(type II alveolocytes). The viral load on the nasal mucosa of patients was higher than on the pharyngeal mucosa [47]. These data indicate that the nasal cavity is the gateway for primary infection [47].

Scientists have studied the expression of genes associated with the target for SARS-CoV-2 - ACE2 and TMPRSS2 (Transmembrane protease, serine 2), using immunohistochemical methods. For the study, we used biopsy material from various tissues of the human body: bronchial tree, cornea, retina, esophagus, ileum, colon, heart, skeletal muscles, spleen, liver, placenta, kidneys, testes, pancreas, prostate gland, brain, skin and tissues of the fetus [35]. Goblet and ciliated cells (providing mucociliary clearance) localized in the nasal mucosa are a reservoir of coronavirus infection and possible sources for the spread of COVID-19 in the population [35]. In addition, the pathogen SARS-CoV-2 was isolated from the tears of a patient COVID-19 and can cause infection by spreading through the nasolacrimal canal into the nasal cavity [8,11]. Thus, these data prove the highly virulent and highly pathogenic nature of COVID-19.

Anosmia is defined as complete loss of olfactory function that can be caused by a variety of causes, with upper respiratory tract infections being the most common cause [15].Among the various pathogens, the most common are members of the coronavirus family [15.33]. It has been proven that the type of coronavirus 229E, a common variant of ARVI, is capable of causing hyposmia in humans [3]. During the coronavirus epidemic in November 2002 in southern China, anosmia was reported in a 27-year-old patient diagnosed with severe acute respiratory syndrome (SARS) [16]. However, the incidence of anosmia caused by SARS-CoV-2 is significantly higher than that of SARS-CoV. Researchers believe that post-infectious olfactory dysfunction is caused by damage to the olfactory epithelium or the nerve of the same name [15].

Eliezer M. et al. in a study [9] gave a clinical case of infection with SARS-CoV-2. A 40-year-old woman had an acute loss of olfactory function without nasal obstruction. The patient underwent a CT-scan of the nasal cavity, which showed bilateral inflammatory obstruction of the olfactory fissures, which was confirmed by magnetic resonance imaging (MRI). This inflammation seriously impaired the olfactory function, preventing the penetration of odorant molecules into the olfactory epithelium of the nasal cavity [9].

Anosmia is a clear sign of SARS-CoV-2 infection [14]. Patients with COVID-19 may present with sudden olfactory dysfunction without any other symptoms [14, 27]. Before the onset of anosmia, other symptoms may be present in the form of a dry cough [19]. In a retrospective study [19], 54 (47%) of 114 COVID-19 patients had anosmia. The data also showed that patients developed anosmia 4.4 days after the onset of SARS-CoV-2 infection, with a duration of 8.96 days, and 98% of patients recovered within 28 days [19]. Olfactory dysfunction in patients with a confirmed diagnosis of COVID-19 is often accompanied by dysgeusia [17, 19].

There have been several cross-sectional studies on the prevalence of OD in COVID-19 patients in countries such as Italy, Spain, United Kingdom, France, Belgium, the United States and Iran [6,12,28,34,44]. These surveys were conducted by non-contact methods such as online questionnaires and telephone polls [6,21,34]. The incidence of anosmia in COVID-19 patients varied widely among these studies, from 33.9% to 68% [6,12,28,34,44]. Survey data showed that people with OD also have a taste disorder, which suggests a possible probabilistic relationship between them [6,12,28,34,44]. In addition, most studies have shown that the incidence of olfactory disorders in patients with COVID-19 is higher in women than in men [6,12,34]. The female predominance in the experiments is consistent with the findings of previous studies, where olfactory dysfunction was also caused by an upper respiratory tract infection [18].

To date, two case-control studies have been conducted on the relationship between OD and COVID-19 [7,29]. Moein et al. [29] conducted an odor identification test at the University of Pennsylvania (UPSIT). The experiment involved 60 patients with diagnosed coronavirus infection and 60 subjects as a control group corresponding to the age and sex of the patient group [29]. The study showed that 59 (98%) of 60 patients with COVID-19 had some olfactory dysfunction, 35 of 60 patients (58%) had pronounced anosmia [29]. Another study, using a questionnaire, analyzed the prevalence of smell and / or taste disorders in 19 patients with COVID-19 and a similar number of patients with influenza [7]. It was proved that the level of the occurrence of OD in patients with COVID-19 was significantly higher than in patients with influenza by 39.2% and 12.5%, respectively [7]. The main drawback of this study is the lack of an odorant test. The results are interpreted only on the basis of the questionnaire data, which may contribute to some bias on the part of the medical community.

The odor identification test is key in the diagnosis of olfactory dysfunction. However, most of the studies did not give patients such tests. After analyzing the literature data, we found only 3 such studies in which odorant tests were carried out [29,30,36].

Ottaviano G. et al. in their study reported that hyposmia was the only, and in some cases the main symptom in 6 patients with COVID-19, confirmed by 6 smells of the olfactory test called “le nez du vin” (collection of wine aromas “Nose wine ") [30].

Moein et al. [29] in their experiment, they first performed a 1: 1 case-control comparison using a 40-odorant odor identification test to obtain more reliable results between groups. The study showed that 59 (98%) of 60 patients with COVID-19 showed different variants of OD, only 21 (35%) of them were aware of this testing, which allowed us to identify a more accurate frequency of occurrence of OD, compared to the previous study [29]. Importantly, these data provide solid evidence that OA is often associated with COVID-19. In a study [36], 33 quarantined patients diagnosed with COVID-19 were remotely assessed. At home, they had to independently conduct an odorant test using a solution of denatured ethyl alcohol with a decreasing concentration [36]. As a result, the results were as follows: chemosensitive dysfunctions were registered in 21 patients (63.6%). Specifically, 13 patients (39.4%) reported combined taste and olfactory disorders. In 4 patients (12.1%), only a decrease in taste (dysgeusia) was observed, while in the remaining 4 patients (12.1%), isolated hypo / anosmia was observed.

Specialists at the Mayo Clinic (USA) used artificial intelligence with advanced technology of deep neural networks to identify and analyze the clinical features of the pathogen SARS-CoV-2 [37]. The study found that the prevalence of anosmia in COVID-19 (+) - patients was 28.6 times higher than in patients with similar respiratory infections, and OD was one of the earliest symptoms of COVID-19 [37]. Susceptibility to SARS-CoV-2 infection depends to some extent on the host genotype; the heritability in anosmia was 47% [38].

According to current research, olfactory dysfunction has a high incidence in patients with COVID-19 in European and American countries, while it is rare in patients in China [19,25].

Lovato A. et al. Reviewed 5 articles on the clinical picture of COVID-19 patients

from China, including 1556 cases; no studies have reported on the manifestation of OD in patients [25]. Mao L. et al. In a study [26] retrospectively analyzed the neurological symptoms of 214 patients in Wuhan, China, and found that 5.1% (n = 11) of patients had a sense of smell impairment [26]. To our knowledge, this article is the only study to date describing OD in COVID-19 patients in China.

Several reasons explain that the incidence of OD in patients varies across countries. First, SARS-CoV-2 is able to mutate, which causes its increased virulence and pathogenicity [43]. In a study [10], the authors performed a phylogenetic analysis of the SARS-CoV-2 genome and found the 3 most common variants with an altered amino acid sequence [10]. Genotypes A and C of SARS-Cov-2 have a significant population of Europeans and Americans, however, type B is the most common genotype in East Asia. It is assumed that strains of type A and C are highly pathogenic for the olfactory epithelium of the human nasal cavity, which leads to an increase in the prevalence of OD in European and American countries. Secondly, a species-specific pathogenetic predisposition to SARS-Cov-2 is possible, and it can also circulate in the population. However, there is no evidence to support this assumption. Third, due to the COVID-19 outbreak that originally occurred in China, doctors were poorly informed about the infection, highlighting only the primary life-threatening symptoms, but leaving out the history of olfactory disorders.

Since anosmia may be the only clinical manifestation of COVID-19 in patients without any other significant signs, it highlights the differentiation of coronavirus infection by an otolaryngologist [14]. Otolaryngologists should always be vigilant when working with outpatients to diagnose COVID-19 on time. To avoid cross-infection, a physician may consider a remote sense of smell assessment for a COVID-19 patient with OD [36]. Also an important problematic issue is that many infectious diseases of the upper respiratory tract, for example, rhinosinusitis are very often accompanied by anosmia / hyposmia [1]. This can contribute to the difficulty in making the correct diagnosis, especially during a pandemic.

Among other things, during the COVID-19 pandemic, it is necessary to remember about the timely detection of rare hereditary pathologies. Thus, in the article [2], the authors describe a clinical case in a patient with Rendu-Osler disease (congenital hemorrhagic telangiectasia). The patient experienced intense nosebleeds during stress.Thanks to timely diagnosis, including detection of telangiectasias on the skin and mucous membranes; family nature of the disease; the absence of pathology of the hemostasis system, the doctors managed to correctly diagnose and begin timely therapy.

Otolaryngology is a high-risk unit for COVID-19, especially for physicians over 60 years of age [32]. The main prerequisite is that it is important for the medical personnel to comply with the rules of personal protection in the first place [46]. With the exception of urgent emergencies, telemedicine in ENT is a good option for reducing COVID-19 cross-infection [27].

Current confusion and future directions. Many current studies lack longitudinal results on olfactory dysfunction in COVID-19 patients from disease onset to full recovery. Therefore, the question of whether OD in a patient with coronavirus infection is temporary or permanent remains to be seen. What is the exact prevalence of anosmia / hyposmia in COVID-19 patients worldwide? Can indicators of olfactory dysfunction be used as a valuable indicator of diagnosis and prognosis of infection?

Uncovering these problems in the future will require more extensive research, both basic and clinical. Macaca mulatta monkeys are a good experimental model for studying the correlation of pathogenesis and olfactory dysfunction in COVID-19. The high similarity with humans lies in the fact that the course of infection with SARS-CoV-2 in monkeys is exactly the same. The olfactory epithelium can be biopsied for ultrastructural observation to better understand the pathology of OD in patients with COVID-19 [18]. Once the COVID-19 pandemic has been contained, an epidemiological study should be conducted involving patients from different countries and races.

Conclusions. OD is a characteristic feature of COVID-19 in patients that can occur on its own or with other symptoms, but its pathogenesis is not well understood. In-depth studies are needed to elucidate the clinical features and pathogenesis of the SARS-CoV-2 pathogen in patients with OD. Otolaryngologists and therapists need to be aware of anosmia in COVID-19 to avoid misdiagnosis and thereby contribute to the COVID-19 pandemic.

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THE ROLE OF ABBERRANT HOXA GENE EXPRESSION IN THE GENESIS OF GYNECOLOGICAL DISEASES

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The review presents current data on the effect of endometrial expression of the homeobox genes *HOXA10* and *HOXA11* on implantation processes in healthy fertile women and in common gynecological diseases. The features of gene expression, potentially unfavorable for implantation and leading to infertility, are described. The variability of receptivity markers expression before and after myomectomy, salpingectomy and the removal of ovarian endometriomas was shown.

Keywords: *HOXA10* and *HOXA11* genes, expression, endometrium, implantation.

Purpose: to determine the role and expression pattern of *HOXA* genes in the genesis of various gynecological diseases.

Homeobox genes «*Hox / HOX*» (Gr. homios – similar) of mammals are homologues of the gene complexes of the fruit fly Drosophila melanogaster Antennapedia and Bithorax.

These selector genes are expressed in the process of organogenesis and regulate the anatomical and functional identity of the body segment structures of the embryo by encoding transcription factors that control a number of «downstream» genes, completely unknown) [48].

The name «*Hox*» is used for the homeosis genes of vertebrates, «*HOX*» for humans. The complete set of 39 *Hox / HOX* genes in humans and mice is carried out in four clusters (*HOXA, HOXB, HOXC and HOXD*), each of which contains 9-13 genes of 7, 17, 12 and 2 chromosomes, respectively. The *Hox / HOX* gene homeobox is represented by a sequence of 183 base pairs encoding a 61 amino acid homeodomain, similar to the bacterial helix / anti-helix model. Homeodomains are a helix-loop-helix-coil-helix structure that is responsible for recognizing and binding specific DNA sequences that regulate the expression of target genes.

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The homeodomain mediates protein binding to the promoter areas of target genes containing the 5 '- TAAT-3' sequence. The *Hox / HOX* genes are characterized by collinearity, indicating expression along the anteroposterior body axis in a sequence identical on the chromosome. The *HOX* axis of the human genital system coincides with that of mice [49].

The specificity of temporal and spatial expression of *Hox / HOX* genes is manifested in the control of the female reproductive tract organogenesis. The *Hox9, Hoxa10, Hoxa11* and *Hoxa13* genes in mammals regulate the differentiation of Mullerian ducts into adult reproductive structures. It was found that they are prematurely and simultaneously expressed in the paramesonephral duct in early embryogenesis (exclusion from the collinearity principle) in the phase when the Mullerian ducts are devoid of stromal or epithelial differences [12,49].

The ability of *Hox / HOX* genes to regulate the morphogenesis of body segments explains the development of abnormalities in homeobox mutations. Loss of the function of the *Hom-C3'labial* gene in the fruit fly leads to impaired involution of the embryo's head segment, salivary glands, and cephalopharyngeal apparatus [40]. It is possible to develop an additional pair of wings instead of a pair of stoppers or to transform the antenna into Drosophila legs [34]. With the dominant position of the gene, which is located behind the 5 'mutated gene, posterior transformation is probable. In case of 3 '*Hox* gene mutation or deletion, changes in the body segment by the type of anterior transformation are likely to happen.

The homology of the *HOX* genes of different mammalian clusters allows to compensate for the loss of function [14]. The *Hoxa10* deletion in mice results in the transformation of the proximal uterine body into a tubular and narrow structure similar to the fallopian tube, controlled by the *Hoxa 9* gene [7].

Several of the *Hox / HOX* genes are involved in the unique transformation of the female reproductive system in the postnatal period – during the menstrual cycle and pregnancy.

HOXA9 is expressed in the fallopian tubes, *Hoxa11* – in the lower segment of the uterus, cervical glands and epithelium, *Hoxa13* – in the ectocervix and the upper part of the vagina (in the epithelium) [18,49].

The *HOXA10* protein is found in the nucleus and cytoplasm of epithelial and stromal cells in the endometrium of mice and baboons [1]. In adults, *Hoxa10 / HOXA10* is expressed in the endometrium during the menstrual cycle [18]. Hybridization of *Hoxa10* mRNA in situ reveals more pronounced expression in the functional layer of the endometrium compared to the basal one, moderate expression in the myometrium and distal intestine [49]. The *Hox / HOX* genes regulate cell differentiation and endometrial proliferation by influencing the relationship of receptors and female steroid hormones, the secretion peak of which occurs during the «implantation window» [1,12,49].

The *HOXA10* expression is also modulated by testosterone and vitamin D [23].

About 40 genes regulated by *HOXA10* have been described, including moderators of endometrial receptivity [41]. The *Hoxa10 / HOXA10* and *Hoxa11 / HOXA11* genes act as important transcriptional moderators that either activate or suppress downstream target genes [25].

Important targets for embryo implantation include cell adhesion molecules, signal transduction factors and metabolic mediators [42].

Emx2 is a divergent homeobox gene that is the mammalian homologue of the Drosophila empty helix gene. The *Emx2* gene of vertebrates is located outside the *Hox* cluster and is expressed in the

developing vertebrate brain and genitourinary system, in the embryo – in the epithelial components of the pronephros, mesonephros, ureteral kidneys, wolffian and Mullerian ducts. In mouse embryos, *Emx2* expression is significantly reduced in the male gonad, but remains in the female gonad. *Emx2* null mutants do not develop kidneys, gonads or a reproductive tract [17].

In adults, *EMX2* is found in the human uterus. *EMX2* expression showed dynamics depending on the phase of the human reproductive cycle [16]. *EMX2* is cyclically expressed in the adult endometrium, having an antiproliferative effect; it doubles in the peri-implantation endometrium [15]. *Hoxa10*-regulated *Emx2* expression is fundamental to embryo implantation. *Hoxa10* / *HOXA10* suppresses the expression of the homeobox gene *Emx2 / EMX2* [37].

The *HOXA10* expression changes in mice and primates are associated with defects in uterine functions leading to infertility [42], due to a decrease in endometrial receptivity, hemorrhage and disorganization in the implantation zone [36]. Violation of protein synthesis occurs due to mutations and epigenetic devations, DNA modification with the same nucleotide sequence. The attachment of a methyl group to cytosine in the CpG dinucleotide at the C5 position of the cytosine ring reduces gene expression, up to «epigenetic shutdown» with the development of endometriopathies, endometriosis and endometrial cancer.

The *HOXA-10* gene regulates the activity of the β3-integrin subunit [25] and the development of endometrial pinopodia [11]. During decidualization, the expression of *HOXA10* and *HOXA11* and the vascular endothelial growth factor *VEGF* increase [8].

An increase in the expression of the *HOXA* genes mRNA in decidual cells transformed from the stroma makes mucosa more susceptible to embryo implantation [42].

Changes in *HOX* genes expression are connected to a decrease in implantation and diseases associated with infertility (of unknown origin, PCOS, endometriosis), and recurrent spontaneous abortions [13,26,28,33]. Endometrial polyps, disorders of blood flow in the vessels of the uterine mucosa and chronic endometritis are also noted among the pathological conditions of the endometrium [20].

The role of *Hoxa / HOXA10* in implantation is confirmed by the aberrantly high expression of HOXA10 mRNA in the fallopian tube mucosa during ectopic pregnancy [47].

A decrease in the level of *HOXA10* mRNA in endometrial biopsies of women with hyperandrogenism is associated with a decrease in fertility in PCOS [30]. Endometrial tissue obtained by pipel biopsy in the secretory phase was immediately placed in liquid nitrogen and stored at -72 ° C; after RNA extraction Northern blot analysis was performed.

In a prospective study, Kara M. et al. (2019) studied the endometrium of 53 women of reproductive age with abnormal uterine bleeding not associated with uterine fibroids and polyps in the proliferative phase of the menstrual cycle. The expression of *HOXA-10* mRNA in glandular epithelial cells of the endometrium in the group with polycystic ovary syndrome (PCOS) (n = 33) was significantly lower than in healthy fertile women (0.60 ± 0.14 versus 1.23 ± 0.21) ($p < 0.05$). A similar tendency was observed for the *HOXA-11* and leukemia inhibitory factor (LIF) genes ($p < 0.05$) [38].

The *HOXA* genes leading role in the genesis of endometriosis is confirmed by data on abnormal endometrial receptivity during the "implantation window" on the background of changes in the DNA methylation profile [43].

In a sample of infertile women with peritoneal endometriosis diagnosed during laparoscopy (n = 31) without drug therapy, a decrease in the expression of *HOXA-10* and *HOXA-11* mRNA in the secretory endometrium was revealed by quantitative real-time PCR (PCR-RT) in the last three months. Western blot analysis also showed a decrease in the expression of endometrial proteins in endometriosis compared to the control group (n = 26) [29].

The analysis of *HOXA-10* expression in the endometrium of women with infertility of idiopathic genesis, various forms of endometriosis, uterine myoma and healthy fertile in the middle of the secretory phase was carried out by PCR-RT, the same protein – by an immunohistochemical method. The *HOXA-10* mRNA expression level in the endometrial glands was significantly lower than in the stroma, but without intergroup differences. No protein expression was found in the glands. Overexpression of the *HOXA-10* protein in the endometrial stroma in women with peritoneal endometriosis was determined more often (100%) than in other groups with infertility (on the background of infiltrative endometriosis (72.7%), ovarian endometriomas (70.0%), uterine fibroids (68.8%), of unexplained genesis (55.6%)) [5].

Violation of epigenetic regulation at the heart of the pathogenesis of endometriosis confirms the hypermethylation of the *HOXA10* gene promoter and a decrease in its expression in the eutopic endometrium of animal models (mice and baboons) [45].

Changes in genomic DNA methylation at the *HOXA10* locus in the stroma of endometrioid heterotopies, including foci on the peritoneum, ovaries and lung parenchyma, are associated with progesterone resistance and increased local estradiol production [48].

The *Hoxa10 / HOXA10* gene in embryogenesis implements the introduction of immature mesenchymal cells into the endometrial tissue; it also determines the endometrioid profile of immature cells among adults. Homeobox genes "switching off", medical or surgical, is considered to be the way to prevent endometriosis. Characteristic of healthy fertile women, a halving of the *Emx2* level from the index in the peri-implantation endometrium is absent in endometriosis [37].

The predominant expression of the *HOXA10* protein was observed in stromal cells in comparison to the glandular eutopic and ectopic endometrium of fertile and infertile women. Szczepańska M. et al. (2010) revealed a significant decrease in the transcript ($p = 0.019$) and protein ($p = 0.048$) of the *HOXA10* gene in women with endometriosis-associated infertility [15].

A later study by the authors (2012) confirmed low expression of mRNA and *HOXA11* protein levels in endometriosis compared to healthy women ($p = 0.003$ and $p = 0.004$, respectively) and tubal-peritoneal infertility ($p = 0.041$ and $p = 0.001$, respectively) in the middle of the luteal phase using Western blotting and PCR-RT [32]. In both studies, excessive methylation of the CpG islet in the 1st exon of the *HOXA11* gene was determined in endometriosis in comparison to other groups ($p < 0.001$) [4,15].

A decrease in the *HOXA10* gene mRNA expression in the eutopic endometrium in the middle of the luteal phase in endometriosis-related infertility was associated with excessive methylation of the promoter in the study by Fambrini M. (2013) [9].

Similar observations took place in women with ovarian endometriomas in the middle luteal phase in comparison to healthy women. There was an increase in methylation of the *HOXA10* promoter in the eutopic endometrium with endometriosis rather than with intact mucosa (8.7% versus 6.2%, $p = 0.037$ and 11.9 versus 9.2%, $p = 0.032$ for sequences 1 and 2, respectively). The methylation level was significantly higher in the eutopic tissue

in endometriosis than in the ectopic tissue: the average difference for sequence 1 and 2 was −3.6 (p = 0.001) and −6.0 (p = 0.0001), respectively [35].

An increase in the expression of microRNA 135b in the secretory phase of the uterus correlates with a decrease in *HOXA-10*, confirming the aberrant receptivity of the endometrium among women with endometriosis [21].

Reduction in the level of *HOXA10* methylation was noted in the blood of fetuses of women with endometriosis who took folic acid (n = 22), in contrast to the control group (n = 15) [6].

A possible mechanism of endometrial receptivity disorder in uterine myoma in the study by Doherty L.F. et al. (2015) [44] was associated with a decrease in the expression of *HOXA10* mRNA due to the activation of transforming growth factor (*TGF-β3*) after applying the medium of cultured myomatous node cells.

Makker A. et al. (2017) evaluated the expression of the *HOXA10* and *HOXA11* genes in the "implantation window" of women with infertility and uterine myoma without cavity deformation (n = 18) in comparison to healthy fertile women (n = 12). Indicators of mRNA and proteins studied by quantitative PCR-RT and immunohistochemistry, respectively, were lower in infertile women, however, a statistically significant decrease was observed only for *HOXA10* mRNA (p = 0.03) and the same protein (p = 0.001) [27].

In the endometrium of women with intramural, submucosal leiomyomas without uterine cavity deformation, there was a tendency towards a decrease in the expression of *HOXA-10* and *HOXA-11* mRNA in the middle of the luteal phase in comparison to the fertile ones and uterine septum, but without statistically significant differences. After myomectomy of intramural nodes, three months later, a statistically significant increase in the expression of *HOXA10* (12.8 times) and *HOXA11* (9 times) was revealed. Removal of submucosal myomas had no significant effect on gene expression [24].

In a case-control study by Alizadeh Z. et al. (2013) no differences in the endometrial expression of *HOXA11* and *HOXA10* mRNA on days 19-23 of the menstrual cycle were found among infertile women with uterine fibroids larger than 5 cm (n = 12). After myomectomy, there was an increase in *HOXA11* by 1.24 times (p = 0.7) and *HOXA10* by 2.39 times, but without statistically significant differences (p = 0.15) [19].

The decrease in the expression of *HOXA-10* and *HOXA-11* mRNA in the secretory endometrium of infertile women with endometrioma (n = 20) and benign ovarian cysts (n = 5) in comparison to healthy women (n = 5) was statistically insignificant. Removal of the endometrioma was accompanied by a considerable rise in the expression of *HOXA-10* mRNA (12.1 times) and *HOXA-11* (17.2 times), in contrast to preoperative parameters (p = 008 and p = 035, respectively) [31].

A number of studies have shown a decrease in *HOXA-10* mRNA in the middle phase of secretion in the endometrium of infertile women with hydrosalpinx as opposed to fertile women. Salpingectomy resulted in a statistically significant 15-fold increase in the *HOXA10* expression in both the glandular epithelium and the endometrial stroma compared to the preoperative indicator [32].

The evaluation of *HOXA10* and *HOXA11* proteins in epithelial and stromal cells of the endometrium on the 7-8th day after ovulation by calculating the histochemical index (h-score) in a prospective study of infertile women (n = 65) did not reveal statistically significant differences in the groups (with low ovarian reserve (n = 22), tubal-peritoneal factor (n = 13), endometriosis (n = 5)), except for the sample with infertility of unknown origin (n = 15) in comparison to healthy patients (p = 0.005) [10].

A statistically significant decrease in the expression of the *HOXA10* gene mRNA was found in the endometrium of women with endometrial polyps (n=21) – by 2.9 times (p = 0.016), *HOXA11* – 5.5 times (p = 0.03) compared to the control (n = 9), regardless of size and quantity [2].

In the samples of the secretory endometrium of women of reproductive age with infertility, a lower expression of *HOXA10* mRNA (p = 0.047) was detected by 0.69 times compared to the control. The expression of miRNA-135b was 1.81 times higher (p <0.01). The *HOXA10* gene expression increases significantly in the middle of the luteal phase and remains high from the moment of implantation to the end of the reproductive cycle [3].

In a cohort study by Yang Y. et al. (2017) 18 healthy women, 12 with habitual implantation failure under the age of 40 (transfer of at least four good quality embryos for at least three fresh or frozen cycles) and 20 – with recurrent miscarriage, evaluated the expression intensity of *HOXA-10* and E-cadherin. The calculation was carried out according to the H-score equation: H score = ∑Pi (i + 1), where i is the intensity of staining (0 = negative; 1 = weak; 2 = moderate; 3 = strong), Pi is the percentage of cells stained at each intensity (0% -100%). H-scores were measured separately in stromal cells and glandular epithelium.

The *HOXA-10* expression was localized in the nuclei of stromal cells and the cytoplasm of glandular epithelium cells. The *HOXA-10* H-scores in the groups with recurrent miscarriage and implantation failures were lower than in the control group, both in the glandular epithelium and in the stroma [16].

Endometrial samples obtained during hysteroscopy of 84 women with gynecological diseases (submucosal (n = 13) and intramural uterine myoma (n = 13), endometriosis (n = 27), uterine septum (n = 6), Asherman syndrome (n = 8), hydrosalpinx (n = 4) or uterine polyps (n = 11)) were distinguished by methylation of at least one CpG cluster in the *HOXA10* promoter area in comparison to healthy controls (n = 7). High methylation in a number of CpG islets of the *HOXA10* gene promoter was detected in endometrial polyps, submucosal and intramural myomas. Women with endometriosis, in contrast to healthy controls, were distinguished by a decrease in methylation. A correlation was found between gene expression in submucous uterine myoma and DNA methylation of 1 CpG cluster in the second intron area (CpG 4.5.6; r = 0.72, p = 0.02), as well as in endometriosis (r = −0.9, p = 0.04) [39].

In 25 biopsies of the eutopic endometrium of women of reproductive age with infertility on the background of chronic endometritis, bisulfite sequencing revealed methylation in the promoter area of the *HOXA10* gene in 84% and the *HOXA11* gene in 64%. The correlation with the duration of infertility was determined: up to one year the methylation level was 5.7%, more than 10 years – it was close to 50% [46].

The methylation status of the *HOXA10* and *HOXA11* genes suggests a probable molecular marker of infertility in various gynecological diseases. The leveling of abnormal DNA methylation of genes is associated with the drugs indole-3-carbinol and epigallocatechin-3-gallate, which indirectly affect endometrial receptivity [22]. The endometrium of women with the most common gynecological diseases is characterized by a specific pattern of the *HOXA10* gene methylation.

It is assumed that data on the nature of the *HOXA* gene expression in various gynecological diseases will help in the development of specific therapeutic agents for fertility management.

The authors declare no potential conflicts of interests

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HDV INFECTION: A PREDICTOR OF SEVERE HEPATIC FIBROSIS (LITERATURE REVIEW)

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Abstract: About a third of the world's population has serological signs of previous or current HBV infection, and 350 million people suffer from chronic hepatitis B. It is generally known that the combined damage of the liver with hepatitis B (HBV) and delta (HDV) viruses significantly increases the risk of adverse outcomes such as liver cirrhosis and hepatocellular carcinoma. However, the lack of official registration of HDV in Russia contributes to the belittling of the threat posed by it. In addition, many aspects of the pathogenesis and improvement of HDV diagnostic methods still require detailed study. The problem is of particular importance for the Republic of Sakha (Yakutia), since the share of chronic hepatitis D in the etiological structure of chronic viral hepatitis accounts for 24.5%, in some areas the number of people with antibodies to HDV infection among HBsAg-positive population reached 31% [8]. In the European part of Russia, antibodies to the HD virus were detected in 1.3-5.5% of individuals with HBsAg [3].

The aim of this study is to study the epidemiological and pathogenetic aspects of HDV infection using the example of the Republic of Sakha (Yakutia).

The rate of progression of chronic hepatitis D infection in patients is not the same, while the factors that determine the unfavorable outcome of this infection need to be clarified [14,16,28]. In addition to the genetic characteristics of the virus, the role of interferon genes in the formation of liver fibrosis was noted, due to their binding to the receptors of host cells and their influence on the process of viral reproduction within the cell. The results of clinical studies indicate their predictive effects in Asian populations (Japan, China, Taiwan). There are works indicating the influence of polymorphism (rs368234815) of the IFNL4 gene on the incidence of hepatocellular carcinoma [17]. Taking into account the above, the clinical and epidemiological situation for hepatitis D can serve as the subject of studying the influence of the virus genotype and polymorphisms of candidate genes on the formation of HDV-associated hepatocellular carcinoma, as well as on the likelihood of achieving a stable virological response and / or spontaneous clearance in patients with different rates of liver disease progression.

Keywords: Hepatitis D virus, hepatitis B virus, chronic hepatitis, epidemiology, predictors, gene polymorphism, INFL 3, INFL 4, liver cirrhosis, hepatocellular carcinoma.

Introduction. Despite some progress in studying the characteristics of the epidemiology of hepatitis D, the risk of its progressive course remains high. Chronic hepatitis D is a severe form of liver disease characterized by an aggressive course and leading to the rapid development of liver cirrhosis and hepatocarcinoma [3, 6, 7].

Due to the lack of an official registration of this disease in the Russian Federation, the epidemiological situation is assessed fragmentarily by based on the available results of selected scientific studies. In hepatitis B, super-infection with the delta virus causes progression of the disease and leads to a more rapid development of liver cirrhosis than mono-infection of hepatitis B. [1, 2, 12].

Purpose of the study: is to study the epidemiological and pathogenetic aspects of HDV infection using the example of the Republic of Sakha (Yakutia).

Research results: The Republic of Sakha (Yakutia) is one of the disadvantaged territories of the Russian Federation in terms of the prevalence of parenteral viral hepatitis [9]. According to the Rospotrebnadzor Administration for the Republic of Sakha (Yakutia), the incidence of chronic viral hepatitis in 2018 amounted to 67.8 people / 100 thousand people, which is higher than the incidence rates in the Far Eastern Federal District (48.1 people / 100 thousand people) and the Russian Federation (42.2 people / 100 thousand people. The frequency of detection of antibodies to hepatitis D virus according to scientific research in Yakutia is heterogeneous, ranging from 17.2% to 31.7% [8]. In the federal register "Chronic viral hepatitis in the Republic of Sakha (Y)", only 15,068 people were registered, of which the share of chronic hepatitis D accounted for 15.5%. Chronic viral hepatitis D is detected more often in men of working age and is characterized by a progressive course, with the development of cirrhosis of the liver (LC) and hepatocellular carcinoma (HCC). In the etiology of all cirrhosis, the proportion of delta infection is 38.4%, and in HCC - 28.5%, among all deaths from viral hepatitis in Yakutia in 2019, 43% suffered from chronic viral hepatitis D. According to the analysis cases of detection of hepatocellular carcinoma in the Republic of Sakha (Yakutia), this pathology exceeds the average incidence in the country, and the incidence of malignant neoplasms of the liver is ten times higher than the average in the Russian Federation both among men and women [5, 9, 11].

A feature of the hepatitis D virus is its ability to replicate in the human body in the presence of hepatitis B virus [4, 25]. The causative agent of HDV infection was first identified by Italian scientists in 1977 when analyzing 83 liver biomaterials in HBsAg carriers [20, 21]. The

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antigen found in liver cells was initially mistaken for a new HBV marker, but further research led to the discovery of the defective hepatitis D virus [22]. The virus particle is formed from the nucleocapsid, which is represented by the only virus protein with an envelope coated with the surface proteins of the hepatitis B virus [5]. The genetic material, in the form of genomic HDV RNA, has a size of 1700 nt and is located inside the nucleocapsid. [24].

The rate of progression of chronic hepatitis D in patients is not the same, and the factors that determine the unfavorable outcome of this infection need to be clarified [25]. It is customary to distinguish 8 main genotypes of the hepatitis D virus, based on the polymorphism of the nucleotide sequences of genomic RNA, the geography of distribution of which is extensive and diverse [18, 26, 27, 28].

The most common variation - genotype 1, covers all continents and is characterized by variety of clinical course. Genotypes 2, 4 are more common in East Asian countries, including the Far East, and have a milder and slower course of the disease than genotype 1. Genotype 3 is common in the more northern countries of South America, such as Peru, Ecuador, Venezuela, Colombia; genotype 3 is often characterized by a fulminant course with the rapid development of cirrhotic changes. Genotypes 5,6 and 7 are poorly studied, found in the countries of Central and West Africa (Table 1) [3,6].

Based on genotyping of blood serum samples from patients with HDV infection living in the Republic of Sakha (Yakutia) and subsequent phylogenetic analysis of hepatitis D virus isolates, the prevalence of hepatitis D virus genotypes 1 and 2 was revealed. Among the indigenous population of the republic, both genotypes were equal in frequency of occurrence, but in the non-indigenous population genotype 1 was found somewhat more often (11.4%) (Table 2). The incidence of liver cirrhosis in both groups is almost the same, 37.1% and 36.6%, respectively. With the 1st genotype, chronic hepatitis in 54.3% of cases proceeded with an active cytolytic reaction, with the 2nd genotype it was slightly less - 36.6%. The proportion of patients in the stage of cirrhosis prevailed in genotype 2 (46%), HCC was observed in patients with only HDV genotype 1 (5.7%) [10].

There are data from the analysis of partial or complete HDV sequences isolated in certain regions of the world [4, 23], including in Russia [8], however, ideas about the evolution of HDV and the relationship of different variants of the virus with the progression of the disease and molecular evolution of HDV are fragmentary character. As a result of the analysis of HDV sequences obtained in dynamics by the Sanger sequencing method, the existence of positive selection in the domains located in the T- and B-cell epitopes of HDAg was shown, as well as a return to the original sequence, i.e. a tendency towards "reverse evolution" of HDV. Apparently, this indicates the selection of more replicatively successful and, possibly, more pathogenic variants, as well as the evasion of the virus from the immune response [22].

From a clinical point of view, in the prospective observation of patients, an association of high levels of viral load was noted HDV with disease progression [1], as well as reactivation in advanced liver disease of HB-virus replication, usually suppressed by HDV [13]. There are also studies of host genetics, especially variants of genes that control immune and inflammatory response pathways. The role of interferon genes is due to binding to cell receptors and participation in the process of viral reproduction inside the cell. N. Ma et al. [19] in their work investigated 3128 people, a homogeneous (Chinese) population, for the possibility of infection with HBV and a number of gene polymorphisms (14 loci) of interferons. The most significant gene variants - IFN associated with HBV elimination were genes IFNAR2, IFNLR1, and the polymorphic variant rs4649203 of the IFNLR1 gene was associated with persistent virological effect (SVR). In addition, the authors point out the connection between the formation of HCC and the polymorphisms of the IFNA1, IFNA2, IFNL4 genes. In the studied groups with cirrhosis and liver cancer, genotypes rs12971396 G, rs8113007 T and rs7248668 A of the IFNL 4 gene, which are associated with a worsening of the course of viral hepatitis B. In addition, the development of liver cancer in polymorphic variants of the IFNA1-rs1831583 and IFNA2-rs649053 genes is indicated. The polymorphic variant rs4649203 of the IFNLR1 gene is designated as a predictor of virus elimination, and the polymorphisms rs1051393, rs12233338 of the IFNAR2 gene as a candidate gene for infection in the studied population [19]. There are studies indicating the relationship between the development of HCC and the polymorphism (rs368234815) of the IFNL4 gene [17, 18]. The IFNL3 gene

Table 1

Distribution of HDV genotypes in world

HDV genotype	Distribution	Pathogenity
1	All around the world, predominantly North America, Middle East, Europe, incl. Russia (Tuwa, Yakutia)	Variable
2	Eastern and Northern Asia (Japan, Taiwan, Yakutia)	Mild
3	Northern part of South America (Brazil, Peru, Columbia, Venezuela, Ecuador)	Severe
4	Japan, Taiwan, China	Mild
5-7	Western and Central Africa	No data
8	Western and Central Africa, Brazil	No data

Table 2

Indicator	Genotype	
	I (n = 36)	II (n = 29)
Mean age (yr)	42±12,4	41,2±8,9
M:F ratio	1 : 1.6	1.3 : 1
Indigenous population share, %	88.6	96.7
LC formation frequency, %	37.1	36.6
Hepatitis activity grade, %: - Chronic hepatitis without activity - Chronic hepatitis with cytolytic activity	 8.6 54.3	 6.6 36.7
- HDV-цирроз	40.0	46.7
- HDV- liver cancer	5,7	-

polymorphism (rs8105790) is associated with spontaneous clearance of hepatitis B [16].

Conclusion: The course of chronic hepatitis D is characterized by its aggressive nature and rapid development of complications such as cirrhosis and liver cancer. The clinical and epidemiological situation for viral hepatitis D in the Republic of Sakha (Yakutia) can serve as an example for studying the influence of candidate genes and their polymorphic variants on the formation of HDV-associated hepatocellular carcinoma or probability of obtaining a stable virological response, spontaneous clearance in patients with different rates of liver disease progression and different activity of the disease. The results obtained will expand the understanding of the course of HDV infection and can be used to form priority groups for the appointment of antiviral therapy and develop a personalized approach to the management of people with HDV infection. To objectively assess the situation with HDV infection and improve the effectiveness of preventive measures, it is recommended to introduce official registration of HDV infection.

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CELLULLAR AND MOLECULAR MECHANISMS OF NEUROSTEROIDS IN DIFFERENT PARTS OF CENTRAL NERVOUS SYSTEM OF RATS (Part 1)

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Neuroactive steroids are a class of endogenous steroids which are synthesized in nervous tissue or\and able to modulate the brain functional activity. The first part of the review is dedicated to less investigated genomic and non-genomic mechanisms of action of pregnenolone, pergnenolone sulfate as well as their synthesized analogs and highlights the peculiarities of their production in different regions of rat central nervous system in ontogenical aspect. Some effects of pregnenolone and pregnenolone sulfate modulation of NMDA, GABA, kainate and AMPA receptors, the inhibition of potential-dependent Ca2+ channels both in vivo and in vitro are presented in the review. We've also made an attempt to systematize basic effects of neurosteroids in dependency with the region of central nervous system.

Keywords: neurosteroids, neurotransmitters, regions of central nervous system, ontogenesis, pregnenolone, pergnenolone sulfate, PREG, PREGS, KK-169, NMDA-receptor, GABAA-receptor, AMPA-receptor.

Abbreviations: AMPA – -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; GABAA – γ-aminobutyric acid's receptor; mEPSCs – miniature excitatory postsynaptic currents; NMDA – N-methyl-D-aspartat; PREG, PREGS – pregnenolone, pregnenolone sulfate; OATP – organic anion transporting polypeptides; TRP-channel - Transient receptor potential channels; CREB - cAMP response element-binding protein.

1. Introduction. Currently, the main neurosteroids are pregnane steroids, which include dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS), pregnenolone (PREG) and pregnenolone sulfate (PREGS). Messenger RNAs, protein components of neurosteroids have been found in neurons of such brain regions as the cerebral cortex, hippocampus, thalamus, amygdala, hypothalamus and nuclei in spinal cord. Detected neurosteroids' concentrations in nervous tissue were several times the size of their concentrations in blood serum, which did not change after adrenal and orchiectomy. Neurosteroids modulate the activity of many neurotransmitters and nuclear receptors without being specific or obligate ligands, that is why their regulatory functions are optional and may be suppressed by other endogenous mediators, many of the targets for the action of neurosteroids have not yet been identified [1].

2. Pregnenolone and pregnenolone sulfate. Pregenolone's (PREG) synthesis from cholesterol takes place in a mitochondrion with an involvement of enzyme named cytochrome P450cc (cholesterol side-chain cleavage enzyme), after that the conversion of PREG to other neuroactive metabolites follows [5]. Noteworthy that limiting stage of steroidogenesis is cholesterol transportation through the mitochondrial membrane, not the P450scc activity. Mechanisms of this process still remain unclear, but it's widely known that at least two proteins - StAR и TsPO take part in the cholesterol's transportation. [8]. Sulfotransferase converts PREG into PREGS, which is one of the most important neurosteroids synthesized in CNS. Consequently, the presence of the PREGS in rats' brain (male rats and mice 2 months old) were confirmed by the tandem of liquid chromatography and mass spectrometry(LC -MS\MS), concentrations in spinal fluid, blood serum, hippocampus, cerebral cortex were 3, 1, 20 и 17 ng\g respectively [1]. The presence of the PREGS in the human was confirmed as well [4].

It should be notified that the PREGS, which effects neurons in the CNS, doesn't always have neuronal origin. PREGS may also be transported to CNS through the BBB by OATP - organic anion transporting polypeptides expressed in the vascular plexus cells from peripheral tissue. Other representatives of the same transporter proteins family OSTα and OSTβ are more specific to PREGS and for this reason contribute to PREGS elimination from neurons in the mice's brain [7].

3. Major molecular targets. In spite of the huge amount of information about PREG's metabolites' molecular mechanisms of action, we lack data about PREG itself. It was identified in the experiment with antagonists of sigma-1 and sigma-2 receptors named DuP734 and Lu28179 respectively that PREG suppresses NMDA-mediated dopamine release in the extrapyramidal system [Table 1] by the interaction with those receptors, because the antagonists' usage led to the total abolishment of this effect. Intracellular cascade, which supports the sigma-receptors' signal transduction, consists of proteinkinase Cβ, which was established by another experiment with the LY379196 enzyme inhibitor. It also resulted in suppressing PREG's effect on dopamine's release [3]

PREGS interacts with different receptors of multiply ligand-activated ion channels both presynaptic and postsynaptic [4]. PREGS is an excitatory neurosteroide and for this reason it is a negative modulator for $GABA_A$, kainate and AMPA

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receptors and a positive one for NMDA receptors, although its binding site differs from the glycine's [3]; also PREGS is an agonist for transient receptor potential channels (TRP-channels) [9] and may provoke a dopamine release in rat's black substance both *in vivo* and *ex-vivo* in nanomolar and picomolar concentrations.

Cellular penetration of Ca2 + mediated by NMDA receptors in the cell increases neuron's activation and its "synaptic power". This process is called long-term potentiation, it's basic for neurons' training, shor-term and spatial memory formation [8]. Also NMDA-receptors' activation leads to the increase in an intracellular cAMP's level, which consistently increases both a concentration and activity of the transcription factor, which binds CRE genome fragments (CREB) and regulates transcription of various neuropeptids, and for this reason also regulates synaptic plasticity. This is the excellent example of genomic PREGS' activity [6]. Studies, focused on the effects of pregnenolone sulfate and its synthesized analogs (KK-169, KK-181) on NMDA-receptors, have serious clinical relevance, because many processes, which are basic for neurons' training in a physiological state, may be impaired in patients with schizophrenia or autism, therefore PREGS and its synthesized analogs may be estimated as a probable cure for this diseases. Application points, molecular mechanisms of PREGS interaction with NMDA receptors have to be defined accurately to use those substances for treatment purposes. It is widely known that sulfated molecules are more hydrophilic that non-sulfated ones, for this reason it's understandable, why PREGS and the other sulfated neurosteroids are more likely to interact with intracellular receptors, not with extracellular receptors. Nevertheless, it was stated that PREGS' synthesized analog called KK-169, which has almost identical effects on NMDA, AMPA receptors and synaptic transmission, may be stored intracellularly despite of its hydrophility. Such things as the presence of intracellular targets for PREGS and KK-169, the influence of their intracellular storage on neuron's functions and its structure, possibility of their usage in clinical practice are still under research. The main restricting factor for their introduction into therapeutical schemes is their negative inhibitory side effect on $GABA_A$ receptors. Furthermore, some of KK-169' features, which may be used in a description of PREGS' mechanisms of interaction with NMDA-receptors, were described. KK-169 changes glutamate's interaction with NMDA-receptor in such a way as the receptor's reaction on glutamate's presence is maximum possible and leads to more continuous and effective neuron membrane's depolarization with the minimum glutamate's quantity possible in a synaptic gap, which prevents receptors from early desensitization. Desensitization takes place only when non-physiological high concentrations of both KK-169 and glutamate are in synaptic gap in the same moment. It doesn't occur if the glutamate's concentration is high and KK-169 is absent from a synaptic gap, which underlines the neurosteroids' regulatory effect on a synaptic functioning. Therefore, the more NMDA-receptors are in a synaptic gap, the less is the PREGS' effect, because in a physiological state this neurosteroid increases the effectiveness of NMDA receptor's antagonist [3]. Interestingly that KK-169 also causes an outstanding increase in the amplitude and decay rate of an action potential in the autapse-synapse. KK-169 decreases time of the interaction between NMDA-receptor and its antagonist called memantine in a presence of N-methyl-D-aspartate, but this effect may be rather ambiguous as it can be explained by both increased ability of ion channels' opening, mediated by the interaction between NMDA-receptors with its antagonists in a KK-169's presence, and increased number of NMDA-receptors in a KK-169's presence [2].

It was showed that PREGS may change the functioning ability of recombinant NMDA receptors in geterological cells, formed by a combination of the NR1 subunit with one of NR2 subunits (NR2A, NR2B, NR2C or NR2D). PREGS modulates ion currents, induced by NMDA, glutamate and glycine through the NR1/NR2A and NR1/NR2B receptors, and inhibits a receptor's activity, if they belong to NR1\NR2C and NR1\NR2D subclasses. Effects of PREGS may change over time and differ from each other in CNS regions or on stages of ontogenesis, because NR2 subunits' expression has its topological features and changes during the ontogenesis as well [2]. Other pregnenone neurosteroids also modulates the NMDA receptor's activity

PREGS may mediate its influence on the NMDA receptor by preventing the an agonist's detachment or effecting kinetics of deactivation or macroscopic NMDA receptor's desensitization [Table 2], which were demonstrated on the transfected HEK-293 culture, NR1a/NR2A и NR1b/NR2B subclasses [3,8]. It is widely known that NMDA receptor's activity is influenced by various endogenous molecules, such as zinc, polyamines and protons. Zinc and polyamines modulates receptor's activity through the increase and decrease of tonic proton inhibition, on the contrary, PREGS' influence does not connected with proton sensor. The region of receptor's subunit NR2B, named as steroid modulatory domain SMD1 and formed by J/K loops in the S2 zone of glutamate recognition site and forth transmembrane region, may mediate both steroid and proton regulatory mechanisms, and in this way it contributes to forming of hydrophobic recess for the interaction with PREGS[9].

PREGS excitory effects on NMDA receptors are rather clear nowadays, but data, which implies on a bidirectional PREGS' action in a presence of high extracellular calcium concentrations (more then 0,5 mmol\l), still exists. PREGS boosted all NMDA-receptor-dependent peak and constant ion currents in perforated whole-cell modification of a patch clamp technique without Ca^{2+}, whereas in a presence of extracellular Ca2+ in concentration of 0,5mmol\l and higher PREGS inhibited ion currents. The same research [10] stated that bidirectional PREGS' effects on constant ion currents are conditioned by an influence on the average time of ion channel opening, which is a constant unit for each receptor type. Data was obtained by the usage of cell-attached modification of patch clamp technique, which allows to analyze an ion current in a single NMDA-receptor-de-

Table 1

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

Location	Basic effect	Required conditions	Receptors	Meaning
Striatum	NMDA-stimulated Dopamine Release (-)***	-	Sigma-1 (+)* Sigma-2 (+)	Influence on the processes of neuronal and behavioral plasticity

Table 2

List of pregnenolone sulfate (PREGS) main effects in various parts of the central nervous system (without developmental characteristics)

Location	Basic effect	Required conditions	Receptors	Meaning
Hippocampus	Spontaneous release of glutamate (o** \ +)	Culture of hippocampal neurons in newborn rat pups. Sections of the hippocampus of 3-4 day old rat pups	Sigma-1 (+)	Regulation of synaptogenesis, formation of synaptic neural networks
	Stimulated release of glutamate (+)	Mature hippocampal neurons	Sigma-1 (+)	Improvement of learning and memorization processes due to paired relief (PPF) in mature hippocampal neurons
	Spontaneous release of γ-aminobutyric acid (-)	Mature hippocampal neurons	Sigma-1 (+)	Modulation of the excitability basal level, enhancement of long-term potentiation, improvement of memory and learning
	Release of acetylcholine (+)	Intraventricular injection, infusion into the medial septal nucleus, bodies of acetylcholinergic neurons		Improvement of memorization processes
	Spontaneous release of norepinephrine (o) NMDA-stimulated norepinephrine release (-)	Hippocampal slices, synaptosomes	Sigma-1 (+)	Influence on the processes of memorization and learning, participation in the pathogenesis of epilepsy
Prefrontal cortex	Spontaneous release of glutamate (+)	Concentration ~20 μM	Sigma-1(+), α1-adrenoreceptors (+), σ1-receptor (+)	Improvement of synaptic transmission, cognitive functions
	Dopamine-stimulated Glutamate Release (-) 5-HT-stimulated Glutamate Release (-) Spontaneous release of glutamate (o)	Concentation ~1 μM	Activation of the Gi-mediated signaling cascade	Suppression of stimulated release of glutamate may play an important role in the pathogenesis of neuropsychiatric diseases
Фронтальная кора	Release of acetylcholine (+)	-	-	Improvement of cognitive processes
Стриатум	Spontaneous release of glutamate (o)	-	-	-
Гипоталамус	Dopamine release (o)	-	-	-
Nucleus accumbans	Dopamine release (+)	Intracerebro-ventricular injection	-	Mediation of behavioral responses (motivation, encouragement)

Note. *(+) stimulating effect; ** (-) inhibitory effect; ***(o) no effect.

pendent ion channel. Ca^{2+} influence on PREGS' effects may vary because of the NMA-receptor structure. If DRPEER and exon-5 motives are present in the GluN1 subunit of the receptor, either Ca2+ binding occurs (as DRPEER is a calcium-binding motive), or changes in the receptor's conformation takes place, which leads to inhibition of ion influence on the receptor (H+, Zn2+, Ca2+). In other words, the presence of any of these motives leads to the potential effect during the interaction between PREGS and NMDA-receptor, even if a Ca^{2+} concentration is high. These results were obtained in the experiment with GluN1-GluN2A modifications of NMDA-receptor [6,10].

Showed that PREGS causes long-term postsynaptic potentiation mediated by AMPA receptors [2]. Patch clamp technique in the culture of CA1 pyramidal neurons was used to find out that short-term 5-minutes PREGS exposition induces long-term potentiation in the hippocampal neurons culture, which was selected from 3 to 5 days old rat pups, but not 6 days old ones. There is information about a transient increase in a glutamate release from presynaptic terminal under the PREGS' influence, which may be the trigger for long-term potentiation of postsynaptic AMPA-receptors. These postsynaptic effects are mediated by NMDA receptors with NR2B in its structure. The mechanism of presynaptic PREGS' activity embraces an intracellular Ca^{2+} increase through the NMDA-receptors with NR2D subunits, the regulation of its expression may be also conducted postnatally. Same effects may be mediated by PREG and PREGS synthetized in hippocampal neurons. Depolarization of hippocampal slices leads to enduring increase in the frequency of minor excitory postsynaptic ion currents, interestingly that these effect occurs only in neurons, selected from 3-4 days old rat pups and was absent in hippocamps of 6019 days old animals [1, 2]. Moreover, preincubation of cultivated slices with antibodies to PREGS led to eradication of the long-

term potentiation effect, which allows to conclude that local production of PREGS after the depolarization trully effects the synaptogenesis process [2].

P450scc is an enzyme, which converts cholesterole into pregnenolone, may be identified in rat's brain in early ontogenesis' stages, whereas maximum NMDA receptors expression is detected much later, therefore, endogenous synthesis of PREG and PREGS is likely to influence a functioning activity of NMDA, AMPA, kainite and $GABA_A$ receptors on the last stages of intranatal development.

Besides of the glutamatergic transmissions, PREGS' influence on both spontaneous and potential-dependent release of GABA [Table 2], main inhibitory mediator of the CNS was identified in hippocampus by decreasing of spontaneous inhibitory postsynaptic ion channels' frequency in PREGS' presence [10]. This effect is mediated by the same mechanisms as spontaneous glutamate's releasing, such as sigma-1 receptor activation and $G_{i/o}$ signaling cascade. PREGS also speeds up receptor's desensitization to glutamate by contributing to disulfide bonding between M2-M3 loops in receptor's subunits (M2 domain covers the ion channel, M3 domain is large intracellular domain) and extracellular cysteine loops 7 and 2. Thus effect is absent if Val256Ser mutation is present in α-subunits of the receptor [10]. Glutamatergic transmission's suppression leads to strengthenig of long-term potentiation in CA1 hippocampal neurons, which may have positive effect on learning processes and neuronal plasticity [3].

4. Functions of PREG and PREGS *in vivo.* PREGS increases convulsive potency of NMDA receptors, extends mice's long-term memory, rat's reproducing memory if PREGS is injected directly into the gigantocellular nucleus. PREGS also prevents lack of NMDA effects [Table 2], induced by its antagonists in the passive avoidance test, and antagonistic dizocilpine-induced rat's amnesia. The necessity of the sulfation is approved in chemical sulfation inhibitory tests. It was found that chronical inhibition of the neurosteroids' sulfatase activity by estrone sulfamate improves memory in the passive avoidance test. Experiments, mentioned above, allow to suggest that animal's behavior correlates with the positive modulation of NMDA receptors.

PREG may have functions, which are not connected with GABA, NMDA or other neurotransmitters' receptors. The pregnenolone-linking protein with high affinity and low capacity was identified in the rodent's brain (both in fetus and a sexually mature individuals) [5], it was calledMAP2. The interaction between pregnenolone and MAP2 is intense and becomes even more intense in an association with tubulin.

Conclusion. Pregnenolone and pregnenolone sulfate are major components of the normal rat's neurosteroid status. Nowadays, such questions as a PREG and PREGS' interaction with NMDA-receptors, both excitory and bidirectional, their influence on releasing of basic neurotransmitters in the CNS (glutamate and GABA), on the CNS' ontogenesis process. obtained results may be successfully used in the description of the normal and pathophysiological human neurosteriod profile, because neurosteroid profiles of rats and human being are alike. Nonetheless, many molecular mechanisms of PREG and PREGS' action as well as their side effects are still not clear, that is why their clinical application is rather controversial.

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POINT OF VIEW

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THE RELATIONSHIP BETWEEN MARKERS OF BONE REMODELING AND THE RISK OF BONE FRACTURE IN PATIENTS WITH RHEUMATOID ARTHRITIS

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The aim of the study was to study the possibility of predicting bone fractures by determining the concentration of markers of bone remodeling in patients with rheumatoid arthritis (RA). **Materials and methods.** We observed 88 patients with a reliable diagnosis of RA. The diagnosis was made based on the classification adopted at the 2010 EULAR / ACR meeting. Depending on the presence of osteoporetic fractures detected by collecting anamnestic data, the patients were divided into 2 groups: RA patients with osteoporotic fractures (n = 11) and RA patients without a history of fractures (n = 77). Commercial ELISA reagent kits were used to determine CrossLaps, P1NP and 25-OH vitamin D in serum.

Results. We examined the diagnostic value of in vitro determination of bone remodeling markers (CrossLaps, P1NP, and serum 25-OH vitamin D) using a characteristic curve (ROC curve). Laboratory determination of type I collagen C-telopeptide for predicting the risk of fractures in RA is of good quality (area under the ROC-curve 0.751, specificity 44.16%, sensitivity 100.0%). Laboratory determination of P1NP is of good quality, which confirms the value of the area under the ROC-curve (0.788), test sensitivity 81.82%, specificity 66.23%. Laboratory determination of 25-OH vitamin D for predicting the risk of fractures in RA has an area under the ROC-curve of 0.753, sensitivity 63.64%, specificity 88.31%.

Findings. Thus, the proposed method for predicting the development of low-energy bone fractures in patients with RA using the laboratory determination of markers of bone remodeling makes it possible to more accurately assess the risk of developing low-energy fractures as one of the complications of RA.

Keywords: rheumatoid arthritis, osteoporosis, markers of bone remodeling

Introduction. According to the WHO, rheumatoid arthritis (RA) ranks second among rheumatic diseases. In the world of RA, about 58 million people are affected [1, 2]. Although the etiology of the disease is not fully understood, the pathogenesis of RA is characterized by the activation of cells of the immune system [3].

According to the data available to date, up to half of RA cases (up to 48.6%) are complicated by the development of osteopenia and osteoporosis (OP), which leads to the appearance of bone pain, fractures with minor trauma, changes in posture, early disability in patients of working age [6 , 8]. The pathogenetic reasons for the development of secondary OP in RA are the intake of glucocorticosteroids (GCS), the presence of a chronic immune-inflammatory process, and low physical activity. Due to insufficient knowledge of the etiology, pathogenesis, lack of specific clinical and laboratory signs of the disease (especially in the early stages), the practitioner often faces difficulties both at the stage of diagnosing AP in RA patients and when choosing the optimal treatment strategy [3].

At the present stage, laboratory, histological and radiation research methods are used to diagnose OP, identify disorders of bone metabolism and predict the development of low-energy bone fractures. In clinical practice, the determination of many parameters in blood serum is used to study the state of bone tissue metabolism, for example, the determination of the concentration of proteins and peptides characterizing resorption and bone formation: C-terminal telopeptide of type I collagen (Cross Laps), osteocalcin, osteoprotegerin, total amino-thermal type I propeptide daprocollagen (P1NP) [4, 5]. The role of vitamin D is of great importance [7]. These techniques are characterized by minimal invasiveness and are available for use in the vast majority of clinical diagnostic laboratories.

The aim of our study was to investigate the possibility of predicting bone fractures by determining the concentration of bone remodeling markers in patients with RA.

Materials and methods. We observed 88 patients with RA (women aged 21 to 81 years). When diagnosing RA, we were guided by the clinical classification adopted at the 2010 EULAR / ACR meeting (European League Against Rheumatism / American College of Rheumatology). The patients were followed up by specialists of the A. B. Rheumatology Research Institute of Clinical and Experimental Rheumatology". The majority of patients were between 41 and 55 years old. According to the degree of RA activity, patients were distributed as follows: with activity 0 (DAS28<2.6) there were 19 patients in the study (21.59%), with a low degree of activity I (2.6<DAS28<3.2) - 10 people (11.36%), with an average degree of activity II (DAS28≥3.2 - 5.1) - 52 people (59.09%), with a high degree of activity III (DAS28>5.1) there were 7 patients (7.96%).

Depending on the presence of osteoporetic fractures, identified by collecting anamnestic data, the patients were divided into groups: 1st - RA patients with osteoporotic fractures (n = 11), 2nd - patients with RA without a history of fractures (n = 77).

To determine Cross Laps, P1NP and 25-OH vitamin D in blood serum, we used commercial ELISA reagent kits (Cross Laps ELISA Kit (IDS, UK), kit Procollagen I N-terminal Propeptide (PINP) SEA-957Hu (CLOUD-CLONE CORP., USA), 25 (OH) Vit D ELISA).

For the statistical processing of the

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data, the software packages "STATISTICA 10.0 for Windows" were used. The significance of differences between groups was compared using statistical variation analysis (ANOVA). The results were considered statistically significant at p <0.05. For the reliably significant studied indicators, an ROC analysis of the curves was carried out, the areas under the curves were calculated, and new points for making a diagnostic decision were selected.

Results. We have investigated the diagnostic value of laboratory determination of markers of bone remodeling in order to predict the risk of bone fractures.

To analyze the diagnostic accuracy of laboratory tests, a characteristic curve (ROC curve) was used, the method for evaluating ROC curves was the calculation of the area under the curve (AUC - Area Under Curve), which varies from 0.5 (no diagnostic effectiveness of the test) to 1.0 (maximum test efficiency). This calculation allows us to conclude about the predictive value of the laboratory test.

The curve for determining the diagnostic value of type I collagen C-telopeptide in blood serum is shown in Figure 1. The data on the area under the curves, the points of making the diagnostic decision (cutoff points) are presented in the tables below (tables 1, 2).

Based on the performed calculations, it can be concluded that the laboratory determination of type I collagen C-telopeptide for predicting the risk of fractures in RA is of good quality, which confirms the value of the area under the ROC-curve (0.751). The point corresponding to the optimal sensitivity / specificity ratio is the C-telopeptide value of type I collagen equal to 0.488 ng / ml. This value corresponds to a specificity of 44.16%, and a sensitivity of 100.0%.

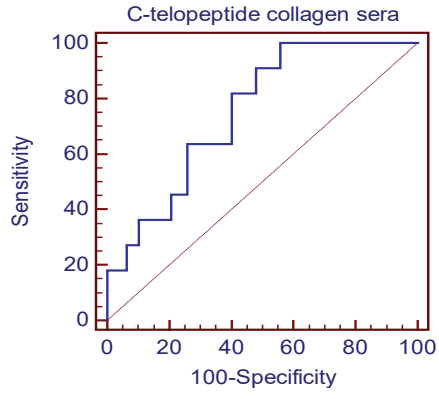
By evaluating similar ROC curves, the diagnostic value of determining type I N-terminal propeptide daprocollagen (P1NP) and 25-OH vitamin D in serum was studied for predicting the risk of fractures in RA.

The laboratory determination of P1NP was found to be of good quality, which confirms the value of the area under the ROC curve (0.788). In this case, the point corresponding to the optimal sensitivity / specificity ratio is the P1NP value equal to 51.76 ng / ml. This value corresponds to a sensitivity of 81.82%, a specificity of 66.23%.

Also, based on the performed calculations, it was concluded that laboratory determination of 25-OH vitamin D for predicting the risk of fractures in RA is of good quality, which confirms the value of the area under the ROC curve (0.753). In this case, the point corresponding to the optimal sensitivity / specificity ratio is the value of 25-OH vitamin D equal to 31.185 ng / ml. This value corresponds to a sensitivity of 63.64%, a specificity of 88.31%.

Findings. It is known that the currently used methods for diagnosing OP and predicting the risk of fractures in RA do not always reflect the true state of bone tissue. Often, low-energy fractures develop in RA patients with normal bone mineral density, assessed by densitometry or absorptiometry, which indicates the insufficient effectiveness of these methods for diagnosing OP. At the same time, the results of our study are consistent with the literature data that the methods for determining markers of bone synthesis and resorption have sufficient sensitivity and specificity for practical use in order to predict the risk of fractures. It can be assumed that the wider clinical use of these markers for diagnostic purposes, including their combined use with instrumental methods (densitometry), will improve the accuracy of predicting the risk of low-energy fractures.

Thus, the proposed method for predicting the development of low-energy bone fractures in patients with RA using the laboratory determination of markers of bone remodeling makes it possible to more accurately assess the risk of developing low-energy fractures as one of the complications of RA. Qualitative diagnos-



ROC-curve characterizing the diagnostic value of the determination of type I collagen C-telopeptide in blood serum for predicting the risk of fractures in RA.

Table 1

Basic descriptive characteristics of the ROC curve

Area under the ROC curve (AUC)	0.751
Standard error [a]	0.0671
95% Confidence interval [b]	0.647 to 0.837
Z statistics	3.738
Significance level P (Площадь = 0.5)	0.0002

[a]DeLongetal.. 1988
[b]Binomial accuracy

Table 2

Criteria values and coordinates of the ROC curve

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	-LR
>=0.2	100.00	71.5 - 100.0	0.00	0.0 - 4.7	1.00	
>0.488	**100.00**	**71.5 - 100.0**	**44.16**	**32.8 - 55.9**	**1.79**	**0.00**
>0.508	90.91	58.7 - 99.8	44.16	32.8 - 55.9	1.63	0.21
>0.547	90.91	58.7 - 99.8	51.95	40.3 - 63.5	1.89	0.18
>0.549	81.82	48.2 - 97.7	51.95	40.3 - 63.5	1.70	0.35
>0.646	81.82	48.2 - 97.7	59.74	47.9 - 70.8	2.03	0.30
>0.667	63.64	30.8 - 89.1	59.74	47.9 - 70.8	1.58	0.61
>0.746	63.64	30.8 - 89.1	74.03	62.8 - 83.4	2.45	0.49
>0.752	45.45	16.7 - 76.6	74.03	62.8 - 83.4	1.75	0.74
>0.786	45.45	16.7 - 76.6	79.22	68.5 - 87.6	2.19	0.69
>0.796	36.36	10.9 - 69.2	79.22	68.5 - 87.6	1.75	0.80
>1.024	36.36	10.9 - 69.2	89.61	80.6 - 95.4	3.50	0.71
>1.062	27.27	6.0 - 61.0	89.61	80.6 - 95.4	2.62	0.81
>1.244	27.27	6.0 - 61.0	93.51	85.5 - 97.9	4.20	0.78
>1.284	18.18	2.3 - 51.8	93.51	85.5 - 97.9	2.80	0.88
>1.621	18.18	2.3 - 51.8	100.00	95.3 - 100.0		0.82
>1.993	0.00	0.0 - 28.5	100.00	95.3 - 100.0		1.00

tics in this case makes it possible to determine an adequate amount of therapy and pharmacological correction of disorders of bone metabolism. The inclusion of bone resorption markers in the standard examination program for RA patients can reduce the risk of developing low-energy fractures and improve the quality of life of patients.

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PERSPECTIVES FOR THE STUDY OF ACTIVE LONGEVITY AMONG RESEARCHERS

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Along with the increase in life expectancy in most countries of the world, including in Russia, the proportion of people in older age groups is growing. The authors carried out a scientific review of the problem of "active longevity" and the prospects for its study in a group of researchers. Currently various components of the question of "active longevity «are widely studied. However, in relation to researchers, most of its problems have not been studied systematically, there are practically no justified preventive measures, as well as measures to normalize the functions that have already been violated in a particular researcher. The development of the problem of active longevity of researchers will help to formulate a set of measures to extend the effective professional longevity of researchers and ensure scientific continuity.

Keywords: active longevity; professional longevity; researchers; scientists; cognitive abilities; quality of life.

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Introduction. Simultaneously with the increase in life expectancy in most countries of the world, including Russia [34-35], the share of older people in the population is growing. Since 2003, the average life expectancy in the Russian Federation for those born in 2020, according to the State Statistics Committee, is 73.4 years [30], and according to the conservative forecast, it will reach 75.4 years by 2035.

One of the areas of activity where the proportion of older workers is high is science. The development of science is one of the priority goals in the Russian Federation. In 2018, the National Project "Science" was approved, the goals of which are [30]:

1) ensuring the presence of the Russian Federation among the five leading countries of the world engaged in research and development in the field of priority areas for scientific and technological development areas;

2) ensuring the attractiveness of work in the Russian Federation for leading Russian and foreign scientists and young promising researchers;

3) outstripping the increase in domestic spending on research and development, compared with the growth of the country's gross domestic product.

To achieve these goals, the project "Science" sets the following tasks [18]:

1) Creation of at least 15 world-class scientific and educational centers based on the integration of universities and scientific organizations and their cooperation with organizations operating in the real sector of the economy;

2) creation of world-class scientific centers, including a network of international mathematical centers and genomic research centers;

3) updating at least 50 percent of the instrument base of the leading organizations performing research and development;

4) development of advanced infrastructure for research and development,

innovation activities;

5) the formation of an integral system of training and professional growth of scientific and scientific-pedagogical personnel, providing conditions for the implementation of scientific research and development by young scientists, the creation of scientific laboratories and competitive teams.

According to the National Research University Higher School of Economics [11], by 2020 the Nauka national project became the leader in budget execution (50.3%, or 21.7 out of 43.1 billion rubles), especially in the sections “Development of scientific and scientific and industrial cooperation "(the budget was executed by 73.8%) and" Development of advanced infrastructure for research and development in the Russian Federation "(44.5%). However, in the section "Development of human resources in the field of research and development" there is a relative lag (15.4%), which requires understanding and analysis of the reasons. In this review article, we tried to analyze the existing scientific data on one of the most important areas in the development of human resources in science - active longevity of researchers in the context of the global problem of active longevity of the population as a whole.

The purpose of this work was to conduct a review of domestic and foreign literature related to professional longevity and analyze the receipt of data in relation to scientific activities, to indicate the prospects for studying this issue.

Materials and methods. A descriptive review of scientific publications for the last 10 years on the main scientific databases (elibrary.ru, dissercat.com, GOOGLE SCHOLAR, MEDLINE, PUBMED) related to the issue of active longevity of working people was carried out. Date of request: January 20, 2021.

Results. *Active longevity of researchers: not necessary or necessary?*

The professional activity of scientists requires a high level of education, a long time for mastering the theoretical and practical aspects of the subject, is characterized by great intellectual workload and has a high social significance. All this determines the importance of maintaining the professional health of scientists [26]. However, a search in domestic and foreign databases (elibrary.ru, dissercat.com, GOOGLE SCHOLAR, MEDLINE, PUBMED) did not allow us to find works devoted to the study of active longevity / healthy aging in individuals engaged in scientific activities. So, a number of domestic works on various aspects that can be attributed to the problem of active longevity of mental workers, included other persons - managers and administrators, engineers and operators - or did not specify at all the nature of mental work [8,21], therefore, obtained in their results can hardly be transferred to researchers. The issues related to the active longevity of scientific workers (or the problems of aging) were not considered even in the voluminous work of AG Alakhverdyan, dedicated to the current state of scientific personnel [1]. The Federal Target Program (FTP) “Scientific and Scientific-Pedagogical Personnel of Innovative Russia” for 2009–2013, as well as the scientific project of the Russian Academy of Sciences (RAS) for 2006–2008, [19] the science of young researchers and completely ignored the issues of retention in science and maintenance of the effectiveness of the work of older scientists. That is why, relying on the found literary sources, this problem can still be approached only indirectly, which will be considered below.

Physical and mental health of researchers. Professional health is a combination of psychophysiological and physiological parameters that ensure high performance and professional longevity. The greater the stock of functional reserves of the body, the higher the efficiency of professional activity and the period of professional longevity [20]. Occupational health consists of emotional, cognitive and behavioral components and directly affects the efficiency of activities, work capacity, as well as the mental, physical and social well-being of the employee [12].

Domestic works on assessing the health status of research workers as a whole, regardless of age, not to mention the elderly, are not enough. So, Savina A. A. [24] revealed an increased growth rate of general and primary morbidity among employees of the Russian Academy of Sciences (RAS), exceeding the average indicators for Russia by 2.7 and 3.2 times, respectively. Thus a high rate of growth of primary morbidity observed in endocrine diseases (61%) tumors (55%), diseases of the urogenital system and diseases, caused external causes (by 42.0%), pathology musculoskeletal system (30.0%) and circulatory system (by 28.0%). Among the reasons for hospitalizations, problems of the circulatory system were in the lead (31.9%), followed by the genitourinary system (14.1%), digestive organs (9.5%), neoplasms (8.9%), pathology of the musculoskeletal system (8.2%) and external causes (6.5%).

In another study, researchers, contact with various occupational hazards, showed an increase in disease risk in 2.6 - 4.6 times, by comparison with those, who had no occupational hazards [3], but a separate analysis of older workers and the control group of another there was no profile in the study. In a series of studies have shown, that the SRI staff, has not yet reached the pre-retirement age (mean age 48.4 and 46.7 years) were more common risk factors, like abdominal obesity, smoking and lack of exercise, while the university staff - stress and disturbance nutrition. Despite the high level of education of the respondents, they were poorly informed about the presence at such risk factors, like high cholesterol and hyperglycemia. These studies also showed a high level of stress in workers of the two studied categories, accompanied by an increase in blood pressure [10,17].

In addition, in the researchers significantly more frequently, than the university staff, it was detected high anxiety, anxious - depressive syndrome and clinically significant depression. [14] However, these studies did not provide for a comparison with similar indicators in people who are not engaged in scientific work, so it is not possible to answer the question of whether the state of health of researchers differs from the population; the older age group was also not studied. Nevertheless, when analyzing these results, it can be assumed that preventive work with the medical component of active longevity of researchers should be started long before they reach the pre-retirement and especially retirement age. Psychological work is likely to be equally important, since as high emotional stress and stress (which were identified in the discussed works) accelerate aging [25].

The importance of the psychological component for the work of researchers is also evidenced by a study on a small number of research institutes of different ages, in which the assessment of psychological well-being and satisfaction with professional achievements turned out to be low, despite the fact that they considered their health to be satisfactory and the level of cognitive functions high. At the same time, managers (and they are, presumably, older than lower-ranking employees) showed a higher level of autonomy, self-efficacy and professional well-being and naturally higher achievements in the implementation of professional goals [26]. Although this study did not specifically target older scientists, it does provide some insight into the possible psychological implications of maintaining active longevity in them.

The subjective assessment of health, based solely on physical well-being, is becoming a key parameter in the new interpretation of aging: It is much more important to feel healthy than to have formal confirmation of it. Apparently, the psychological state also has a very large influence on the subjective assessment of the state of health. When studying patients with chronic somatic diseases (diabetes mellitus, obesity, arterial hypertension) aged 45 to 80 years, it turned out that the subjective assessment of their own health was inversely related to such indicators of psycho-emotional state as the level of depression, reactive anxiety and personal anxiety [28]. Moreover, a positive orientation towards the socially active and rewarding aspect - a favorite job - was associated with a more positive and stable psycho-emotional state, lower levels of depression and anxiety, and higher social achievements. Of the options proposed (health status, family well-being, material security, favorite job, principles and beliefs), only the choice of a favorite job as the main value orientation correlated with a higher health-related QoL [28]. D.M. also speaks of a similar influence. Rogozin [23]: in his opinion, the most significant factors that determine the subjective perception of health are not age, but higher education, professional employment, and a variety of labor practices. Erudite people who manage their free time on their own (flexible hours), according to the study, assessed their state of themselves much better than those who were looking for external causes of their problems (pinning their hopes only on guardianship and care from the state).

Dynamics of the number of scientific personnel and its age structure. The number of scientists in various branches of science gradually increased in the second half of the 20th century, while in the post-Soviet period (1994-2010) the number of scientific personnel decreased significantly in almost all areas (in medical sciences by 11%, in natural sciences - by 22%, in technical sciences - by 33%, social - by 27%, agrarian - by 26%), with the exception of the humanities (an increase of 41%. [1] In the period up to 2018, the number of personnel of scientific organizations in Russia continued to decrease (by 7.3 % compared to 2010) and amounted to 682.5 thousand people, which was 3.6% compared to 2017 (Table 1) [22].

We can see in the table that, although there is a decline in headcount across all categories, it has affected support personnel more, which may be due to low wages and growing skill requirements. Due to the outflow of young personnel in the period from 1990 to 2010, among Russian scientists, the share of researchers in the older age group increased significantly, while the percentage of young and middle-aged scientists decreased. At the time of 2010, 25.2% of scientists in the Russian Federation were aged 60 and older [1]. The main reason for this age bias is the outflow of a young and active part of research workers abroad or, if they stayed in Russia, leaving science as an industry funded on a leftover basis and having unclear development prospects. However, this is not the subject of this article; we will be more interested in the trends of the last decade. In the past three years, the number of researchers with higher scientific qualifications - candidates and doctors of sciences - has also decreased: in 2016 compared to 2020 - by 2.8%, in 2017 - by 4.7% and in 2018 by 2.9%. 2010 to 2018 this indicator decreased from 105.1 to 100.3 thousand people. (by 4.6%). Ratay and Tarasenko believe that since 2010, the average age of researchers has decreased from 48 to 47 years (candidates of sciences - from 53 to 51 years, persons without a scientific degree - from 45 to 44 years), and doctors of sciences - increased from 62 to 63 years old [22]. We believe that the age difference of 1 or 2 years is unlikely to indicate a trend that has taken shape.

2010 to 2018 the number of the age group of 30-39 years increased by 1.5 times, and the share of scientists of this age in the total number of researchers - from 16.2 to 26.5%. The share of the age group under 39 years old has remained practically unchanged since 2017 (Table 1) [22]. According to other data, by 2018 scientists under 39 years old accounted for almost 44%, the share of scientists over 60 years old was not much less, but the share of the middle generation (40 - 59 years old) was characterized by a "failure" [2]. By 2018, the number of researchers over the age of 70 was 33.5 thousand out of 360 thousand, that is, at least a tenth of all researchers, or 3.5 thousand more than in 2008 [6].

Age-related aspects of scientific activity. The aging problem can be viewed from different points of view: passport age, biological age, social age, mental age. They do not always coincide. For different work activities, the age defining an employee as “elderly” is apparently not the same and can vary over a wide range. In particular, mental and social old age among mental workers and creative professions can occur at a rather old age [32]. The influence of the general workload and the level of education on the professional longevity of older people is clearly traced [7], hence the potential for continuing professional activity among researchers is undoubtedly higher than among people of many other professions. Indeed, employment of older people is higher in areas with a high level of utilization of advances in science and technology, and science workers, along with doctors and teachers, have high employment rates for older people [31].

Sociologists and psychologists of science have actively studied the relationship between aging and scientific performance [42]. The work of researchers may be assessed by several criteria, the main of which are:

The number of employees of scientific organizations by category, taking into account the dynamics of age groups [22]

	Number of pateints (n)			Growth rate, percent	
	2010	2017	2018	2018 к 2010	2018 к 2017
Total	736 540	707 887	682 541	-7.3	-3.6
Researchers	368 915	359 793	347 847	-5.7	-3.3
Technicians	59 276	59 690	57 716	-2.6	-3.3
Support staff	183 713	170 347	160 577	-12.6	-5.7
Other personnel	124 636	118 057	116 401	-6.6	-1.4
Age groups of researchers					
Total	368915	359793	347847	-5.7	-3.3
≤ 29 years old	71194	66376	60634	-14.8	-8.7
30-39 years old	59910	91429	92106	+53.7	+0.7
40-49 years old	54113	51149	52800	-2.4	+3.2
50-59 years old	88362	59893	54830	-37.9	-8.5
60-69 years old	60997	57414	54076	-11.3	-5.8
≥ 70 years old	34339	33532	33401	-2.7	-0.4

1) creativity is large ("breakthrough") scientific achievements and the creation of new directions in science; 2) productivity; 3) influence on other scientists and (often judged by citation).

With regard to scientific creativity, two categories of research can be distinguished with completely opposite results. The first group of studies examined extraordinary scientific achievements such as Nobel Prize winners; they showed that the studies subsequently awarded these prizes were mainly performed by people under 40 [41,52,55]. When analyzing 414 Nobel prizes for the period from 1901 to 1992, it was proved that over the years the age distribution of laureates in the field of physics, chemistry, medicine in different years is approximately the same [51]. In physics, the time of maximum creativity occurs at the age of 31-35 years, in medicine - 31-35 years, in chemistry - 36-40 years. About 7% of the physics laureates make their discoveries before the age of 25. Only 6% of all Nobel laureates have made breakthrough discoveries after the age of 50. In the 19th century, the psychologist Beard [38] studied the relationship between creativity and age and concluded that young people have a lot of enthusiasm, but little experience, and the old enthusiasm has little, but experience more than enough, therefore, creativity will reach its maximum at the intersection of these two factors - experience and enthusiasm. This partly explains why the peak of creativity in different fields of activity occurs at different ages. It is possible that there is an age shift from scientific research to fulfilling other roles in science (for example, transfer of experience, teaching), more typical for researchers of the "average" and less typical for the most prominent scientists [51].

In terms of scientific productivity, some studies have also shown that young researchers are more productive than older researchers [43,45,50]. However, there are other results as well. Thus, even in the early works of Lehmann [47-48], it was established that the peak of productivity of scientists falls on 30-39 years, although in different branches of science it differs somewhat: physicists - 32-33 years, mathematicians - 23 years, physiologists - 35 -39 years old, astronomers - 40-44 years old. In the future, no fewer studies have shown that the most productive and most influential on science are not young researchers, but middle-aged and older scientists [37,39,40,46,53,54].

The influence of scientists on the professional environment through publications is the main factor of recognition in the scientific community and a direct consequence of the activity and effectiveness of scientific activity; subsequently, the citation index and the so-called impact factor were added to the number of publications. In an international project in the late 1970s. with the participation of more than 10,000 researchers, two peaks of the publication activity of scientists in the natural sciences were identified - at the age of 45-49 and 55 [16]. In some studies, higher productivity and impact factor were noted among young scientists. However, others have shown a higher productivity and impact on the scientific environment among older scientists, confirms Merton's theory of "accumulation of benefits": researchers who are active in scientific work from a young age accumulate more "scientific capital" and thereby subsequently get the opportunity to do better. access to finance, equipment, etc., which in turn help them stay productive longer [44,49]. Recently, it turned out that middle-aged scientists publish no fewer high-quality papers, but at the same time, they publish as co-authors a lot of papers with a lower impact factor, which in general somewhat “dilutes” their average performance. After 50 years, researchers reduce the number of publications, but their scientific influence (impact factor) rises again. In other words, if a researcher does not leave his scientific activity, then his productivity, measured by the number of high-quality publications, only increases throughout his career [44]. All the precipitated work was carried out on foreign scientific material. In domestic scientometrics, this direction has been studied extremely insufficiently. Thus, the position, length of scientific work and the Hirsch index were identified as predictors of the 5-year publication activity of domestic medical scientists; although the contribution of scientific work experience was about 10%, this can be considered an indirect confirmation of the fact that persons working in science for a long time do not lose their effectiveness [29].

Advantages and importance of older age in the research team. A large number of researchers in older age groups are employed in research institutes, departments of universities, institutions of postgraduate education and often constitute the "backbone" of scientific schools [4]. For obvious reasons, the level of scientific qualifications and research experience among employees of older age groups, on average, exceeds that of young people [5]. To ensure scientific continuity and transfer of experience without loss, professional growth of young people, mentoring by the older generation of researchers is necessary [9, 15], and to create full-fledged scientific schools, 2-3 generations are required [21].

The experience of teamwork in a team, the ability to independently make important decisions and be responsible for them are associated with the activation of business potential, which increases in some employees with age, while others do not, depending on psychological characteristics [33]; this position can be fully attributed to the peculiarities of scientific activity. Compared to young people, older people are better prepared for the development of complex multi-level projects, multifactorial expert assessment, making informed decisions, counseling, etc. Changes can make a significant contribution to improving the effectiveness of scientific work of older age groups and increasing their professional activity. working conditions: for example, the expansion of digitalization, the possibility of "remote work", the formation of working teams from people from different regions and countries, as well as the expansion of access to databases and information resources. Young, but less qualified workers (secretaries, assistants, trainees, junior employees) can undertake support and technical support for the work of such specialists [27]. So, the scientific potential and efficiency of a researcher may depend not only and not so much on the calendar age, but on his “professional health” and the ability to restructure the priorities of his scientific activity in accordance with changing age abilities and experience. Interestingly, the change in the role of a particular scientist in the scientific hierarchy abroad is traced in the fact that with age, scientists (professors) in the list of co-authors of publications move from the first position to the last, since the first author, as a rule, is the main performer of the work, and the leader more often it is indicated by the last [27,36] in Russia such a tendency in the list of authors is just being formed. In this regard, perhaps one of the indicators of achieving the goals in the national project "Science" is not optimal, which says that with the advanced development of science, it is planned to rely exclusively on the young. According to the project, the share of scientists under the age of 39 should reach 50.1%. However, according to experts, the age structure of “effective” science should be different, with the dominance of the 40-60 age group and equivalent, approximately 20%, groups under

39 and over 60 [2]. All the arguments in favor of the high importance of senior researchers, presented by us above, also confirm this point of view.

Conclusion. Thus, productivity and impact on the scientific environment is not a simple function that diminishes with age. Effective strategies for the development of science must take into account the characteristics of scientific research as a collective activity and focus not only on the ability of individuals to "enlighten" and discover. Science has come a long way from the individual talents and properties of individual great scientists to teamwork, coordinated work, the dynamic progress of which depends on researchers of all ages. Currently, there is a fairly large proportion of interested youth in the composition of scientists and a significant group of elderly people with qualifications, degrees and titles, while a relatively small section of middle-aged people. But it is the smaller link that is considered the most productive, having both strength and sufficient experience in the profession.

Moreover, in ten years - this small link of middle age will move into the category of older age, which, accordingly, will become more and more small in number. This will make it more relevant than ever to preserve the active longevity of this group of scientists, to preserve the active working capacity of researchers as long as possible, which requires an effective solution to the following problems: maintaining a satisfactory state of physical health, high working capacity, reducing fatigue, maintaining a high quality of life, preventing and reducing the severity of disorders anxiety-depressive circle; special attention is required to maintain high mental performance, that is, the preservation of cognitive functions.

Conclusions. Currently various components of the issue of "active aging" are being actively studied. However, in relation to research workers, most of his problems have not been studied systematically, there are practically no justified preventive measures, as well as measures to normalize functions that have already been impaired in a particular researcher. Working out the problem of active longevity of research workers will help formulate a set of measures to prolong the effective professional longevity of research workers and ensure scientific continuity.

In connection with the active longevity of researchers, further study of the following aspects is necessary to develop sound, expedient and effective methods of prevention and rehabilitation:

1. The state of health and measures to maintain it: a) risk factors and diseases common to the population of pre-retirement age b) nosological units and risk factors more typical of researchers of pre-retirement and retirement ages c) organizational, methodological and medical measures for protection health of researchers

2. The state of functions associated with the implementation of scientific activities, such as performance and fatigue, cognition, disorders and disorders of the anxiety-depressive circle, professional burnout, knowledge of computer technology, knowledge of foreign languages

3. The state of the adaptation reserve: effective interaction with colleagues and communicative competence, satisfaction with the position in the team, financial well-being, recognition in the scientific community (correspondence of the position / title to ambitions, achievements), the attitude of colleagues (presence of the label “old man”), leadership and organizational qualities, mentoring and transfer of scientific skills to young employees.

A holistic vision of the above processes, their impact on life expectancy, risk factors and triggers, as well as measures for their prevention among researchers has yet to be studied and developed.

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FEATURES OF CLINICAL MANIFESTATIONS AND CYTOKINE REGULATION IN GASTRITIS IN SCHOOLCHILDREN WITH FAMILIAL PREDISPOSITION TO THE PEPTIC ULCER DISEASE

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Aim: To study the features of clinical manifestations and indicators of circulating cytokines in gastritis in schoolchildren with a family predisposition to peptic ulcer disease.

Material and Methods: 3343 schoolchildren of 7-17 years old were examined in Siberia (Tyva, Evenkia, Aginsky Buryat National District, Krasnoyarsk). 463 children with gastrointestinal complaints underwent esophagogastroduodenoscopy with biopsy of the gastric mucosa. In accordance with the Sydney classification, the morphological diagnosis of gastritis and the presence of Helicobacter pylori was carried out. Blood serum was also taken to determine the level of cytokines (IL-2, IL-4, IL-8, IL-18, IL-1β, IFN-α, TNM-α) by ELISA. The studies were approved by the ethics committee and the consent of the patients and their parents was obtained.

Results: Schoolchildren with family predisposition to peptic ulcer disease with gastritis more often have dyspeptic manifestations. It was found that in children with a familial predisposition, gastritis proceeds with the involvement of the systemic level of cytokine regulation (expression of IL-4, IFN-α). Moreover, in children with H. pylori infection, the cytokine regulation of the inflammatory process is specific (TNM-α expression).

Conclusion: The features of the course of gastritis in children with a familial predisposition to peptic ulcer disease have been established, and they can be considered markers of the unfavorable course of the pathology.

Keywords: children; gastritis; peptic ulcer; hereditary predisposition; cytokines; DS; GERD.

The problem of diseases of the stomach and duodenum in childhood does not lose its relevance [7], which is largely due to the lack of dynamics to reduce their prevalence and weighting the course. The age of formation of the pathology of the gastroduodenal zone in the majority falls on the school and preschool periods of life, which is represented mainly by gastritis [2, 5, 7], which is, with further progression, the morphological basis of diseases such as peptic ulcer (PUD). YaB belongs to the category of multifactorial. The most unfavorable stage in children, characterized by the growth of the disease, is the period of schooling [1]. Familial predisposition to pathology also has a pronounced negative effect on the formation of ulcer [14]. Predisposition to ulcer, as shown by numerous studies, is based on the features of neurohumoral regulation and morpho-functional characteristics of the stomach, which have a genetic basis. As such, an increase in the formation of pepsinogen-1, an increase in the mass of parietal cells and their hypersensitivity to gastrin, congenital deficiency of mucus fukomucoproteins, and a number of others are considered [3]. In recent years, the features of the cytokine profile in persons with peptic ulcer disease have been shown [10, 11]. The participation of cytokines in the inflammatory process is multifaceted: it is also the regulation of intercellular and intersystem interactions that determine the differentiation and survival of cells, the functional activity of proliferation and apoptosis [4]. There are features of the course of the inflammatory process in the gastric mucosa (GM), initiated by Helicobacter pylori (H. pylori) infection, which induces the production of a number of cytokines. It is known that IL-1β, IL-6, tumor necrosis factor (TNF) -α, TGF-β1, IL-17, IL-18, IL-21 and IL-22 are actively involved in this pathological process [19].

In persons with a family predisposition to peptic ulcer disease, morpho-function-

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al features of the stomach and features of regulatory mechanisms are created, which create the prerequisites for a decrease in the level of the body's resistance to the effects of adverse environmental factors [9]. In such individuals, the disease progresses faster to atrophy and metaplasia [15]. In addition, they have a higher risk of developing diseases for which they have a predisposition. In this regard, deviations in the course of the disease in persons with a predisposition should be considered as prognostic markers [6].

Aim: To study the features of clinical manifestations and indicators of circulating cytokines in gastritis in schoolchildren with a familial predisposition to peptic ulcer disease.

Material and methods. The research was carried out in different regions of Siberia in two stages. At the first stage, a cross-sectional screening examination was carried out with the collection of gastrointestinal complaints and anamnestic data on the presence of peptic ulcer disease in relatives of 1 and 2 degrees of relationship in 3343 schoolchildren aged 7-17 years (1502 boys and 1841 girls; junior schoolchildren 7-11 years old - 1602; senior schoolchildren 12-17 years old - 1741). Examined in Tyva (Turan, Saryg-Sep village) - 1535 children; in Evenkia (Baykit village) - 842 children; in the Aginsky Buryat National Okrug (Aginskoe settlement) - 790 children. In addition, similar data were obtained from 176 schoolchildren in Krasnoyarsk who are being examined and treated at the clinic of the Research Institute of Medical Problems of the North for problems with the gastrointestinal tract.

Then, by the method of random selection, groups of schoolchildren with gastroenterological complaints were formed, who underwent endoscopic examination of the upper gastrointestinal tract with sampling of biopsies from the mucous membrane of the antrum and the body of the stomach for subsequent morphological examination. All children in Krasnoyarsk also underwent endoscopic and morphological examination of GM. In total, instrumental examination with morphological confirmation of the diagnosis of gastritis was carried out in 463 schoolchildren. The criteria for excluding children from the study were: 1. the age of the child under 7 and over 17; 2. the presence of acute inflammatory diseases during the last month; 3. the presence of chronic diseases of other organs in the acute stage; 4. functional failure of organs and body systems. 5. the presence of allergic diseases. 6) the absence of morphological signs of gastritis.

The diagnosis of dyspepsia syndrome was carried out in accordance with the definition of the Committee on Functional Diseases of the World Congress of Gastroenterologists (Rome IV), which is a complex of disorders including pain or discomfort in the epigastrium, a feeling of fullness in the epigastric region after eating, and early satiety. Clinically, two variants of its course were distinguished: 1) epigastric pain syndrome; 2) postprandial distress syndrome [13].

The diagnosis of gastroesophageal reflux disease (GERD) in children was made in accordance with the child's consensus on pathology [8]. At screening, the presence of weekly heartburn in the subjects was taken as a diagnostic criterion for GERD. Heartburn was understood as a burning sensation in the retrosternal region.

Endoscopic and morphological diagnostics of gastritis was performed in accordance with the modified Sydney classification [12, 17]. Morphological evaluation of the presence and severity of the inflammatory process in the gastric mucosa included light microscopy of biopsy sections after staining with hematoxylin-eosin. The degree of gastritis activity was assessed by the intensity of neutrophilic infiltration of the epithelium and / or the lamina propria of the gastric mucosa. Morphological diagnosis of Helicobacter pylori (H. pylori) infection was carried out after staining biopsy sections according to Giemsa.

For the procedure of enzyme immunoassay (ELISA), a 5 ml blood sample was taken from the subjects. The blood sample was centrifuged, the serum was separated and stored at -20 ° C. The concentration of cytokines in blood serum (IL-2, IL-4, IL-8, IL-18, IL-1β, IFN-α, TNM-α) was determined using the standard test systems "Vector-Best" (Novosibirsk).

In accordance with Article 24 of the Constitution of the Russian Federation and the Helsinki Declaration of the World Medical Association (1964), all examined children and their parents were familiarized with the goals, methods and possible complications during the research with the signing of informed consent to participate before their inclusion in the study.

Statistical processing of the results was carried out using the SPSS version 23.0 software (IBM, Inc). The analysis of the statistical significance of differences in qualitative features was carried out using the - test with Yates' correction in the presence of a feature in the group of less than 10, and less than 5 - two-sided Fisher's exact test. The significance of differences in quantitative traits was analyzed using the Mann-Whitney test. The results of the study are presented for samples that do not obey the normal distribution law, median (Me) and interquartile range (C25-C75). The statistical significance of the differences in signs was assessed at $p<0.05$.

Research results and discussion. Undoubtedly, clinical manifestations are one of the most important indicators of the course of the disease. The study of this issue showed that in schoolchildren with a family predisposition for peptic ulcer disease, there was an increase in the frequency of complaints characterized as dyspepsia syndrome (Table 1). Schoolchildren with a family predisposition to peptic ulcer disease noted dyspeptic complaints in 55.5% and 39.2% ($p <0.001$) without predisposition. At the same time, there was no predominance of a specific clinical variant of the course of dyspepsia. They had epigastric pain in 28.0% compared to 18.3% in children without family burden ($p <0.001$). As for the postprandial variant of the clinical course of dyspepsia, it was also more often present in the complaints of children with a familial predisposition to ulcer: in 27.6% and in 20.9% in children without it ($p = 0.001$). The formation of this variant of dyspepsia is largely associated with motor disorders of the gastrointestinal tract. It should be assumed that several leading links of pathogenesis involved in the formation of the disease (acid, changes in visceral sensitivity and motor disorders) increase in children with a family predisposition to ulcer. A definite confirmation of this is the increase in the prevalence of GERD among schoolchildren with a familial predisposition, in the formation and progression of which similar pathogenetic mechanisms are considered. The frequency of GERD was 13.4% among schoolchildren with familial burden of ulcer and 7.7% in the absence of it ($p = 0.001$).

As characteristics of the progressive course of gastritis to the state of atrophy, its activity is considered. This is especially true for children. An analysis of the question of the association of hereditary susceptibility to ulcer with gastritis activity in the surveyed contingent of Siberian schoolchildren showed the absence of such (Table 2). So high (2-3 degrees) activity of antral gastritis had 48.4% of children with a family predisposition for peptic ulcer disease and 54.6% in its absence ($p = 0.235$). There was no increase in the activity of gastritis in the body of the stomach: in schoolchildren with a family

Table 1

The frequency of clinical syndromes in the examined children, depending on the presence of a family predisposition to peptic ulcer disease

Syndrome		Predisposition to peptic ulcer				p-level (significance of feature differences)
		+ (n=479)		– (n=2864)		
		n	%	n	%	
DS		266	55.5	1123	39.2	0.001
Clinical variants of DS	Epigastric pain	134	28.0	525	18.3	0.001
	Postprandial Distress Syndrome	132	27.6	598	20.9	0.001
GERD (weekly heartburn)		64	13.4	220	7.7	0.001

Note: GERD - gastroesophageal reflux disease; DS - dyspepsia syndrome.

Table 2

The activity of gastritis in the examined children, depending on the presence of a family predisposition to peptic ulcer disease

Gastritis activity		Predisposition to peptic ulcer				p-level
		+ (n=126)		– (n=337)		
		n	%	n	%	
Antral department	1. 1st degree	65	51.6	153	45.4	0.235
	2. 2-3 degree	61	48.4	184	54.6	0.235
Body	3. 1st degree	44	72.1	186	71.0	0.859
	4. 2-3 degree	17	27.9	76	29.0	0.859
p-level (significance of feature differences)	1-2	0.614		0.017		
	3-4	0.001		0.001		

predisposition of 2-3, the degree of activity was determined in 27.9% and 29.0% in its absence (p = 0.859).

It is known that familial predisposition to ulcer affects various regulatory mechanisms of the inflammatory process, including the immune response. Cytokines are largely involved in this. The study of indicators of the cytokine profile in gastritis in schoolchildren with a familial predisposition to ulcer showed certain features. For example, they showed IL-4 expression (p = 0.020) (Table 3). The functional parameters of the participation of IL-4 in the inflammatory process are diverse: suppression of macrophage activity, the production of a number of cytokines (in particular TNF (tumor necrosis factor), IL-6). It is known about the regulatory effect of IL-4 on proliferative processes [4]. In people with peptic ulcer disease, as well as with a predisposition to the disease, there is an imbalance in the protective and aggressive factors of the stomach (local level). And an increase in circulating IL-4, obviously, should be regarded as the activation of a cascade of metabolic, immune events in the body, aimed at ensuring the optimization of proliferative processes in GM. In addition, an increase in IFN-α was noted in children with a familial predisposition to peptic ulcer disease (p = 0.001). It is a cytokine that functionally triggers the body's immune responses to damage [20]. IFN-α has not only antiviral, but also immunomodulatory effect due to the effect on the expression of receptors of the major histocompatibility complex (MHC). In recent years, it has been shown that IFN-α also has an antibacterial effect, which is based on the ability of IFN-α to induce in the affected cell the activity of a number of enzymes with antibacterial activity: in particular, indolamine-2,3- and NO-synthetase [18]. Schoolchildren with a history of ulcer disease showed a tendency to transition to the systemic level of cytokine regulation.

Among other established features of cytokine regulation, there was one more: differences in the expression of interleukin 1β, which was higher in schoolchil-

Table 3

Indicators of cytokines in blood serum in children with gastritis with a family predisposition to peptic ulcer disease

Cytokines	Predisposition to peptic ulcer						P
	+ (n=73)			– (n=106)			
	Me	C_{25}	C_{75}	Me	C_{25}	C_{75}	
1. IL-2	0.1	0.1	0.5	0.1	0.1	0.1	0.108
2. IL-4	1.6	0.9	2.0	1.1	0.4	1.8	0.020
3. IL -8	16.4	0.1	73.9	13.5	0.1	82.8	0.652
4. IL -18	131.2	58.4	186.8	127.8	54.7	212.9	0.859
5. IL-1β	0.1	0.1	0.1	0.2	0.1	0.3	0.024
6. IFN-α	0.7	0.1	1.8	0.1	0.1	1.0	0.001
7. TNM-α	0.1	0.1	0.2	0.1	0.1	0.1	0.203

Table 4

Indicators of cytokines in blood serum in children with gastritis with a familial predisposition to peptic ulcer disease and the presence of *H. pylori*

H.pylori	Cytokines	Predisposition to peptic ulcer						P
		+			–			
		Me	C_{25}	C_{75}	Me	C_{25}	C_{75}	
H.pylori + (nPU+ = 31; nPU– = 60)	1. IL-2	0.1	0.1	0.5	0.1	0.1	0.1	0.234
	2. IL-4	1.5	0.5	2.1	1.0	0.5	1.8	0.208
	3. IL-8	22.6	1.4	86.4	18.6	0.1	91.2	0.487
	4. IL-18	124.6	38.9	194.9	138.1	82.7	227.3	0.445
	5. IL-1β	0.1	0.1	0.1	0.1	0.1	0.4	0.051
	6. IFN-α	0.5	0.1	1.6	0.1	0.1	1.1	0.017
	7. TNM-α	0.2	0.1	0.5	0.1	0.1	0.1	0.048
H.pylori – (nPU+ = 36; nPU– =41)	8. IL-2	0.1	0.1	0.4	0.1	0.1	0.1	0.249
	9. IL-4	1.6	1.0	2.0	1.1	0.4	1.9	0.061
	10. IL-8	8.3	0.3	74.3	7.6	0.1	84.5	0.575
	11. IL-18	117.9	86.7	173.9	117.7	24.1	155.3	0.575
	12. IL-1β	0.1	0.1	0.1	0.1	0.1	0.1	0.133
	13. IFN-α	0.6	0.1	1.8	0.1	0.1	1.1	0.001
	14. TNM-α	0.1	0.1	0.1	0.1	0.1	0.1	0.337
p-level (significance of feature differences)	1-8	0.976			0.929			
	2- 9	0.445			0.807			
	3-10	0.369			0.641			
	4-11	0.927			0.289			
	5-12	0.108			0.240			
	6-13	0.713			0.531			
	7-14	0.125			0.215			

dren without a family history of peptic ulcer disease (p = 0.024). Increased levels of IL-1β are observed in some infectious diseases and inflammatory processes of a non-infectious nature [16]. The pathophysiological meaning of this needs to be studied. Evaluating the results obtained for the state of the cytokine profile and the expression of some of them in gastritis in schoolchildren with a burdened family history of ulcer, a natural question arises that the revealed changes could be the result of different levels of H. pylori infection in children with and without a predisposition to ulcer, which is the primary cause of inflammatory process. Consequently, the existing differences in the expression of cytokines should reflect to a greater extent the characteristics of the inflammatory process in GM of infectious genesis.

When analyzing the level of cytokine expression in schoolchildren with confirmed H. pylori infection, peculiarities were noted in the presence of familial ulcer problems (Table 4). In particular, the expression of TNM-α (tumor necrosis factor), the main role of which is the induction of the inflammatory process. These are the production of IL-1, IL-6 and TNF itself, stimulation of the processes of adhesion and antibody formation, participation in the redistribution of immunocompetent cells, etc. [4]. It is possible that the systemic level of TNF expression is involved in the redistribution of immunocompetent cells from the peripheral blood to the inflammation focus. In persons with a family predisposition to ulcer, the local level of protection of the gastric mucosa is reduced. Under conditions of H. pylori invasion, systemic defense mechanisms are involved, including through TNF.

In addition, the replication of IFN-α, noted in schoolchildren with a hereditary predisposition, was observed both in the presence of H. pylori infection and in its absence. This emphasizes the dominant role of genetic mechanisms in changes in cytokine regulation. Regardless of the presence of infection in children with a familial predisposition to ulcer, replication of IL-4 persisted, but in the form of a trend that was more pronounced without the presence of H. pylori.

Conclusion. Thus, schoolchildren with a family predisposition to ulcer have features of the clinical and molecular manifestations of gastritis. In such children, the systemic level of cytokine regulation increases, the functional orientation of which is associated with the activation of plastic processes and immune reactions. In H. pylori-associated gastritis, they increase the expression of cytokines associated with the induction of a systemic immune response. Whereas there were no changes in gastritis activity indicators in children with a family predisposition to ulcer.

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CLINICAL CASE

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A CASE OF THE COMPLICATED COURSE OF DRUG-RESISTANT TUBERCULOSIS IN A 14-YEAR-OLD ADOLESCENT

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The article presents a clinical case of the development of drug-resistant tuberculosis in a teenager. The peculiarity of the clinical case is due to the fact that the adolescent was observed for local tuberculosis, was removed from the register for cure, but a year later he entered the anti-tuberculosis hospital with widespread tuberculosis with extensive drug resistance of mycobacterium tuberculosis (MBT) to 8 anti-tuberculosis drugs, with poor tolerance to chemotherapy . All these factors led to a complicated course of tuberculosis and was an indication for surgical treatment, for superior lobectomy with atypical resection of C6 of the left lung with decortication. In this regard, it is important to further improve the work of the general medical network among patients who have undergone tuberculosis, as well as the alertness of pediatricians on this pathology.

After the child was removed from dispensary observation, tuberculosis developed with a complicated course and extensive drug resistance of MBT to 8 anti-tuberculosis drugs, which led to upper lobectomy with atypical C6 resection of the left lung with decortication. Further improvement of the organization of anti-tuberculosis measures among patients with drug-resistant forms of tuberculosis is urgent. Pediatricians are advised to be wary of children who come into contact with people with drug-resistant TB.

Keywords: tuberculosis, adolescents, drug resistance, lobectomy, tuberculosis of the intrathoracic lymph nodes, X-ray examination.

Introduction. One of the serious problems of modern phthisiology is the increase in the incidence of tuberculosis caused by a drug-resistant pathogen - Mycobacterium tuberculosis (MBT). In 2010-2011. a multicenter study in 23 centers in 16 European countries found primary MDR MBT in 52.4% of cases. At the beginning of treatment, the tested MBT strains had resistance to pyrazinamide in 59.7%, to injectable drugs - in 26.6%, to fluoroquinolones - in 17.6%, to other anti-TB drugs - in 6.8% of cases.

In 2012-2014 in Saudi Arabia, MBT is mono-resistant to isoniazid in 1.8% of cases, to rifampicin - in 1.4%, to streptomycin - in 1.9%, to ethambutol - in 1.1% and to pyrazinamide - in 2.1% , primary MDR MBT was detected in 4% of cases.

The incidence rate of tuberculosis among children in the Russian Federation at the age of 0-14 years was 9.6 in 2017; 2018 - 8.3, per 100,000 children. Total in Russia in 2018 got sick for the first time - 65234 children (2017-70861 (-5627 cases) (2016 - 78121) (- 7261); including: 0-14 years old 2153 (2017-2475) (-322); (in 2016 -2865) (- 390), 15-17 years old: 765 (2017-868) (-103); (in 2016 - 964) (-96).

In the Republic of Sakha (Yakutia), according to the State Budgetary Institution of the Republic of Sakha (Yakutia) SPC "Phthisiology", the proportion of patients with multidrug resistance (MDR-MDR) among newly diagnosed patients with bacillary forms of TOD (respiratory tuberculosis) was 28.1% in 2019 (79 people), in 2018 - 29.8% (86 people), in 2017 - 32.8% (96 patients). In the Republic of Sakha (Yakutia), the incidence rates of tuberculosis in children aged 0-14 years remain higher than in the Russian Federation. In 2017. amounted to 17.3 per 100 thousand population. In 2018. amounted to 15.0 per 100 thousand population, which is also higher than in the Russian Federation. In 2019, the incidence in children was 14.1, which is 2 times higher than in the Russian Federation (14.1 per 100 thousand population)

The morbidity rate of the adolescent population during the study period decreased by 20.7% - from 51.1 to 40.5 per 100 thousand of the adolescent population, the average rate of decline was -0.6%. This indicator is characterized by the absence of a downward trend (R2 = 0.03), and the rate of its growth (decline) fluctuated within significant limits - from 51.1% in 2017 to -29.1% in 2018 [6]

The most important indicator characterizing the quality of the organization of diagnosis and treatment of tuberculosis is the frequency of primary multidrug resistance (MDR) in newly diagnosed patients [1]. In contrast to the two above-mentioned indicators, the incidence of tuberculosis with MDR of the pathogen in the Republic of Sakha (Yakutia) over the past 5 years has not tended to decrease.

Tuberculosis of the intrathoracic lymph nodes (IHLN) is the main clinical form of primary tuberculosis in children, adolescents and young people aged 18-24 years (up to 80-90% of cases) [4,5].

Most complications (up to 70%) are observed before the age of 3 years and in adolescence, in connection with the pubertal period, against the background of hormonal changes in the body [3]. The presence of close, long-term contact in the family, with relatives, patients with drug-resistant tuberculosis, in combination with unfavorable social factors, lead to the development of complicated processes in children and adolescents and to a positive dynamics in treatment [2].

Clinical example. Patient A. born in 2001 (14 years old) applied in May 2015. about the bend test Mantoux with 2 TE -9 mm. The Mantoux test was delivered at the school. The child was sent for X-ray examination. X-ray examination of the chest organs revealed changes in the intrathoracic lymph nodes.

Epidemic history: Family contact - with my mother in 1998. pulmonary tuberculosis was diagnosed, drug resistance of mycobacterium tuberculosis (MBT) was not revealed. Since 2000 to the present, the child's mother is not registered with the dispensary, on the chest X-ray from May 2017: metatuberculosis changes.

Anamnesis of life: A child from the 4th pregnancy, which lasted 1 half - toxicosis, in 2 - the threat of termination of pregnancy, Delivery on time, birth weight 4280g, height - 56 cm. Breastfeeding up to 1 year. Vaccinated at the maternity hospital, post-vaccination scar 5 mm.

Past diseases: chickenpox, acute respiratory viral infections.

He lives in a complete family, in a three-room comfortable apartment. Two

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Table 1

Result of microbiological research

Material	Дата	By the "LM" KUM method	By seeding method	DNA research	Drug sensitivity
sputum	26.06.17	Detected3+	3+	-	Stability H, R, Rb, S, E, Et, Am, Ofl
sputum	13.09.17	single units	1+	-	-
sputum	10.10.17	negative	-	-	-
flushing from the nasopharynx	08.11.17	Not detected н	Not detected	Not identified	-
flushing from the nasopharynx	09.11.17	Not detected	Not detected	Not identified	-
flushing from the nasopharynx	10.11.17	Not detected	Not detected	Not identified	-
flushing from the nasopharynx	13.12.17	Not detected	Not detected	Not identified	-
flushing from the nasopharynx	18.01.18	Not detected	Not detected	Not identified	-

*LM- luminescent microscopy

adults and three children live with the girl.

After collecting the anamnesis and analyzing the examination data, the girl was taken for dispensary registration in group III A with a diagnosis of Tuberculosis of the intrathoracic lymph nodes. Received treatment: isoniazid-0.6 (600 mg), ethambutol - 1.2 (1200 mg), pyrazinamide - 1.5 (1500 mg / kg), a total of 136 doses in the hospital of the department of pediatric tuberculosis No. 2. Withdrawn from dispensary registration in October 2016 with clinical cure.

06.03.17. there were complaints of increased body temperature up to 38.6, weakness, barking cough, I turned to a pediatrician, treatment was prescribed. I went to the doctor on 14.04.17 with complaints of a wet cough, runny nose, weakness, decreased appetite, was diagnosed with pharyngitis, treatment was prescribed. 20.04.17. I went to the pediatrician with complaints of pain in the chest, the girl's mother noticed a deformity in the sternum, turned to the traumatologist and pediatrician. The pediatrician has appointed a sputum test for acid-resistant Mycobacterium tuberculosis (KUM).

According to the radiograph from 28.06.17.: Deformity of the sternum at the point of transition to the handle, without signs of destruction. Infiltrative tuberculosis of the upper lobe and S-6 segment of the left lung in the phase of decay and seeding (Figure 1). Treatment was prescribed from 30.06.17. - 03.08.17. according to the IV chemotherapy regi-

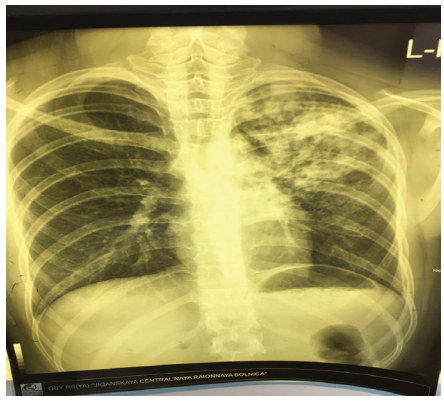

Fig. 1. Overview X-ray of the chest organs from 28.06.17.

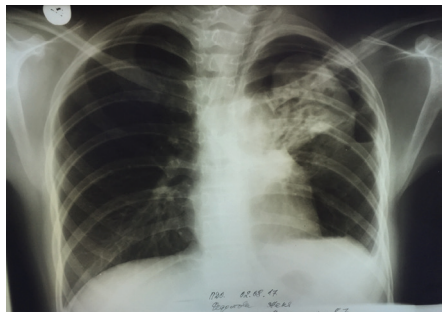

Fig. 2. Plain X-ray of the chest organs from 05.09.17.

men-pyrazinamide (Z) (1500 mg), amikacin (Am) (750 mg), levofloxacin (Lfl) (450 mg), prothionamide (Pto) (500 mg), cycloserine (Cs) (500 mg), PASC (9.0 g), received 35 doses. The condition worsened on 02.08.17. There were complaints of pain in the side, an increase in body temperature to 38.0 - 38.5 C. On the repeated review radiograph of the chest organs from 02.08.17.: the upper lobe of the left lung, which collapsed due to the presence of air in the pleural cavity up to 1.5 cm. The upper lobe and S6 of the left lung are inhomogeneous and darkened with the presence of multiple different-caliber cavity shadows.

The right lung is transparent. The right root is structural, with the presence of calcifications. This picture corresponded to the picture of a partial pneumothorax on the left.

On the plain chest x-ray from 09/05/17. (Fig. 2): the upper lobe of the left lung is reduced in volume, unevenly darkened, with multiple dense shadows, in dynamics there is a partial improvement in the form of pathological changes. A small amount of air is retained apically in the pleural cavity. In S6 of the left lung, a decay cavity with a diameter of 1.5-2.0 cm with perifocal infiltration around is preserved. The lower lobe of the right lung is compensatory emphysematous sub-in-

Table 2

Dynamic of the patient's MANTOUX test, 2013-2016

year	Results
2013	Отр.
2014	10 мм
2015	9 мм
2016	14 мм

Table 3

Dynamics of the general blood test

Date	gemoglobin	red blood cells	ESR	white blood cells	rod-shaped	segmentonuclear	lymphocytes	monocytes	eosinophils	platelets
23.06.17.	87	4.13	9	12.73	10	68	9	12	-	664
13.11.17.	120	4.56	37	8.7	8	55	22	8	7	536
08.02.19.	83	4.5	26	6.2	1	51	35	9	4	551

Table 4

Dynamics of biochemical blood analysis

Date	Total bilirubin	Bilirubin direct	ALT	AST	creatinine	urea	glucose	calcium	potassium	chlorides
23.06.17.	7.1	3.72	17.5	2.16	-	-	-	-	-	-
09.01.17.	8.0	-	94.3	164.1	-	-	4.8	-	-	-
17.01.18.	7.0	-	125.6	176.1	51	2.3	-	2.73	108	4.4
18.02.19.	8.1	-	142.5	115.8	55	3.5	-	2.43	97	-

flated. Conclusion: Caseous pneumonia of the upper lobe and S6 of the left lung. Partial pneumothorax of the upper lobe of the left lung (Fig. 2).

According to the results of the ATR test in 2017, an increase in the size of the papule from 17 mm to 30 mm with necrosis was revealed, which is a sign of high sensitization of the body.

In the biochemical analysis of blood, the presented results show an increase in ALT from 17.5 units from 23.06.17 to 142.5 from 18.02.19, which indicates a hepatotoxic reaction to anti-tuberculosis drugs. According to the results of clinical, laboratory and X-ray examination (Tables 1-4 and Fig. 2), a consultation was held on 05.09.17 and a clinical diagnosis was made: Caseous pneumonia of the upper lobe and S6 of the left lung. Mycobacterium tuberculosis (+). Drug resistance to isoniazid, rifampicin, rifobutin, streptomycin, ethambutol, ethionamide, amikacin, oflaxocin (H, R, Rb, S, E, Et, Am, Ofl). Intoxication syndrome. Normochromic anemia 1 art.

The treatment was corrected. The condition has stabilized. In 22.09.17. medical documents were sent for correspondence consultation to the "Central Research Institute of Tuberculosis" to determine the further treatment tactics. Hospitalization in the adolescent department is recommended. Sputum analysis for Mycobacterium tuberculosis is negative from 09.10.17.

In the adolescent department of the Central Research Institute of Tuberculosis, taking into account the clinical, laboratory and radiological data of the examination, it was decided to conduct surgical treatment.

Treatment before surgery: individual chemotherapy regimen, taking into account XDR MBT (H, R, Rb, S, E, Et, Am, Ofl) and drug tolerance. Due to the increase in transaminases in the biochemical blood test from 09.11.17 g, hepatoprotective therapy was prescribed for 1.5 months (phosphogliv, 5% glucose). From 05.12.17 g - 11.12.17 g (8 doses): cycloserine 0.5 (500 mg); capreomycin 0.8 (800 mg) (lymphotropic); pyrazinamide 1.5 (1500 mg); PASC -9.0 g; zenix 0.6. From 22.12.17 – - 04.07.18. (195 doses): cycloserine 0.5 (500 mg); pyrazinamide 1.5 (1500 mg); PASC 9.0; bedaquiline (according to the scheme-is prescribed at a dose of 400 mg once a day daily for 2 weeks, then 200 mg 3 times a week for 22 weeks). A total of 201 doses were received before the operation. The tolerability of the drugs is unsatisfactory (hepatotoxic reaction to zenix; allergic reaction to capreomycin - eosinophilia in the hemogram of 18%).

05.07.18. the operation was performed-upper lobectomy with atypical resection of the C6 left lung with decortication. Computed tomography of the lungs from 06.07.18.: A picture of multiple tuberculomas in the progressive phase with moderate signs of tuberculosis inflammation activity. Bronchiectasis. Phe-

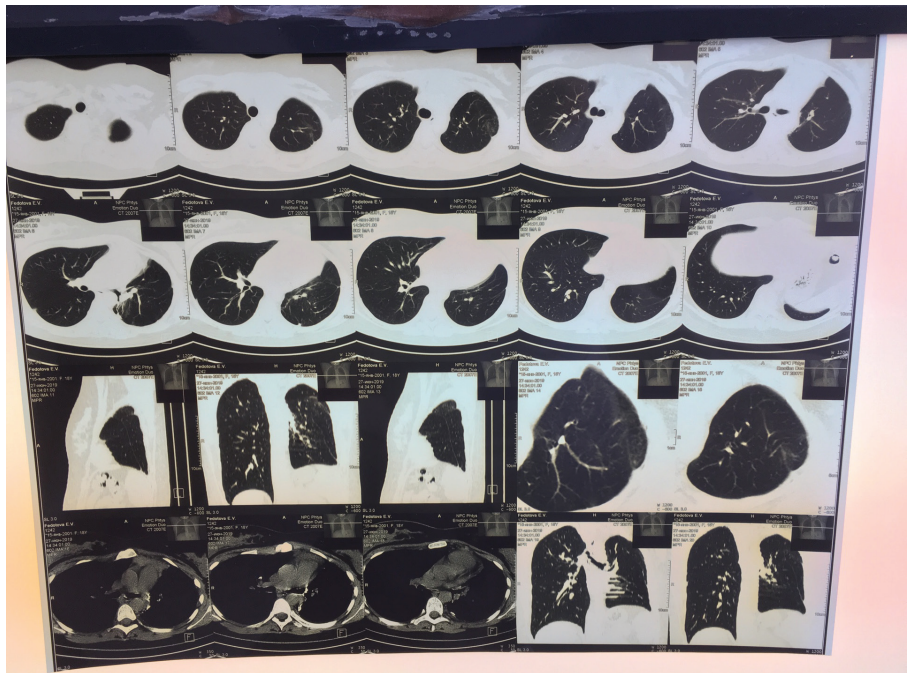

Fig. 3. Chest KT scan from June 27, 2019.

nomena of exogenous alveolitis. Tuberculous lymphadenitis.

Treatment after surgery according to an individual chemotherapy regimen: from 05.07.18. - 23.10.18. (111 doses) cycloserine 0.5 (500 mg); pyrazinamide 1.5(1500 mg); PASC 9.0; bedaquiline (according to the scheme). The total number of doses received in the adolescent department is 314. Pathogenetic therapy: prednisone –(7.5 mg per day); essentiale, methionine, glycine, glutamic acid; up to 30.07.18 g. - inhalation with ambrobene 2 r/day; ferrum lek. From 06.07.18. - 06.08.18. air insufflation into the abdominal cavity (800 ml) was performed – 4 procedures.

Clinical diagnosis: Tuberculosis of the intra-thoracic lymph nodes of the bronchopulmonary group on the right in the calcination phase, MBT (-), XDR (broad drug resistance) MBT (H, R, Rb, S, E, Et, Am, Ofl). Condition after upper lobectomy with atypical resection of C6 of the left lung with decortication from 05.07.18 for caseous pneumonia of the upper lobe and C6 of the left lung.

Chest KT scan from June 27, 2019. The state of the VATS resection of the upper lobe of the left lung, in the basal part, the chain of the tantalum suture is determined. The lung is reduced in volume, the lower lobe is compensatorily pulled up, with the presence of fibrous strands. In the upper part of the upwardly displaced lower lobe, pneumosclerosis, small foci. The left root is deformed. The mediastinum is shifted to the left.

The right lung is in full volume, without focal and infiltrative changes. The bronchovascular pattern in the right lung is not changed. The bronchi are traced to the subsegmental level, their lumen is not changed. The right root with calcinate in the upper group of bronchopulmonary nodes.

In the upper mediastinum, enlarged lymph nodes are not detected. There is no free fluid in the pleural cavity.

Conclusion. Condition after VATS resection of the upper lobe of the left lung for caseous pneumonia. No recent tuberculosis changes were detected.

At this time, the patient is observed at the place of residence. Since 2018, there have been no relapses of the disease. He is under the supervision of a phthisiologist in the III group of dispensary registration, at the place of residence.

Conclusion. The problem of contact of children with patients with drug-resistant tuberculosis in the republic is quite relevant. We present a clinical example of the development of tuberculosis in a teenager with broad drug resistance of MBT to 8 anti-tuberculosis drugs (H, R, Rb, S, E, Et, Am, Ofl), with unsatisfactory tolerability of anti-tuberculosis drugs in the form of increased transaminases in the biochemical blood test. All these factors led to a complicated course of the tuberculosis process and then to the surgical method of treatment - upper lobectomy with atypical C6 resection of the left lung with decortication. In this regard, it is important to further improve the work of the general medical network among patients who have suffered from tuberculosis, as well as the alertness of pediatricians for this pathology.

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SURGICAL TREATMENT OF MYXOMA OF THE STOMACH: A CASE REPORT

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Plexiform angiomyxoid myofibroblastic tumor of the stomach is a rare mesenchymal tumor that was first described in 2007. The tumor is very rare and for nowday, no more than 115 cases, confirmed by immunohistochemical studies, have been described in the literature. The age of patients with myxoma of the stomach ranges from 7 to 75 years. The disease is equally common among men and women, manifests itself in the form of ulcers of the gastrointestinal tract mucosa and is accompanied by arrosive bleeding. Tumors in all cases are localized in the antrum of the stomach, represent a lobular submucosal or transmural mass with a low potential for malignancy. Currently, there is no information in the world literature about the recurrence and distant metastasis of myxoma of the stomach. This article presents a clinical observation of a patient with myxoma of the antrum of the stomach with severe concomitant pathology, in connection with which surgical endoscopic treatment was performed. The positive results of the performed treatment indicate the high efficiency of endoscopic submucosal resection of the gastric myxoma.

Keywords: myxoma of the stomach, endoscopic submucosal tumor resection.

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Introduction. Plexiform angiomyxoid myofibroblastic tumor (PAMT), also known as plexiform fibromyxoma, was first described in the early 21st century by Y. Takahashi [9]. This rare tumor of mesenchymal origin is considered a benign tumor according to the WHO classification of Tumors of the Digestive System (2010). PAMT is characterized by spindle cells, plexiform growth pattern and abundant myxoid capillary rich extracellular matrix [10, 11]. The size of PAMT of the stomach ranges from 19 mm to 150 mm, with a mean value of 63 mm [3]. Most commonly symptoms include abdominal discomfort and pain, nausea, vomiting, pyloric stenosis, and weight loss. For 2016 year, the literature provided information on 59 morphologically verified cases of PAMT [6], by 2019 the total number of patients with this pathology reached 113 [8], and in 2020 single cases of the disease were additionally described [5]. The most common problem in clinical practice is the differential diagnosis between PAMT and gastrointestinal stromal tumors (GIST), since, despite similar clinical manifestations, these neoplasms has differences in prognosis [1]. In this situation, with an unclear histological view, the final diagnosis is made on the immunohistochemical analysis of mutations in the KIT and PDGFRA genes [2], which allows confirming plexiform fibromyxoma [7].

In this study we report a rare case of myxoma of the stomach that was initially suspected as GIST. A 61-year-old male patient presented to the Cancer Research Institute (Tomsk) for further evaluation of the lesion suspected as gastrointestinal stromal tumor. In September, 2020, the patient experienced abdominal discomfort, nausea and hematemesis. Since June 2020, he was under the supervision of a cardiologist with a diagnosis of acute myocardial infarction with damage to the anterior lateral wall of the left ventricle, NYHA IIA heart failure. Gastroscopy revealed a submucosal mass measuring approximately 25 mm in diameter at the lesser curvature of the gastric antrum, with an elastic structure and actively bleeding granulations (Figure 1). Tumor biopsy was not performed due to the risk of bleeding.

The additional examination including endoscopic ultrasound of the lesion, chest X-ray, CT of the abdominal organs and ultrasound of the pelvic cavity revealed no signs of synchronous tumors and metastatic lesions of target organs. Based on the examination, a clinical diagnosis was suspected to be GIST of the angle of the stomach, Gr I $T_1N_0M_0$. Due to severe cardiovascular disease, gastric resection was contraindicated and, therefore, endoscopic

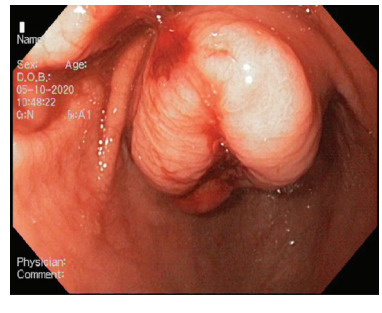

Fig. 1 – Endoscopic image of myxoma of the stomach

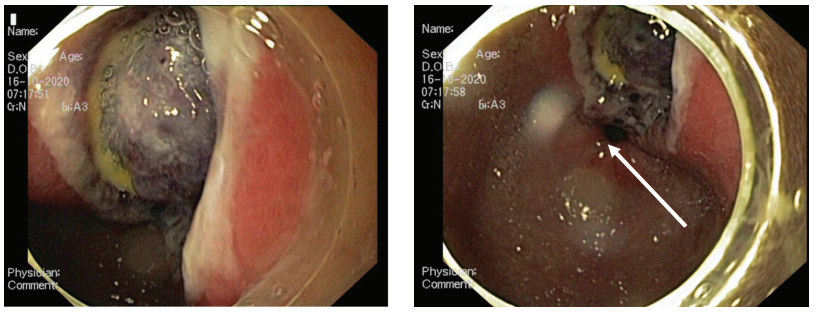

Fig. 2. (A, B). Endoscopic submucosal dissection procedure. Intraoperative endoscopic images after tumor excision. The mucosal defect is 30 mm in diameter. The bottom of the ulcer is confined by the muscle layer of the stomach. The arrow indicates the pyloric canal

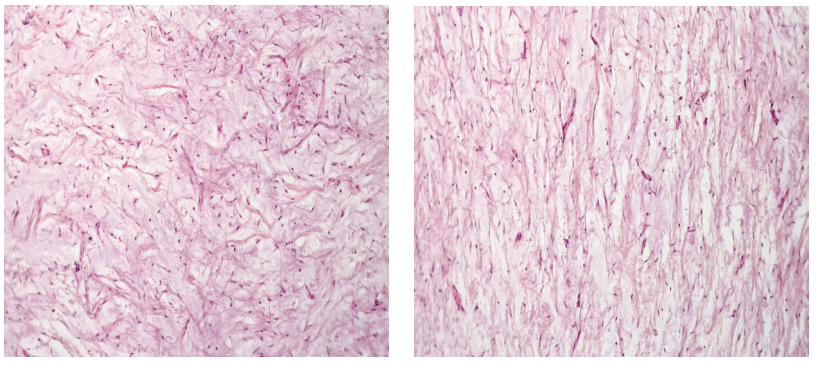

Fig. 3. (A, B). Pathological evaluation of mixoma of the stomach. Microscopic evaluation (hematoxylin and eosin staining, x200)

treatment was offered to the patient. Subsequently, the patient underwent endoscopic submucosal dissection for gastric lesion in PEMR ESD modification (fig. 2).

The postoperative course was favorable without any complications. Oral feeding was initiated on day 2 after surgery; restoration of the gastrointestinal tract motility occurred on day 3 after surgery; and the patient was discharged from the hospital on day 4 after surgery. Pathological examination of the resected specimen revealed plexiform growth of spindle cells with oval nuclei and eosinophilic cytoplasm separated by abundant myxoid stroma rich in small blood vessels. The histological conclusion was myxoma of the stomach with no mucosal invasion. Resections margins were histologically tumor-free (fig. 3).

Thus, the final clinical diagnosis was myxoma of the stomach complicated by ulceration and bleeding. The patient underwent endoscopic dissection of submucosal lesion in the gastric antrum using PEMR ESD technique (October, 2020). At 2-months follow-up, the esophagogastroduodenoscopy showed a scar in the gastric angle, measuring approximately 13 mm in the length. No evidence of inflammation and recurrence was found.

Conclusion. We have reported a case of myxoma of the stomach, a rare mesenchymal gastric tumor that requires differential diagnosis from GIST and other mesenchymal tumors of the stomach. The final diagnosis of myxoma of the stomach is only made after pathological evaluation of endoscopically resected specimen. Endoscopic electroresection is a treatment option for patients not suitable for radical resection because of severe concomitant diseases.

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