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Some Serologic Markers of *Toxoplasma gondii*, Cytomegalovirus and Rubella Virus in Pregnant Women in Saint Petersburg

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ABSTRACT

BACKGROUND: Currently, research investigating specific immunity to various TORCH pathogens (*Toxoplasma gondii, cytomegalovirus, rubella virus*) in pregnant women remains relevant.

AIM: The aim of the study was to evaluate the detection rates of the main serologic markers of TORCH in pregnant women in Saint Petersburg, Russia.

METHODS: A retrospective analysis of the screening of pregnant women (n = 6605) was performed for the presence and levels of specific anti-*Toxoplasma gondii*, anti-rubella, and anti-cytomegalovirus immunoglobulins M and G in the blood.

RESULTS: The rubella seroprevalence rate was 98.2% (n = 4428), with the highest number of seronegative women in the age group of 31 to 35 years. In 8.4% of cases, the serum level of anti-rubella immunoglobulin G was below the protective level. Anti-cytomegalovirus immunoglobulin G was found in 1632 (81.9%) pregnant women, with a significant age-related increase in the number of seropositive women (p < 0.001). Specific anti-*Toxoplasma gondii* immunoglobulin G was found in 28% (n = 1375) of pregnant women. The percentage of seropositive women also increased with age (p < 0.001), with the highest level reported in women aged > 40 years (40.74%).

CONCLUSION: The high level of rubella seroprevalence in pregnant women suggests that testing for anti-rubella antibodies should be performed only in the second trimester. Anti-cytomegalovirus screening is recommended for pregnant women with known risk factors for congenital cytomegalovirus infection. It is recommended to continue anti-*Toxoplasma gondii* screening because of the high percentage of *Toxoplasma gondii* seronegative pregnant women in Russia.

Keywords: pregnant women; TORCH; Toxoplasma gondii; cytomegalovirus; rubella virus.

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Некоторые серологические маркеры *Toxoplasma gondii*, цитомегаловируса и вируса краснухи у беременных в Санкт-Петербурге

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АННОТАЦИЯ

Обоснование. В настоящее время остаются актуальными исследования, направленные на изучение наличия специфического иммунитета к различным возбудителям TORCH-комплекса (*Toxoplasma gondii*, цитомегаловируса, вируса краснухи) среди беременных.

Цель — изучить частоту выявления основных серологических маркеров TORCH-комплекса у беременных в Санкт-Петербурге.

Материалы и методы. Проведен ретроспективный анализ результатов скринингового тестирования беременных (n=6605) на наличие и уровень специфических иммуноглобулинов M и G к антигенам токсоплазм, вирусов краснухи и цитомегаловируса в крови.

Результаты. Уровень серопревалентности к вирусу краснухи составил 98,2% (n=4428), наибольшее количество серонегативных женщин зарегистрировано среди пациенток в возрасте 31–35 лет. В 8,4% случаев уровень иммуноглобулинов G к вирусу краснухи в сыворотке крови был ниже защитного. Иммуноглобулины G к цитомегаловирусу выявлены у 1632 (81,9%) беременных с достоверным увеличением количества серопозитивных женщин с возрастом (p <0,001). Специфические иммуноглобулины G к *Toxoplasma gondii* обнаружены у 28% (n=1375) беременных. Доля серопозитивных лиц также увеличивалась с возрастом (p <0,001), самый высокий уровень зарегистрирован у женщин старше 40 лет и составил 40,74%.

Заключение. Высокий уровень серопревалентности беременных к вирусу краснухи позволяет предложить ограничить обследование на наличие антител к этому вирусу во II триместре гестации. Скрининг беременных на антитела к цитомегаловирусу рекомендовано проводить женщинам с известными факторами более высокого риска развития врожденной цитомегаловирусной инфекции. В связи с высоким уровнем серонегативных беременных по токсоплазмозу в Российской Федерации целесообразно продолжить скрининг на наличие/отсутствие антител к токсоплазме.

Ключевые слова: беременные; TORCH-комплекс; токсоплазмоз; цитомегаловирус; краснуха.

Как цитировать

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BACKGROUND

The study of the role of infectious pathology in preventable infant mortality is an urgent matter because of the wide spread of infections with a high risk of ante-/intranatal transmission of pathogens from mother to fetus and development of congenital infectious diseases.

The pathogens of congenital infectious diseases include human immunodeficiency virus (HIV), hepatitis B and C, cytomegalovirus (CMV), herpes simplex virus, rubella, parvovirus B19, varicella-zoster virus, syphilis, toxoplasmosis, and listeriosis (approximately 50 infectious agents) [1, 2].

The risk of developing congenital infectious diseases is determined by several factors, such as the distribution of the population of women of childbearing age (including pregnant women) according to the presence/absence of serological markers of these diseases (mainly specific IgM and IgG antibodies) [3–5].

Seroepidemiological studies on diseases of the classic TORCH syndrome (toxoplasmosis, rubella, herpes simplex virus, and CMV) have been conducted have been conducted in the world for a long time. Depending on the aims and materials of the studies, the results reflect the frequency of the detection of serological markers in the entire population [6–8], considering sex and age [7-10]; test results in different countries [11, 12] and separate regions within one country [13, 14], geographical area, or one nosological form [15, 16]; and the participants' social and economic status [17].

The results of the studies conducted using screening immunochemical tests (i.e., solid-phase enzyme immunoassay and immunochemiluminescent assays) in the women of reproductive age cohort allow to approximately estimate a relative and absolute number of people at the highest risk of acute infection with the transmission of the pathogen to the fetus (without antibodies, seronegative ones), having a certain immunity level (seropositive according to the presence of specific IgG), and having laboratory signs of acute (reactivation, superinfection) infection (seropositive with specific IgM). The obtained data can be used in the justification and development of the programs for primary screening and monitoring of pregnant women to understand the risk of developing congenital infectious diseases, identify target groups for in-depth examination, and reduce the frequency of congenital infectious pathology [9, 17–19].

In Russia, the number of publications about levels of seroprevalence among pregnant women has been extremely limited, and the number of examined participants has not reached 1000 [3, 6, 8], highlighting the need for larger projects.

The study is aimed at estimating the frequency of the detection of the main serological markers of the TORCH complex (*Toxoplasma gondii*, CMV, and rubella virus) in pregnant women in Saint Petersburg.

MATERIALS AND METHODS

The inclusion criteria were age >16 years and pregnancy. The results of screening for the presence and level of specific IgM and IgG to toxoplasma antigens, rubella virus, and CMV in blood serum samples sent by women's consultations of Saint Petersburg to the Interdistrict Centralized Clinical Diagnostic Laboratory of Saint Petersburg State Medical Institution "City Polyclinic No. 107" during the period from March to June of 2019 were obtained by continuous sampling. Women who were not pregnant and did not have HIV, viral hepatitis B and C, and syphilis were excluded. Overall, 6605 women were enrolled in the study, accounting for 10.2% of the total number of pregnant women in Saint Petersburg in 2019.

Specific antibodies were determined by immunochemiluminescence analysis (IHLA) with ADVIA reagents (IgM and IgG to toxoplasma antigens, rubella virus, and CMV) using a laboratory track (Siemens, Marburg, Germany) and immuno-enzyme analysis (IgM and IgG to herpes virus antigens of types 1 and 2, in total) with a set of reagents DS-IFA-ANTI-HSV-1,2-M and DS-IFA-Anti-HSV-1,2-G (NPO Diagnostic Systems, Nizhny Novgorod, Russia). The sensitivity and specificity of the test systems claimed by the manufacturers is more than 99%.

The participants were ranked by age (16–20, 21–25, 26–30, 31–35, and 36–40 years and older). Statistical analysis was conducted using descriptive statistics, by comparing the frequency of occurrence of signs using Microsoft Excel modules, and using a software package for statistical data processing *StatSoft Statistica 7.0*. The relationship between the signs was evaluated using the nonparametric criterion χ^2-Pearson.

Within the framework of this study, the clinical and life records of the course and outcomes of pregnancies were not analyzed.

RESULTS

The results of screening testing of 6605 women for the presence and level of specific IgM and IgG to toxoplasma antigens, rubella virus, CMV and herpes simplex types 1 and 2 of blood serum samples, which accounted for 10.2% of the total number of pregnant women in Saint Petersburg in 2019, were analyzed.

Rubella

IgG and IgM antibodies to rubella was noted in 4510 women: 98.2% (n = 4428) had positive specific IgG. Different levels of antibodies were found in pregnant women: in 1.8%, antibodies were not detected, and in 8.4%, the level of IgG to rubella virus in the blood was lower than the protective one in accordance with generally accepted indicators in Russia

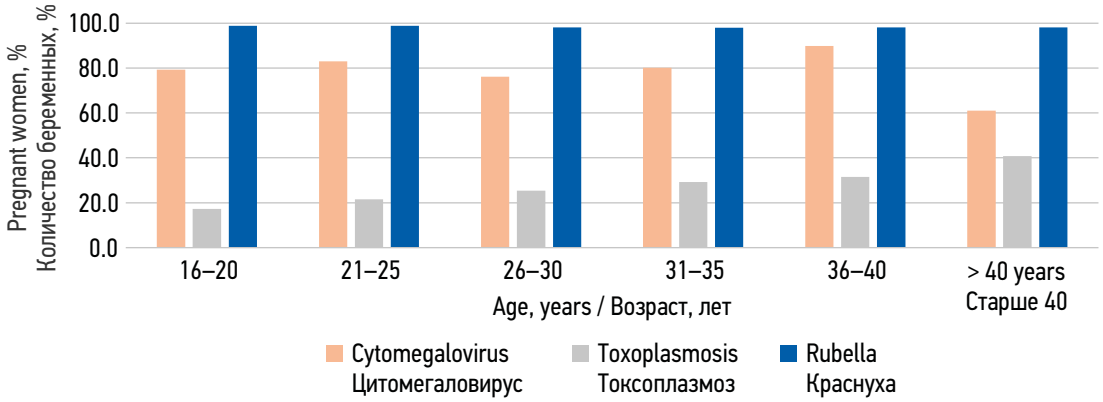

Fig. 1. Rates of cytomegalovirus, toxoplasmosis, and rubella infections in pregnant women by age group.
Рис. 1. Показатели инфицированности цитомегаловирусом, токсоплазмозом и краснухой беременных в возрастных группах.

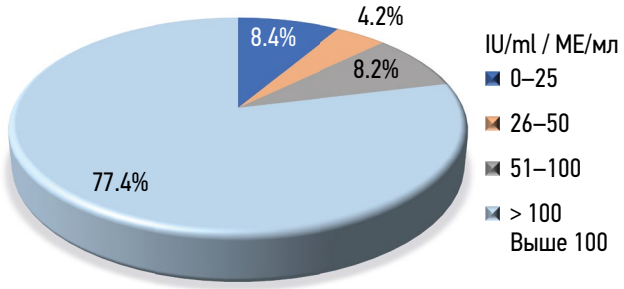

Fig. 2. Distribution of blood anti-rubella immunoglobulin G levels in pregnant women.
Рис. 2. Распределение концентрации иммуноглобулинов G к вирусу краснухи в крови беременных.

(<25 IU/ml) (Fig. 1, 2). Additionally, 82 women were susceptible to rubella, which can lead to infection during pregnancy.

The largest percentage of seronegative women was registered at the age of 31–35 years (Table 1), amounting to 1.99% (n = 31). In the other age groups, the percentage of seronegative women was approximately the same (1.23%–1.94%). IgM was detected in 0.2% (n = 11) of women, and IgG (μ300,31 IU/ml) was determined in all of them, which indicates a false positive result.

Specific IgM was revealed in 0.2% (n = 11) of women, and IgG was registered in all women. All women were referred to undergo extra examination at the polyclinic department of an infection hospital. In all the cases after receiving negative PCR results, IgG avidity estimation, immunoblotting results, data about the vaccination performed, and results of the previous examinations (if the documents were available), the situations were regarded as false-positive IgM by commission-decision making. No follow-up cases of congenital rubella infection or congenital rubella syndrome were registered.

No significant relationship was noted between the age of the pregnant women and frequency of IgG detection (χ^2 = 1.8; p = 0.94).

CMV infection

Among 1993 pregnant women examined, 1632 (81.9%) were found to have IgG to CMV (Table 2). A significant relationship was found between age and detection of IgG to CMV (χ^2 = 45.6; p < 0.001). IgM was detected in 26 (1.3%) women. Moreover, 22 women had specific IgG at the same time. IgG was not detected in four women, and doubtful IgM was detected in them during repeated examination.

Of the total number of examined pregnant women, 361 (18.2%) were seronegative to CMV. Among pregnant women aged 16–40 years, the number of seronegative

Table 1. Rates of anti-rubella immunoglobulin G detection in pregnant women by age group
Таблица 1. Частота обнаружения иммуноглобулинов G к краснухе у беременных различных возрастных групп

Age groups	Seropositive		Seronegative		Total
	abs	%	abs	%	
16–20	80	98.77	1	1.23	81
21–25	515	98.85	6	1.15	521
26–30	1307	98.12	25	1.88	1332
31–35	1528	98.01	31	1.99	1559
36–40	794	98.15	15	1.85	809
>40 years old	202	98.06	4	1.94	206
Total	4428		82		4510

Table 2. Rates of anti-cytomegalovirus immunoglobulin G detection in pregnant women by age group
Таблица 2. Частота обнаружения иммуноглобулинов G к цитомегаловирусу у беременных различных возрастных групп

Age groups	Seropositive		Seronegative		Total
	n	%	*n*	%	
16–20	23	79.31	6	20.69	29
21–25	166	83.00	34	17.00	200
26–30	434	76.14	136	23.86	570
31–35	559	80.09	139	19.91	698
36–40	342	89.76	39	10.24	381
>40 years	105	93.75	7	6.25	112
Total	1629		361		1993

Table 3. Rates of anti-Toxoplasma gondii immunoglobulin G detection in pregnant women by age group
Таблица 3. Частота обнаружения иммуноглобулинов G к токсоплазмозу беременных различных возрастных групп

Age groups	Seropositive		Seronegative		Total
	n	%	*n*	%	
16–20	14	17.28	67	82.72	81
21–25	124	21.49	453	78.51	577
26–30	368	25.38	1082	74.62	1450
31–35	487	29.21	1180	70.79	1667
36–40	272	31.52	591	68.48	863
>40 years	110	40.74	160	59.26	270
Total	1375		3533		4908

women was approximately at the same level and higher than that in the group aged >40 years, ranging from 17% to 23.86%.

Toxoplasmosis

Among 4910 pregnant women examined (Table 3), 1374 (28%) had specific immunity to *T. gondii*. The proportion of seropositive women increased with age ($\chi^2 = 49.9$; $p < 0.001$); the highest level was registered in women aged >40 years and amounted to 40.74%. IgM class antibodies were detected in seven (0.1%) pregnant women and IgG class antibodies in six. Only one pregnant woman had no specific IgG class antibodies with positive IgM (not mentioned in Table 3).

Furthermore, 72% ($n = 3533$) of pregnant women were seronegative. The percentage of seronegative pregnant women was highest in the 16–20 years age group (82.7%).

DISCUSSION

Congenital infectious diseases are a crucial cause of disability and mortality worldwide. Infection of a woman during pregnancy can lead to miscarriage, fetal death, stillbirth, or the birth of children with congenital infection.

Rubella is a critical congenital infectious disease that leads to multiple malformations, among which is the Gregg triad (i.e., congenital cataracts, heart defects, and deafness), which is the most well-known [19]. Rubella can be prevented using a highly immune vaccine. The vaccine potentially eliminates congenital rubella syndrome (CRS) in various countries globally. Specific immunity against rubella varies from 66% to 100% in different regions worldwide [19–22].

In Russia, a low incidence of rubella was recorded in 2014–2018 (in 2014, the target for the elimination of infection was reached in Russia for the first time—less than 1 case per 1 million population; in 2016, the number of cases amounted to 38 people and in 2017 and 2018, 5 cases), which allowed the WHO Regional Commission for Europe to recognize the elimination of rubella in Russia in 2017–2018 [20]. However, in 2019, 34 cases of rubella were registered, and the incidence was 0.03 cases per 100 thousand population [21], indicating a potential danger for women of reproductive age and the birth of children with congenital rubella syndrome. Currently, Russia again met the WHO criteria as a rubella-free country [22].

The results of our study in Saint Petersburg show that the level of protective antibodies was 98.2% and that the indicator did not significantly change with age: 16–20 years old, 98.8%, and >40 years old, 98.1%. Most pregnant women (89.8%) had IgG titers >25 IU/ml, indicating a high immune layer and a low risk of congenital rubella syndrome. Additionally, 9% had a low level of specific

IgG – <25 IU/ml (below the protective titer in accordance with international criteria), which may be associated with a decrease in postvaccination immunity over time. The lowest proportion of seronegative women was detected among women aged 21–25 years who were vaccinated twice (1.15%).

The anamnesis of rubella-seronegative women is unknown. However, the lack of protective immunity in pregnant women was possibly due to the lack of rubella vaccination in older people (rubella vaccination was introduced into the National Vaccination Schedule only in 1997), violation of the vaccination timing, or voluntary refusal of vaccination. Under these conditions, the existing system in Saint Petersburg for extra examination of pregnant women with IgM to rubella virus antigens by relevant methods during specialized care in the *Infectious diseases* institution with commission decision-making on the presence/absence of the disease justifies itself regarding risk assessment of CRS.

Congenital cytomegalovirus infection (CMV) is the leading cause of central nervous system damage, sensorineural deafness and psychomotor developmental delay, which determines its relevance.

The frequency of primary CMV during pregnancy does not exceed 5%, whereas the prevalence of intrauterine infection of the fetus reaches 40%, and 5%–18% of infected children develop a manifest form of the disease. Severe CNS lesions occur when the virus is transmitted during the early stages of gestation from a primary infected mother. A more favorable prognostic sign is recurrent CMV-infection in which the probability of infection of fetus is lower and amounts 0.2%–2.2%. However, recurrent CMVI in the mother does not exclude the development of a manifest form of congenital infectious diseases in the fetus and newborn [9].

Study results show that IgG to CMV was registered in 81.9% of pregnant women, and the proportion of seropositive women increased with age and reached 93.7% among those surveyed over 35 years of age. The resulting seroprevalence index was lower than that in developing countries (China, 98.7%; Nigeria, 91.1%; and Turkey, 97.3%) [9, 13, 23, 24] and higher than that in developed countries (USA, 70%; Norway, 72.1%; and Japan, 69.1%) [25]. Notably, the level of infection of women with CMV can be influenced by various factors: age, number of pregnancies, children, professional employment, socioeconomic living conditions, migration, and geographical and ethnic affiliation. It has been found that living in rural areas and a lower level of education are significant risk factors for CMV infection during pregnancy. The level of awareness on the effect of CMV on the fetus is slightly higher among women living in megacities [26–28].

In the present study, IgG to CMV was absent in 18.2% of women, mainly pregnant women aged 26–30 years, who belonged to the high risk group for transmission of CMV to the fetus. Transplacental transmission of CMV is possible throughout pregnancy. Although routine screening for CMV in pregnant women is currently not performed, serological examination can be recommended at the stage of prepregnancy preparation for all pregnant women. Acute infection can occur in any situation: in immune pregnant women, against the background of physiological immunosuppression; it may result from reactivation of an early acquired infection or infection with another strain of CMV, the frequency of which and the percentage of development are unknown [29–31].

The basis of prevention is educational work. Prenatal counseling leads to a change in women's behavior and a decrease in CMV infection risk during pregnancy. Medical professionals should inform women about possible sources and routes of CMV transmission (e.g., close contact with saliva and urine of preschool children and sexual contact) and the significant risk of fetal infection with primary maternal infection. Moreover, educational films, posters, and brochures can be used in women's clinics and maternity hospitals to provide up-to-date information about CMV [32].

Great hopes are pinned on the development of an effective vaccine against CMV. For over 50 years, attempts have been made to develop a vaccine with the potential to be used before and during pregnancy. Owing to the features of CMV (i.e., the ability to replicate without damaging the cell and evade the immune response, causing lifelong persistence in the human body) and limited number of animals for clinical research, developing an effective vaccine is challenging. To date, none of the vaccines have been licensed. Several CMVI vaccines are undergoing (or have already completed) the second phase of clinical trials: gB/MF59, ASP0013, PePVax, Triplex, V160, and Hookipa HP-101 [33]. There is good tolerance and absence of serious adverse reactions (except for local skin). The target group for the study were recipients of bone marrow and organs. A clinical trial of the V160 vaccine is ongoing among CMV seronegative women who are in close contact with preschool children at home or at work [34].

Another relevant issue is *toxoplasmosis*, which is associated with the complexity of diagnosis and treatment and a high percentage of adverse outcomes. In Russia, the incidence of congenital toxoplasmosis varies between 1 per 1000 and 8000 newborns. Mortality in congenital manifest toxoplasmosis is, on average, approximately 12% [35–37].

The incidence and prevalence of toxoplasmosis are heterogeneous and vary significantly between different regions of the world and countries and within the same country. The level of seropositivity to *T. gondii* among the adult population of most countries of Western Europe, Africa, and South and Central America is 50%–95%, and in the USA,

more than 60 million people are chronically infected with toxoplasma [31]. The infection rate of *T. gondii* in women of reproductive age in Russia is 20%–30% [36, 37].

Transplacental fetal involvement can occur only if the mother is infected during pregnancy, as a result of which fetal damage occurs, accompanied by various clinical presentations and severity of the course. In the case of repeated pregnancies, there is no transplacental transmission of the pathogen [38–40]. Currently, no screening for antibodies to *T. gondii* has been established in Russia.

Based on our results, the infection rate was 28%. A significant increase in the proportion of seropositive women was noted with age: 40.74% in the older age group, compared with 17.28% in the 16–20 years age group, which is explained by the natural course of the epidemic process.

Women who are seropositive for toxoplasmosis do not require follow-up monitoring. As a result of a serological examination, it was found that 72% of women are at risk of infection with toxoplasma during pregnancy. Seronegative pregnant women need advice on infection prevention to reduce the risk of primary invasion (e.g., limiting contact with infected cats, observing personal hygiene rules, and prohibiting the use of raw minced meat and meat dishes without sufficient heat treatment) and serological monitoring every 8–12 weeks until the end of pregnancy [41–43].

CONCLUSIONS

1. Overall, 98.2% of pregnant women in Saint Petersburg have serological markers of immunity against the rubella virus, indicating the effectiveness of routine vaccination in accordance with the National Vaccination Schedule. The rarity of acquired rubella and absence of cases of registration of the congenital form of the disease over the past 7 years allow to recommend limiting the examination of pregnant women for the presence of antibodies to this virus in the second trimester of pregnancy if the women had antibodies detected during initial examination. This reduces the number of repeated studies by 90%.
2. Among pregnant women in Saint Petersburg, prevalence rates of up to 81.8% of IgG to CMV were noted. A decrease in the number of seronegative women was observed along with an increase in the age of the examined participants. Currently, total serological screening of pregnant women for antibodies to CMV is not recommended, and the examination may be limited to pregnant women with higher risk for the development of congenital CMV.
3. Moreover, 72% of pregnant women in Saint Petersburg are at risk of infection with toxoplasmas. The expediency of screening pregnant women in Russia for the presence/absence of antibodies to toxoplasma requires further investigation.

ADDITIONAL INFORMATION

Author contributions: *I.V. Markin:* formal analysis, writing the draft; *V.V. Vasilev:* conceptualization, review and editing; *N.V. Rogozina:* review and editing; *R.A. Ivanova:* writing the draft; *A.E. Nikolaeva:* investigation, review and editing; *A.Yu. Zolotarev:* investigation, review and editing. All authors approved the version of the manuscript to be published, and agreed to be accountable for all aspects of the work, ensuring that questions related to the accuracy or integrity of any part of it are appropriately reviewed and resolved.

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Data availability statement: All study data are available in the article.

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Provenance and peer-review: This paper was submitted unsolicited and reviewed following the standard procedure. The peer review process involved two members of the editorial board.

ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ

Вклад авторов. *И.В. Маркин* — формальный анализ, написание черновика рукописи; *В.В. Васильев* — разработка концепции, пересмотр и редактирование рукописи; *Н.В. Рогозина* — пересмотр и редактирование рукописи; *Р.А. Иванова* — написание черновика рукописи; *А.Е. Николаева* — проведение исследования, пересмотр и редактирование рукописи; *А.Ю. Золотарев* — проведение исследования, пересмотр и редактирование рукописи. Все авторы одобрили рукопись (версию для публикации), а также согласились нести ответственность за все аспекты работы, гарантируя надлежащее рассмотрение и решение вопросов, связанных с точностью и добросовестностью любой ее части.

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Источники финансирования. Отсутствуют.

Раскрытие интересов. Авторы заявляют об отсутствии отношений, деятельности и интересов за последние три года, связанных

с третьими лицами (коммерческими и некоммерческими), интересы которых могут быть затронуты содержанием статьи.
Оригинальность. При создании настоящей работы авторы не использовали ранее опубликованные сведения (текст, иллюстрации, данные).
Доступ к данным. Все данные, полученные в настоящем исследовании, доступны в статье.
Генеративный искусственный интеллект. При создании настоящей статьи технологии генеративного искусственного интеллекта не использовали.
Рассмотрение и рецензирование. Настоящая работа подана в журнал в инициативном порядке и рассмотрена по обычной процедуре. В рецензировании участвовали два внутренних рецензента из состава редакционной коллегии.

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