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ASSESSMENT OF BIOMARKERS IN BIOLOGICAL FLUIDS AND NEUROIMAGING CHANGES IN PATIENTS WITH ALZHEIMER'S DISEASE AND GLAUCOMA

Anna N. Bogolepova[1,2], Ekaterina V. Makhnovich[1,2]✉, Ekaterina A. Kovalenko[1,2], Nina A. Osinovskaya[1], Mikhail M. Beregov[1], Olga V. Lyang[1]

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Introduction. Alzheimer's disease (AD) and primary open-angle glaucoma (POAG) are gradually progressive neurodegenerative diseases leading to disability. According to literature data, POAG can be a predictor of AD development. Early diagnosis of these diseases contributes to a timely initiation of treatment and, as a result, a reduction in the disability of patients.

Objective. To study biomarkers of early diagnosis in biological fluids and neuroimaging changes based on the results of MR morphometry in patients with AD and POAG and to conduct their comparative analysis.

Materials and methods. In total, 90 patients with proven diagnosis of AD (group 1) and POAG (group 2) were examined. The study participants were divided into two groups according to their diagnosis: group 1 — 45 patients (9 (20%) men and 36 (80%) women) with AD; group 2 — 45 people (17 (37.8%) men and 28 (62.2%) women) with POAG. Neuropsychological testing included Mini-mental State Examination (MMSE), Montreal Cognitive Assessment (MoCA), and a ten-words recall test. The beta-amyloid (Aβ) Aβ42/Aβ40 ratio in the blood and sirtuin Sirt1, 3, 5, and 6 in saliva were assessed by enzyme immunoassay (ELISA). In addition, MR morphometry of the brain was performed.

Results. In group 1, cognitive impairments (CI) reaching the degree of dementia were detected; in group 2, pre-dementia CI were observed ($p < 0.001$). According to the neuropsychological examination, similar changes were noted in both groups, in particular, memory impairment of the hippocampal type. The results of the blood and saliva ELISA with the determination of biomarkers in the groups under comparison did not reveal statistically significant differences. At the same time, the parameters of both volumes and thicknesses according to MR morphometry were lower in group 1 ($p < 0.05$), which may reflect neurodegenerative progression. In group 1, a direct correlation was found between a decrease in the saliva level of Sirt3 and a deterioration in direct reproduction (fifth reproduction) according to the ten-words recall test ($R = 0.43$; $p = 0.003$). Correlations between changes in neuropsychological parameters and MR morphometry data, including a decrease in the volume of the entorhinal cortex, were noted in both groups. In groups 1 and 2, a decrease in the Aβ42/Aβ40 ratio in blood plasma was associated with a decrease in the thickness or volume of the entorhinal cortex, which is common for both groups with different CI severity. Taking into account the association with neuropsychological and blood parameters, including in patients with pre-dementia CI from the POAG group, the determination of the volume and thickness of the entorhinal cortex can be regarded as a significant early marker of the neurodegenerative process.

Conclusions. The established association between the volume and thickness of the entorhinal cortex with neuropsychological and blood parameters, including in patients with pre-dementia CI from the POAG group, makes the determination of the volume and thickness of the entorhinal cortex a significant early marker of the neurodegenerative process. A comprehensive assessment of the results obtained by neuropsychological, laboratory, and neuroimaging diagnostic methods, as well as the search for diseases associated with the development of AD, such as POAG, are promising research areas, requiring larger cohort studies.

Keywords: Alzheimer's disease; dementia; cognitive impairment; primary open-angle glaucoma; MR morphometry; blood biomarkers; saliva biomarkers; sirtuins; Aβ42/Aβ40 ratio

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ОЦЕНКА БИОМАРКЕРОВ В БИОЛОГИЧЕСКИХ ЖИДКОСТЯХ И НЕЙРОВИЗУАЛИЗАЦИОННЫХ ИЗМЕНЕНИЙ У ПАЦИЕНТОВ С БОЛЕЗНЬЮ АЛЬЦГЕЙМЕРА И ГЛАУКОМОЙ

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Введение. Болезнь Альцгеймера (БА) и первичная открытоугольная глаукома (ПОУГ) относятся к постепенно прогрессирующим нейродегенеративным инвалидизирующим заболеваниям. В научной литературе отмечено, что ПОУГ может быть предиктором развития БА. Необходима ранняя диагностика данных заболеваний, которая будет способствовать своевременному назначению лечения и, как следствие, позволит снизить инвалидизацию пациентов.

Цель. Изучить биомаркеры ранней диагностики в биологических жидкостях и нейровизуализационные изменения по результатам МР-морфометрии у пациентов с БА и ПОУГ и провести их сравнительный анализ.

Материалы и методы. Обследовано 90 пациентов с установленным диагнозом БА и ПОУГ. Участники исследования были разделены на 2 группы в соответствии с диагнозом: группа 1 — 45 пациентов (из них 9 (20%) мужчин и 36 (80%) женщин) с БА; группа 2 — 45 человек (из них 17 (37,8%) мужчин и 28 (62,2%) женщин) с ПОУГ. Проведено нейропсихологическое тестирование: краткая шкала оценки психического статуса (MMSE), Монреальская шкала оценки когнитивных функций (MoCA), тест запоминания 10 слов. Всем пациентам определяли соотношение бета-амилоидов

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(Аβ) крови Аβ42/Аβ40, в слюне — сиртуин Sirt-1,3,5,6 с проведением иммуноферментного анализа (ИФА), а также выполнялась МР-морфометрия головного мозга.

Результаты. В группе 1 были выявлены когнитивные нарушения (КН), достигающие степени деменции; в группе 2 — додементные КН ($p < 0,001$). По результатам проведенных нейропсихологических методик в двух группах были отмечены схожие изменения, в особенности нарушение памяти по гиппокампальному типу. Результаты проведенных ИФА крови и слюны с определением биомаркеров в двух сравниваемых группах не показали статистически значимых различий. При этом показатели как объемов, так и толщин по данным МР-морфометрии были ниже в группе 1 ($p < 0,05$), что может быть отражением прогрессирования нейродегенеративного процесса. В группе 1 выявлена прямая корреляционная связь снижения уровня Sirt3 в слюне с ухудшением непосредственного воспроизведения (5 воспроизведение) по тесту запоминания 10 слов ($R = 0,43$; $p = 0,003$). В обеих группах отмечены корреляционные связи между изменением нейропсихологических показателей и данными МР-морфометрии, в том числе уменьшением объема энторинальной коры. Как в группе 1, так и в группе 2 выявлено, что снижение соотношения Аβ42/Аβ40 в крови ассоциировалось с уменьшением толщины или объема энторинальной коры, что является общим для обеих групп с разной выраженностью КН. Учитывая наличие ассоциации с нейропсихологическими показателями и данными лабораторного анализа крови, в том числе и у пациентов с додементными КН из группы ПОУГ, определение объема и толщины энторинальной коры может быть расценено как значимый ранний маркер нейродегенеративного процесса.

Выводы. Выявлено наличие ассоциации с нейропсихологическими показателями и данными лабораторного анализа крови, в том числе и у пациентов с додементными КН из группы ПОУГ, в связи с чем определение объема и толщины энторинальной коры может быть расценено как значимый ранний маркер нейродегенеративного процесса. Комплексная оценка нейропсихологических, лабораторных и нейровизуализационных методов диагностики, а также поиск заболеваний, ассоциированных с развитием БА, таких как ПОУГ, является актуальным направлением, в связи с чем требуется дальнейшее проведение более крупных когортных исследований.

Ключевые слова: болезнь Альцгеймера; деменция; когнитивные нарушения; первичная открытоугольная глаукома; МР-морфометрия; биомаркеры крови; биомаркеры слюны; сиртуины; соотношение Аβ42/Аβ40

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INTRODUCTION

Alzheimer's disease (AD) is a gradually progressing neurodegenerative disease leading to disability and being the most common cause of dementia. At the same time, according to statistics, the number of patients with dementia is steadily increasing, which attracts the research attention toward the search for possible solutions [1].

Minimally invasive and noninvasive biomarkers of AD remain to be unavailable for widespread use, which frequently results in delayed diagnosis. When diagnosing AD in routine practice, the patient may already have clinical symptoms of dementia, which indicates the irreversible loss of 30–60% of the neurons in the temporal cortex, in particular the hippocampus [2]. This significantly limits the therapeutic capabilities of the clinician in affecting the course of the disease, leading to untimely initiation of treatment and irreversible changes. In addition, authors attribute the difficulties of creating a pathogenetically-based effective AD therapy to prescribing treatment only at the stage of clinical manifestations. At the same time, pathological proteins, in particular beta-amyloid (Aβ) that triggers the AD continuum, begin to accumulate long before the primary clinical symptoms of the disease appear [1]. This highlights the relevance of timely AD diagnosis and explains the current interest in identifying early diagnostic biomarkers and searching for other pathologies and conditions that may trigger or be associated with AD development.

Various studies have shown that AD is often associated with glaucoma, another progressive neurodegenerative disabling disease common among the population. About 74% of the patients suffer from primary open-angle glaucoma (POAG), which is characterized by thinning of the retinal nerve fiber layer and peripheral vision loss, up to complete blindness [3]. Glaucoma is assumed to be a disease that occurs in the brain, although being clinically manifested as an ophthalmological pathology. This is confirmed by the growing amount of information about retinal damage as a result of retrograde transynaptic degeneration caused by neurodegenerative processes [4]. Recent observational cohort studies have confirmed that glaucoma is a risk factor in the development of dementia among the adult population [5].

AD and POAG share numerous similarities, both in clinical and pathophysiological aspects. Thus, both diseases are associated with cognitive impairments (CI) of a neurodegenerative nature with damage to memory functioning as one of the most important cognitive domains [6].

A sufficient number of studies have confirmed the presence of similar links in the pathogenesis of AD and POAG. The cardinal signs in the pathogenesis of AD are extracellular accumulation of Aβ and intracellular deposits

of hyperphosphorylated tau protein (p-tau), which leads to progressive death of neurons. Aβ and p-tau accumulation in the main type of retinal cells — ganglion cells (GCs), affected by POAG, along with concomitant inflammation indicate coincident pathological processes in AD and POAG [3]. Therefore, Aβ and p-tau continue to be actively studied as early diagnostic biomarkers of these two diseases.

The gold standard of lifetime diagnosis of AD involves determination of markers in the cerebrospinal fluid (CSF) using positron emission tomography (PET) of the brain. However, this approach fails to meet the criteria of widespread availability and minimally invasiveness, making the search for other early diagnostic biomarkers for AD highly relevant. It should be emphasized that, since 2023, the revised AD criteria of the National Institute for Aging and the American Alzheimer's Association (National Institute on Aging, NIA-AA) have already included more widely available and less invasive accurate blood tests. Thus, the following are currently indicated as the main AD biomarkers in blood plasma: p-tau 217, the ratio of p-tau 217/np-tau 217 [7].

The Aβ42/Aβ40 blood plasma ratio is increasingly attracting attention as an important diagnostic indicator. Investigations showed that a lower Aβ42/Aβ40 ratio in blood plasma corresponded to a higher level of amyloid cortical load. Clinically and according to neuropsychological testing, a more pronounced cognitive dysfunction was observed, followed by an increased risk of dementia [8, 9]. Research into the diagnostic accuracy of this biomarker is underway, making the study of the Aβ42/Aβ40 ratio in the blood of POAG patients particularly relevant.

In addition to the classical amyloid theory of pathogenesis, which explains the development of AD and, to a lesser extent, POAG, other pathogenetic links should also be investigated. Recent studies into the processes underlying neurodegenerative diseases have shown that neuron death is influenced by a number of factors, including excitotoxicity, oxidative stress, mitochondrial dysfunction, inflammation, and apoptosis. Various cell groups of the body, including the central nervous system, comprise a family of sirtuin proteins (Sirt) that are involved in almost all of the above links; disruption of the Sirt contributes to the development of degenerative processes.

Seven mammalian sirtuins — Sirt 1–7 — are involved in the regulation of metabolism in many tissues, presumably playing an important role in the pathology of AD and POAG [10]. For example, the induction of Sirt1 expression is believed to weaken neuron degeneration and death in AD animal models. Thus, the authors investigating ophthalmological pathologies [11] found that increased Sirt1 expression protects against diseases associated with eye damage due to oxidative stress, including optic nerve degeneration in patients with glaucoma. Sirt3 plays a protective role in AD and ophthalmological pathology, ensuring the normal functioning of mitochondria [12]. In addition, Sirt6 is known to alter neurogenesis in the hippocampus in adults, affecting the number of glial and neuronal cells, and thus may also contribute to the development of AD. A study of Sirt in the pathogenesis of glaucoma found that Sirt6 is highly expressed in GCs. Removal of Sirt6 in GCs led to a progressive loss of these cells and optic nerve degeneration [13]. In this context, the study of Sirt in biological fluids as early diagnostic biomarkers for AD and POAG can be considered as an important research area.

Neuroimaging, MR morphometry in particular, is another essential element in the early diagnosis of AD. Interestingly, similar such changes were observed in AD and glaucoma. Thus, according to a number of authors [14, 15], glaucoma affects not only the central visual cortex, but also other areas of the brain (e.g., the temporal lobes) that intersect with the areas affected in patients with AD, which also suggests a relationship between the two diseases.

This study is aimed at studying early diagnostic biomarkers in biological fluids and neuroimaging changes based on the results of MR morphometry in patients with AD and POAG followed by their comparative analysis.

MATERIALS AND METHODS

The research sample included 90 patients with confirmed diagnoses of AD or POAG, aged from 40 to 90 years, who are native speakers of the Russia language. The patients were lucid, without pronounced impairments of motor (on the Medical Research Council (MRC) Scale for Muscle Strength at least 4 points in the leading arm) and speech functions, without chronic diseases decompensation and other clinically significant neurological pathologies, history of mental disorders, without the brain MRI absolute contraindications and anxiety-depressive disorders according to the Hospital Anxiety and Depression Scale (HADS) [16, 17].

Following diagnostics, the study participants were divided into two groups: group 1 — 45 patients (9 men and 36 women, 20% and 80%, respectively) with AD; group 2 — 45 people (17 men and 28 women, 37.8% and 62.2%, respectively) with POAG. There were no statistically significant age differences when comparing the two groups: in group 1, the average age was 71 [66; 77] years, compared to 66 [61; 71] years in group 2.

The neurological status all patients was assessed. The state of cognitive functions was evaluated using a set of standard neuropsychological techniques. The Mini Mental State Examination (MMSE) and Montreal Cognitive Assessment (MoCA) were used for integrative assessment of cognitive functions. The MMSE scale includes the following indicators: orientation, registration, attention and calculation, recall, and language. The maximum number of points on the MMSE scale is 30. The scale was used as a screening tool for cognitive impairments (CI): 28–30 points — normal, 25–27 points — moderate CI, 24 points and less points — severe CI (dementia). The MoCA scale is more sensitive for the diagnosis at the stage of moderate CI. On the MoCA scale, the following were assessed: executive and visuospatial function (include clock drawing test), naming, attention, language, abstraction, delayed recall, orientation. The maximum score on the MoCA scale was 30 points; the total score of less than 26 was regarded as CI [18, 19]. Auditory and verbal memory was assessed using a ten-words recall test (A.R. Luria). Normally, after the first memorization, the patient should reproduce

at least five words, after the 5th memorization — at least 9 words. The difference between the last immediate and delayed reproduction in healthy individuals is usually no more than one word [20]. In order to include patients with anxiety and depressive disorders in the study, the Hospital Anxiety and Depression Scale (HADS) was used during the initial screening.

Blood and saliva samples were collected from all the patients. For biomarker assessment of the Aβ42/Aβ40 ratio in blood plasma, enzyme immunoassay kits were used: for determining of beta-amyloid peptide 1–40 (CEA864Hu Enzyme-linked Immunosorbent Assay Kit For Amyloid Beta Peptide 1–40 (Ab1–40) Cloud-Clone Corp) and for determining of beta-amyloid peptide 1–42 (CEA946Hu Enzyme-linked Immunosorbent Assay Kit For Amyloid Beta Peptide 1–42 (Ab1–42)).

For saliva biomarker assay, enzyme immunoassay kits were used: Sirt1 (SEE912Hu Enzyme-linked Immunosorbent Assay Kit For Sirtuin1 (Sirt1)), Sirt3 (SEE913Hu Enzyme-linked Immunosorbent Assay Kit For Sirtuin 3 (Sirt3)), Sirt5 (SEE915Hu Enzyme-linked Immunosorbent Assay Kit For Sirtuin 5 (Sirt5)), Sirt6 (SEE916Hu Enzyme-linked Immunosorbent Assay Kit For Sirtuin 6 (Sirt6)).

For laboratory tests, blood was taken from the peripheral vein using a vacuum system into Vacutest vacuum tubes with K3EDTA (4 mL) anticoagulant in the morning on an empty stomach. Tubes with a blood sample (no later than 30 min) were subjected to centrifugation. Blood for plasma production was centrifuged on an Awel CF108-R centrifuge for 15 min at 4000 rpm at a temperature of 2–8°C. After centrifugation, aliquoting was performed into 1 mL Eppendorf tubes, after which they were placed in a cryostat for subsequent freezing and storage at a temperature of minus 80°C for further laboratory analysis.

Saliva was collected on an empty stomach after brushing teeth in a plastic centrifuge tube (2–3 mL). To obtain the filler liquid, the biomaterial tube was centrifuged for 20 min at 4000 rpm. The filler liquid was taken using a Pasteur pipette and transferred to 1.5 mL Eppendorf tubes. Prior to the laboratory examination, the samples were stored in a cryostat in a frozen state at a temperature of minus 80°C.

All subjects underwent an MRI scanning of the brain followed by morphometric processing. The studies were performed on a 3.0 Tl Discovery MR750w tomograph (GE Healthcare, USA) using a 32-channel head coil. The scanning took place according to a single protocol for all participants. T1-, T2-weighted images, DWI, DWI, and T2-FLAIR were used to evaluate the brain structure and their selection for the study. T1-weighted images (when the patient was turned on) were used for morphometry. The IR-FSPGR sequence with an isotropic voxel of 1×1×1 mm was used. Parameters: TR 7.7 s, TE with a Min Full optimization, deviation angle 11°, bandwidth 31.25 Hz. The processing was performed automatically in the FreeSurfer software, which generated maps of gray and white matter based on the Desikan-Killiany and Destrieux atlases [21–24]. A volumetric assessment of brain regions and measurement of the thickness of various cortical regions were also performed.

The statistical analysis was performed using the RStudio environment, version 2023.09.1 Build 494, and the R programming language, version 4.3.2. The following statistical indicators were calculated for quantitative variables: arithmetic averages, standard deviations ($M \pm SD$), median and quartiles (Me [$Q1$; $Q3$]. When analyzing the differences between the two groups, the parametric Student's criterion or the nonparametric Mann–Whitney criterion were used for quantitative variables. Depending on the normality of data distribution, the Pearson correlation coefficient or Spearman correlation coefficient was used to study the relationships between the two quantitative variables. The qualitative variables between the groups were compared using the χ^2 (Chi-squared) criterion or the exact Fisher criterion if the expected frequencies were less than 5. All the differences were considered statistically significant at a significance level of $p < 0.05$.

RESULTS

The conducted assessment of cognitive functions revealed a statistically significant CI of varying severity in patients of both groups. Thus, according to the MMSE scale in group 1, the indicator was 18.6 ± 4.8 points, which corresponds to severe CI (dementia); in group 2 — 27.8 ± 2.1 points, which corresponds to moderate CI ($p < 0.001$). The overall score according to the MoCA scale in group 1 was also lower and amounted to 15.1 ± 4.4, which corresponds to severe cognitive impairment (dementia), while in group 2 moderate CI was also noted — 24.6 ± 1.9 points ($p < 0.001$). Both groups of patients were characterized by impairments in the cognitive domain of memory, with difficulties in reproducing (especially delayed) and recognizing the previously presented material, reflecting a defect in capturing, consolidating, and extracting information.

In the ten-words recall test, the following results were obtained: in group 1, with direct playback (1 and 5 playback), 2.6 ± 0.9 and 4.4 ± 1.4 words, respectively; in group 2, with direct playback (1 and 5 playback) — 5 ± 1.2 and 8 ± 1.6 words, respectively. In both groups, there was a decrease in direct reproduction of words, while in group 1, the decrease was more significant with both 1 and 5 reproductions ($p < 0.001$). With delayed playback of 10 words in the memorization test, a more pronounced decrease in the level of memorization was also recorded in group 1 compared to patients from group 2: 1.3 ± 1.5 words and 6.4 ± 2 words, respectively ($p < 0.001$).

Table 1 shows the results of blood and saliva ELISA with biomarker assessment in the two compared groups. According to the comparative analysis, no statistically significant differences of biomarkers in biological fluids were obtained. At the same time, it should be noted that the blood plasma Aβ42/Aβ40 ratio of patients from group 1 is lower than in patients from group 2.

Table 2 shows the results of MR morphometry in the two compared groups. Statistically significant differences ($p < 0.05$) were revealed for all the studied MR morphometry parameters. At the same time, both the volume and thickness of brain structures were lower in group 1.

In the course of the study, an assessment of correlational relationships was carried out. In group 1, a decrease in the level of Sirt3 in saliva correlated with a deterioration in direct reproduction (fifth reproduction) in the ten-words recall test ($R = 0.43$; $p = 0.003$). In group 2, there were no correlations between the studied neuropsychological parameters and biomarkers in biological fluids ($p > 0.05$).

Unlike biomarkers in biological fluids, the analysis of the relationship between cognitive indicators and the results of MR morphometry revealed the presence of a significantly larger number of correlations.

In group 1, a decrease in the overall MMSE score was associated with a decrease in the volume of the right ($R = 0.31$ $p = 0.038$) and left hippocampus; the right ($R = 0.41$; $p = 0.006$) and left entorhinal cortex ($R = 0.34$; $p = 0.022$); the thickness of the left cingulate gyrus ($R = 0.36$; $p = 0.017$) according to MR morphometry data. Two of these neuroimaging parameters were correlated with the MoCA school: the volume of the left hippocampus ($R = 0.31$; $p = 0.04$), and the thickness of the left cingulate gyrus ($R = 0.36$; $p = 0.016$).

In group 2, a decrease in the overall score on the MMSE scale correlated with the volume of the right entorhinal cortex ($R = 0.39$; $p = 0.007$); on the MoCA scale, the volume of the right entorhinal cortex ($R = 0.34$; $p = 0.024$), the volume of the left ($R = 0.44$; $p = 0.003$) and the right cingulate gyrus ($R = 0.37$; $p = 0.012$).

In patients with AD (group 1), the results of the ten-words recall test (decreased direct reproduction) were correlated with a decrease in the volume of the left entorhinal cortex ($R = 0.31$; $p = 0.04$); in group 2 — with a decrease in the volume of the right entorhinal cortex ($R = 0.48$; $p < 0.001$). A decrease in delayed playback according to the ten-words recall test in patients from group 1 with AD was associated with a decrease in the volume of the right hippocampus ($R = 0.34$; $p = 0.021$), the volume of the left hippocampus ($R = 0.5$; $p < 0.001$), the volume of the left entorhinal cortex ($R = 0.32$; $p = 0.035$), the thickness of the left the entorhinal cortex ($R = 0.43$; $p = 0.003$).

A correlation analysis of the parameters studied in biological fluids and the obtained MR morphometry data was performed for the two examined groups. In group 1, a correlation was found between a decrease in the blood Aβ42/Aβ40 ratio and a decrease in the following indicators according to the results of MR morphometry of the brain: the volume of the right hippocampus ($R = 0.33$; $p = 0.028$), the thickness of the right ($R = 0.37$; $p = 0.012$) and the left entorhinal cortex ($R = 0.38$; $p = 0.01$), thickness of the right

Table 1. Comparative characteristics of biomarkers in biological fluids in two groups

Parameter	Group 1, $n = 45$	Group 2, $n = 45$	p-value
Aβ42/Aβ40 ratio	0.129 ± 0.097	0.164 ± 0.106	0.104
Sirt1, ng/mL	0.22727 ± 0.1649	0.21932 ± 0.18647	0.648
Sirt3, ng/mL	0.064 ± 0.022	0.086 ± 0.127	0.601
Sirt5, ng/mL	0.0191 ± 0.0151	0.0192 ± 0.017	0.886
Sirt6, ng/mL	0.1342 ± 0.0694	0.1182 ± 0.0586	0.398

Table prepared by the authors using their own data

Note: Data is presented in the form of a mean value and a standard deviation ($M \pm \delta$).

Table 2. Comparative characteristics of MR morphometry results in two groups

Parameter	Group 1, $n = 45$	Group 2, $n = 45$	p
Volume, mm^3			
Right hippocampus	3208.2 ± 486.3	3862.4 ± 630.2	<0.001
Left hippocampus	3100.2 ± 523.8	3824.5 ± 610.6	<0.001
Right entorhinal cortex	1284.6 ± 545.4	1633.4 ± 379.1	<0.001
Left entorhinal cortex	1238.4 ± 477	1778 ± 389.7	<0.001
Right cingulate gyrus	1972 ± 331.3	2194.2 ± 359.8	<0.001
Left cingulate gyrus	2170.9 ± 283.5	2344.8 ± 361.5	0.002
Thickness, mm			
Right entorhinal cortex	2.7011 ± 0.5304	3.2073 ± 0.3903	<0.001
Left entorhinal cortex	2.4711 ± 0.5169	3.1506 ± 0.3561	<0.001
Right cingulate gyrus	2.0273 ± 0.188	2.2267 ± 0.2379	<0.001
Left cingulate gyrus	2170.9 ± 283.5	2344.8 ± 361.5	<0.001

Table prepared by the authors using their own data

Note: Data is presented in the form of a mean value and a standard deviation ($M \pm \delta$).

cingulate gyrus (R = 0.3; p = 0,042). In group 2, a correlation was found between a decrease in the blood Aβ42/Aβ40 ratio and a decrease in the volume of the right entorhinal cortex (R = 0.31; p = 0.037). There were no correlations between the levels of Sirt1, 3, 5, and 6 in saliva and the results of MR morphometry.

DISCUSSION

According to a number of publications, the relationship between the development of AD and POAG has been known for quite a long time. Thus, changes in the eye reflect pathological processes in the brain, including those associated with neurodegenerative diseases such as AD, have been identified. Due to the presence of several characteristics in common, AD and glaucoma are hypothesized to be manifestations of the same pathological process with heterogeneous manifestations. Indeed, the frequency and severity of both conditions increase with age, and the prevalence of glaucoma is higher in patients with AD than in the general population [5, 6]. The above highlights the relevance of studying the relationship between these two diseases, including with respect to the search for common biomarkers.

In this work, we set out to assess the cognitive sphere of patients in two study groups and confirmed the previous data that patients with both POAG and AD demonstrate CI of various levels. At the same time, CI were less pronounced in patients with POAG. However, despite significant statistical differences between groups 1 and 2 in the severity of cognitive dysfunction, similar impairments were noted according to the results of neuropsychological techniques. In particular, this concerns memory impairment of the hippocampal type, which demonstrates the neurodegenerative nature of CI for both diseases [6].

In our study, ELISA was used to determine the Aβ42/Aβ40 ratio in blood plasma; no statistical differences were found between the two groups. However, in group 1, AD patients had a lower plasma ratio of Aβ42/Aβ40. Our results agree well with other studies that consider POAG, a neurodegenerative ophthalmological disease, to be a possible predictor of AD development [25–27]. Our data are consistent with most of the studies conducted, which postulate that a lower Aβ42/Aβ40 ratio in blood plasma is associated with a higher amyloid cortical load according to PET data and an increased risk of dementia in AD [9]. At the same time, higher Aβ42/Aβ40 ratios in patients with POAG can be explained by the difference in the severity of cognitive deficits.

The diagnostic accuracy of AD biomarkers in the blood is still inferior to those in cerebrospinal fluid, having a number of disadvantages and thus requiring further work in this direction. However, the appearance of accurate plasma analyses in the revised NIA-AA AD criteria of 2023 [7] gives hope that blood biomarkers may shortly become a promising screening method for determining the risk of developing AD, as well as, possibly, an early diagnosis tool.

It should be emphasized that saliva is another potential biological substrate used in the diagnosis of neurodegenerative diseases. Numerous studies have addressed the issue of using various biomarkers of neurodegenerative pathologies, including in AD. Similar to our study, the work by Pukhalskaia et al. [28] studied the level of Sirt in saliva. It was found that the levels of Sirt1, Sirt3, and Sirt6 were significantly lower in the group of AD patients compared to the group of healthy individuals, while the levels of Sirt5 did not differ significantly. Our study included patients diagnosed with both AD and POAG, i.e., two neurodegenerative diseases. This may explain the absence of statistical differences in the concentration of Sirt in these groups. It should be noted that evaluation of biomarkers in saliva is still associated with a number of disadvantages, which need to be overcome in the future for saliva biomarkers to be considered potential diagnostic tools. Currently, the literature data in this field remains contradictory.

Our work produced interesting results regarding the correlation between Sirt3 and the ten-words recall test, which evaluates auditory-verbal memory, one of the most important cognitive functions that suffer in AD. Some authors believe that Sirt3 plays a key role in the AD pathogenesis [29]. The current mechanisms of Sirt3 action in AD mainly include an increase in the level of ATP in mitochondria and stimulation of mitochondrial biosynthesis, activating and enhancing mitochondrial dynamics, countering oxidative stress, and regulating neuron excitability. Sirt3 plays a protective role in AD. Animal studies have demonstrated a correlation between a decrease in the concentration of Sirt3 in blood plasma and a decrease in cognitive function in mice [30, 31].

The analysis of MR morphometry data in group 1 of patients showed a statistically significant decrease in the volumes of the right and left hippocampus, right and left entorhinal cortex and cingulate gyrus, as well as the thicknesses of the right and left entorhinal cortex and cingulate gyrus relative to group 2. The results obtained seem reasonable, since these structures are of key importance in the realization of cognitive functions and their disorder is an AD pathognomonic sign. Some publications provide evidence that neuroimaging biomarkers for diagnosis and progression of basal medial temporal structures of the brain have been recently adopted. However, the most vulnerable parts of the medial temporal structures of the brain, which are responsible for the progression of the neurodegenerative process, from the stage of moderate CI to dementia in AD, are atrophy of the hippocampus, entorhinal cortex, and cingulate gyrus [32]. This is consistent with the results of our study. It should be noted that our data on the statistically significant differences between groups 1 and 2 in terms of MR morphometry may demonstrate the progression of the neurodegenerative process and depend on the severity of CI.

In our work, correlations were identified between neuropsychological tests and MR morphometry data. Thus, in AD patients, a decrease in the total score on integrative scales (MMSE, MoCA) correlated with a decrease in the volume of the right and left hippocampus, the volume of the right and left entorhinal cortex, and the thickness of the left cingulate gyrus. In POAG patients, a decrease in the total score on integrative scales (MMSE, MoCA) correlated with a decrease in the volume of the right entorhinal cortex, the volume of left and right cingulate gyri. In both groups, a decrease in the naming of the number of words on the ten-words recall test was associated with a decrease in

the volume of the entorhinal cortex. At the same time, both in group 1 (patients with AD) and group 2 (patients with POAG), a decrease in the blood Aβ42/Aβ40 ratio was associated with a decrease in the volume of the left entorhinal cortex and the volume of the right entorhinal cortex, respectively.

The entorhinal cortex is located in the cortical region adjacent to the hippocampus, occupying most of the parahippocampal gyrus. It plays the role of a connecting link in the information exchange between the associative regions of the neocortex and the hippocampus. It should be noted that the entorhinal cortex forms connections not only with the hippocampus, but also with the cingulate gyrus. The entorhinal cortex, in turn, receives highly processed information from all sensory modalities, contributing to cognitive processes, memory in particular. Recent MRI studies have revealed that the entorhinal cortex is affected primarily in AD [33–34]. Given the established correlation in our two groups between a decrease in the volume of the entorhinal cortex and both a decrease in the Aβ42/Aβ40 ratio and neuropsychological parameters, this neuroimaging parameter can be regarded as a significant early marker of the neurodegenerative process.

CONCLUSION

The results obtained allow us to assume a similar nature of the neurodegenerative process in both AD and POAG. Therefore, POAG may be considered as a predictor of AD development. Considering the above, all patients with POAG may be recommended to undergo a neuropsychological examination in order to diagnose cognitive disorders early and receive timely therapy.

At the same time, the conducted laboratory analysis indicates a relationship between the two neurodegenerative pathologies. Moreover, the study of the Aβ42/Aβ40 ratio in blood plasma seems highly promising. Our findings show that this parameter, unlike Sirt, may reflect the progression of the neurodegenerative process. Research in the direction of searching for AD biomarkers in biological fluids and clarifying their diagnostic accuracy should be continued.

In combination with neuropsychological testing, MR morphometry should be carried out, which allows for a quantitative analysis of the volumes and thicknesses of brain structures, thereby increasing the diagnostic potential of MRI in the early stages of AD and POAG. It should be noted that the statistical differences between the AD and POAG groups based on the results of MR morphometry may be related to the stages of the neurodegenerative process and depend on the CI stage. In these diseases, MR morphometry can be used as a diagnostic screening method for the progression of the neurodegenerative process.

The revealed correlations between neuropsychological parameters and MR morphometry data in patients with AD and POAG once again emphasize the responsibility of the studied brain structures for the implementation of cognitive processes. It is important that neurodegenerative changes based on the results of MR morphometry can be identified at earlier stages of the disease, when CI is minimally different from the norm during neuropsychological testing, as confirmed by our study in patients with POAG. A decrease in the volume and thickness of the entorhinal cortex can be regarded as a significant early marker of the neurodegenerative process.

It can be concluded that a comprehensive assessment of the state of cognitive functions, laboratory and neuroimaging diagnostic methods, as well as diseases associated with the development of AD, such as POAG, represents an important scientific direction. Further studies on larger cohorts of patients are required.

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DIFFERENTIAL DIAGNOSIS FEATURES OF RAPIDLY PROGRESSIVE ALZHEIMER'S DISEASE

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Introduction. The range of pathologies and conditions that can lead to the development of rapidly progressive dementia (RPD) is rather extensive. Alzheimer's disease (AD) is considered the most common cause of dementia. However, there are other pathologies that, unlike AD, are curable, and, given accurate diagnosis, allow a complete regression of pathological symptoms to be achieved. This highlights the importance of differential diagnosis of rapidly progressing AD from other causes of RPD.

Objective. To determine the differential features of rapidly progressing AD and to study the main causes predisposing to the development of RPD but not related to neurodegenerative pathology.

Discussion. Rapidly progressing AD differs from typical AD in the rate of cognitive decline. On average, rapidly progressing AD is associated with a loss of three points or more scores on the Mini-Mental State Examination (MMSE) test within six months and a faster (in 2–3 years) achievement of the terminal stage of the disease. In case of typical AD, this period is longer, lasting for about 8–10 years. Other major causes of RPD include prion diseases, neurodegenerative diseases of non-prion etiology (including rapidly progressing AD), vascular diseases, infectious diseases, inflammatory and autoimmune diseases, oncological diseases, metabolic and deficiency disorders, endocrine disorders, toxic and iatrogenic disorders, mental diseases, and cerebrovascular pathology.

Conclusions. Identification of the RPD cause requires a detailed and comprehensive examination of the patient using various laboratory and instrumental research methods, which is the key to accurate diagnosis and further successful drug correction of terminal diseases. Positron emission tomography of the brain and such biomarkers as beta-amyloid and hyperphosphorylated tau protein in the cerebrospinal fluid play a major role in the diagnosis of rapidly progressive AD and differential diagnosis from other RPD causes.

Keywords: rapidly progressing Alzheimer's disease; rapidly progressive dementia; cognitive impairment; differential diagnosis; cerebrovascular diseases; autoimmune encephalitis

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ОСОБЕННОСТИ ДИФФЕРЕНЦИАЛЬНОГО ДИАГНОЗА БЫСТРОПРОГРЕССИРУЮЩЕЙ БОЛЕЗНИ АЛЬЦГЕЙМЕРА

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Введение. Спектр патологий и состояний, которые могут приводить к развитию быстропрогрессирующей деменции (БПД), достаточно обширен. Наиболее распространенной причиной развития деменции является болезнь Альцгеймера (БА), однако существуют и другие патологии, которые, в отличие от БА, являются излечимыми, и при верной постановке диагноза возможно достижение полного регресса патологической симптоматики. Вышесказанное повышает значимость дифференциальной диагностики быстропрогрессирующей БА с другими причинами БПД.

Цель. Определить дифференциальные особенности быстропрогрессирующей БА и изучить основные причины, предрасполагающие к развитию БПД и не связанные с нейродегенеративной патологией.

Обсуждение. Быстропрогрессирующая БА отличается от типичной БА скоростью когнитивного снижения: в среднем при быстропрогрессирующей БА отмечается потеря 3-х баллов или более по Краткой шкале оценки психического статуса в течение шести месяцев и более быстрое (за 2–3 года) достижение терминальной стадии заболевания, в то время как при типичной БА этот период длительнее и составляет порядка 8–10 лет. К другим основным причинам БПД относятся прионные заболевания, нейродегенеративные заболевания неприонной этиологии (в том числе быстропрогрессирующая БА), сосудистые, инфекционные, воспалительные и аутоиммунные, онкологические заболевания, метаболические и дефицитарные нарушения, эндокринные расстройства, токсические и ятрогенные нарушения, психические заболевания, цереброваскулярная патология.

Выводы. Выявление причины БПД требует детального и всестороннего осмотра пациента с проведением различных лабораторных и инструментальных методов исследования, что является залогом верной постановки диагноза и дальнейшей успешной медикаментозной коррекции курабельных заболеваний. Основную роль в постановке диагноза быстропрогрессирующей БА и дифференциальной диагностике с другими причинами БПД играет позитронно-эмисионная томография головного мозга и уровень биомаркеров, таких как бета-амилоид и гиперфосфорилированный тау-протеин, в цереброспинальной жидкости.

Ключевые слова: быстропрогрессирующая болезнь Альцгеймера; быстрогрессирующая деменция; когнитивные нарушения; дифференциальная диагностика; цереброваскулярные заболевания; аутоиммунный энцефалит

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INTRODUCTION

Dementia is a global challenge for healthcare systems. Dementia is a neuropsychiatric syndrome characterized by a marked decrease in cognitive functions and the development of professional, household, and social maladaptation of the patient [1]. The World Health Organization (WHO) lists dementia as one of the most disabling diseases, the prevalence of which has recently been increasing worldwide [2].

The literature and statistical data confirms Alzheimer's disease (AD) to be the most common cause of dementia. However, typical AD refers to diseases characterized by a gradual progression of cognitive deficits. At the same time, there appear more diagnostic cases associated with a rapid progression of cognitive decline to the degree of dementia. These cases also include a large proportion of AD patients [3].

Although various authors identify prion diseases, Creutzfeldt-Jakob disease (CJD) in particular, as the most common etiology of rapidly progressive dementia (RPD) [4, 5], the current literature mentions other neurodegenerative diseases (nonprion), including AD, as the cause of RPD development. For example, a five-year comparative study [6] showed that neurodegenerative diseases of a nonprion etiology were the cause of RPD in 38% of cases, while prion diseases occurred in only 19% of cases. In addition to CJD and AD, the range of pathologies and conditions that can lead to the development of RPD is quite extensive (see Table).

At the same time, the importance of timely and accurate diagnosis is emphasized the possibility of a partial or complete elimination of cognitive impairment (CI) and other neurological symptoms in some pathologies that can lead to the development of RPD. In a two-year retrospective cohort study conducted in China, the authors [7] demonstrated that out of 310 patients hospitalized with RPD, 68 (21.9%) had viral encephalitis, followed by AD — 45 (14.5%) and autoimmune encephalitis — 28 (9.0%) patients. CJD was detected in only 22 (7.1%) patients. Another research group [8] published a prospective observational study, in which 86 (55.5%) of 155 patients with RPD had potentially treatable causes, such as autoimmune encephalitis (n = 52), vascular diseases (n = 14), neoplastic syndrome (n = 7), toxic/metabolic disorders (n = 7), psychiatric (n = 4), and other diseases (n = 12). The median age of the onset of RPD symptoms in that study was 68.9 years (ranging within 22.0–90.7 years). At the same time, the age of the onset of symptoms <50 years was one of the parameters that were most typical of patients with curable causes of RPD [8]. This indicates that some cases with a rapid progression of cognitive deficits are observed in young people of working age and, in the absence of timely treatment, may lead to professional disablement.

In the light of the above, research into the causes of RPD development is of particular importance. In this connection, our aim was to determine the differential features of rapidly progressing AD and to study the main causes predisposing to the development of RPD that are not related to neurodegenerative pathology.

Table. Causes of rapidly progressive dementia

Group	Deseases
Neurodegenerative diseases*	Prion diseases, rapidly progressive AD, rapidly progressive Lewy body dementia (LBD), frontotemporal dementia (FTD), progressive supranuclear palsy, corticobasal degeneration, multisystem atrophy, Huntington's disease
Cerebrovascular diseases	Multi-infarct dementia, stroke in areas strategic for cognitive functions, cerebral amyloid angiopathy, mitochondrial encephalopathy with stroke-like episodes and lactate acidosis (MELAS syndrome), CADASIL syndrome, CNS vasculitis, cerebroretinal microangiopathy with calcifications and cysts, posterior reversible encephalopathy syndrome, venous thrombosis
Infectious diseases	Meningitis and encephalitis of various etiologies (e.g., tuberculosis, herpes simplex virus, fungal), neurosyphilis, neuroborreliosis, HIV infection, progressive multifocal leukoencephalopathy, CNS toxoplasmosis, Whipple's disease
Inflammatory and autoimmune diseases	Autoimmune encephalitis, Hashimoto's encephalopathy, multiple sclerosis, acute multiple encephalomyelitis, neurosarcoidosis, celiac disease, autoimmune GFAP astrocytopathy
Cancers	Primary CNS tumors, CNS lymphoma, metastatic solid tumor, meningeal carcinomatosis, paraneoplastic syndrome
Metabolic and endocrine disorders	Hepatic encephalopathy, uremic encephalopathy, thyroid gland pathology associated with changes in thyroid-stimulating hormone levels, increased or decreased endocrine activity of the parathyroid glands, adrenal insufficiency
Deficiency disorders	Vitamin B deficiency (B_1, B_3, B_9, B_{12}), electrolyte disturbances, pellagra
Toxic and iatrogenic disorders	Degeneration of the nervous system caused by alcohol; poisoning with bismuth, mercury, lithium, arsenic, lead; neuroleptic malignant syndrome, serotonin syndrome
Mental illness	Psychotic disorders, depression, bipolar disorder, simulation disorder, conversion disorder

Table prepared by the authors using data from references [5–11]

Note: * — the group includes prion and nonprion neurodegenerative diseases; LBD — Lewy body dementia; MELAS — mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes; CADASIL — cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy.

MATERIALS AND METHODS

The scientific literature search was conducted using electronic bibliographic databases in the Russian (eLibrary, CyberLeninka) and English (Web of Science, Scopus, PubMed) languages. The search queries included the following keywords or phrases: rapidly progressive dementia; rapidly progressive Alzheimer's disease; cognitive impairment; differential diagnosis (in Russian); rapidly progressive Alzheimer's disease; rapidly progressive dementia; cognitive impairment; differential diagnosis (in English). The search depth was 10 years.

The inclusion criteria were systematic literature reviews and meta-analyses with information on the causes of RPD, rapidly progressive AD, and diagnostic methods used to identify the disease or condition underlying the development of RPD; original articles reporting the study of RPD causes. The exclusion criteria were theoretical models, theses, and conference materials.

RESULTS AND DISCUSSION

Characteristics of rapidly progressive dementia (RPD) and its underlying reasons

Most researchers refer to RPD as the development of cognitive decline and its rapid progression to the state of dementia over a relatively short period of time, lasting for several weeks or months and no more than two years in most cases [8–10]. However, it should be emphasized that there are no uniform criteria for RPD, with the information about RPD frequency and its main etiology being rather diverse.

Thus, prion diseases, neurodegenerative diseases of a nonprion etiology (including rapidly progressing AD), vascular diseases, infectious diseases, inflammatory and autoimmune diseases, oncological diseases, metabolic and deficiency disorders, endocrine disorders, toxic and iatrogenic disorders, and mental illnesses are distinguished among the main causes of RPD. The Table above shows the diseases and pathological conditions leading to RPD, which does not exclude the presence of other, rarer pathologies that may be the cause of its development [8–11].

The presence of a wide range of pathologies capable of causing the development of RPD dictates the need to differentiate rapidly progressing AD not only from CJD and neurodegenerative diseases (Lewy body dementia (LBD), frontotemporal dementia (FTD), corticobasal degeneration), but also from many other diseases. In this regard, the knowledge of medical professionals about rapidly progressing AD and its differential diagnosis from other significant etiologies of RPD, such as cerebrovascular pathology, infectious diseases, inflammatory and autoimmune diseases, requires elucidation.

AD as a underlying cause for RPD development

AD is a common neurodegenerative disease, which is believed to be among the main causes of dementia. Therefore, AD is to be considered in the differential diagnosis of RPD [12].

It should be noted that the clinical manifestations of AD can be different, depending on the time of the onset of the disease and its form. Thus, AD with the early onset (before the age of 65) differs from that with the late onset (after the age of 65). The guidelines of the International Working Group (IWG) as of 2014 distinguish the following forms of AD: typical, atypical (frontal variant of AD, logopenic variant of primary progressive aphasia syndrome, posterior cortical atrophy), and mixed [13].

The typical form of AD course, which can be referred to as classical, is characterized by slow progression. According to neuropsychological testing, cognitive functions demonstrate a decrease in the Mini-Mental State Examination scores (MMSE; normal levels of 28–30 scores) on average from two to four or more per year. After 8–10 years, this gradual deterioration leads to the terminal stage of the disease, i.e., severe dementia (10 or less MMSE scores) [14, 15]. However, AD may have another, rapidly-progressing form. Thus, the expert group [16] conducted a systematic review of 61 articles and published a consensus document, which proposed to use the loss of three or more score during the period of six months as an empirical definition of rapid cognitive decline and to apply this definition in routine medical practice for clinical decision making in patients with a mild to moderate severity of AD. At the same time, the achievement of the terminal stage (severe dementia) and disability of the patient with the loss of independence in RPD occurs much faster, on average, over 2–3 years.

According to [17], a significant proportion of patients may experience a rapid progression of AD. Thus, in a longitudinal two-year study, 686 patients with mild to moderate AD were observed. In this study, 30% of patients showed a decrease in cognitive functions by MMSE exceeding three scores per year, which was twice as fast as the average of the entire cohort. This demonstrates the high prevalence of this type of AD course.

In general, such factors as medical and social support for the patient, genetic predisposition, as well as concomitant cerebrovascular and other comorbid pathologies, especially at the stage of decompensation, may have an impact on the rate of AD progression. For example, the presence of a history of strokes and/or chronic cerebral ischemia (CCI) can significantly exacerbate the clinical manifestations of AD and lead to a faster loss of independence of the patient. In this case, the rate of disease progression was shown to be higher [17, 18].

However, it should be emphasized that in most cases, rapidly progressive AD occurs in patients under 65 years of age, i.e., with an early onset of the disease. AD is a disease that is frequently inherited. Thus, hereditary forms account for 10% of the total number of patients with AD (the remaining cases are sporadic). The presence of pathological genes is mainly noted in patients with the early onset of the disease (under 65 years of age).

Accordingly, it can be assumed that genetically-determined disorders that lead to early neuronal damage and synaptic dysfunction are among the main factors in the development of rapidly progressing AD. The study [18] observed a cohort of patients with rapidly progressing AD and found the frequency of the Apoliprotein E (*ApoE ε4*)

gene allele of 23.1%. *ApoE* is the most important genetic risk factor for sporadic AD, affecting the timing of the onset of the disease. It is assumed that the *ApoE* allele *ε4* gene is not widespread among patients with a rapidly progressive form of AD. Nevertheless, the question of whether the *ApoE* genotype is associated with the progression of AD is still a matter of debate [16].

Most researchers note the presence of severe amyloid angiopathy as a reason underlying the rapid progression of AD. This condition contributes to a faster damage to brain neurons, which is clinically manifested by a rapid decrease in cognitive functions [19, 20] compared with the classical form of AD development.

It should be noted that rapidly progressing AD is characterized by a diffuse brain damage, which primarily affects the cortical regions [21]. In this regard, the AD clinical picture, in addition to the presence of rapidly progressive cognitive impairments (CI), is characterized by other neurological symptoms, including motor disorders with damage to the pyramidal and extrapyramidal systems (to a greater extent) or emotional-volitional disorders [22]. According to [23], patients with rapidly progressing AD may have earlier behavioral and psychotic disorders, while the clinical manifestations of rapidly progressing AD may mimic CJD, which causes diagnostic difficulties.

One difficulty in establishing the diagnosis of rapidly progressing AD is associated with its highly diverse clinical picture. For this reason, changes in both neuroimaging and laboratory biomarkers can be used as distinctive differential features of rapidly progressing AD from RPD of another etiology. Positron emission tomography (PET) with beta-amyloid (Aβ) or tau protein (tau) ligands and PET with ^{18}F-fluorodeoxyglucose (^{18}F-FDG) are among the most accurate neuroimaging diagnostics methods. At the same time, brain MRI in rapidly progressing AD does not have clearly specific signs. Given the lesion nature in rapidly progressive AD, neuroimaging analysis (brain MRI) shows diffuse atrophic changes that rapidly increase under dynamic observation. In the Russian Federation, PET with beta-amyloid (Aβ) ligands or tau protein (tau) remains, unfortunately, a poorly accessible diagnostic method, unlike PET with ^{18}F-FDG, which is also sensitive to changes in AD. The research team [24] noted that positron emission tomography (PET) with ^{18}F-fluorodeoxyglucose (^{18}F-FDG) is capable of identifying regionally specific hypometabolism in the left angular gyrus and the left temporal cortex.

Currently, laboratory biomarkers play a leading role in the AD diagnosis, including rapidly progressing AD. These are biomarkers in biological fluids, with the most accurate diagnostic indicators being in cerebrospinal fluid (CSF): Aβ42, p-tau217, p-tau181, p-tau231, ratios of p-tau181/Aβ42, t-tau/Aβ42, and Aβ42/Aβ40. At the same time, low levels of Aβ42 in CSF, lower levels of the Aβ42/Aβ40 ratio, as well as increased indicators of the p-tau181/Aβ42 ratio may be associated with a faster decline in cognitive functions. In addition, patients with rapidly progressing AD showed higher levels of p-tau than patients with typical AD [22].

Blood plasma parameters in the AD diagnosis, including rapidly progressing AD, are also being actively studied. Thus, the longitudinal study [16], which included 122 patients with AD observed for an average of 4.2 (2.6) years, found a link between blood plasma biomarkers and the rate of disease progression. It was noted that lower levels of Aβ40 and Aβ42 were associated with a significantly faster cognitive decline.

Currently, no pathogenetic treatment for rapidly progressing AD, as well as for a typical form of AD, exists. Therefore, standard anti-dementia therapy aimed at slowing the progression of the disease is used. In rapidly progressing AD, an earlier transition to a combination therapy by cholinesterase inhibitor and memantine is recommended [25, 26].

Cerebrovascular pathology as a cause of RPD development

Cerebrovascular pathology, along with neurodegenerative diseases, is a common cause of severe cognitive dysfunction (dementia). Most vascular diseases of the brain, including multi-infarct dementia, strokes in strategic areas crucial for cognitive functions, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) and vasculitis of the central nervous system (CNS), can cause the development of RPD [26].

It should be noted that in the first post-stroke year, more than half of patients develop CI. However, in the absence of other concomitant vascular, neurodegenerative, or somatic pathologies, the CI progression, as a rule, does not occur. On the contrary, there may be a positive trend against the background of ongoing rehabilitation measures. At the same time, in the presence of repeated strokes or comorbid pathology, a rapid cognitive decline may occur [27]. Secondary post-stroke complications, such as seizures, can also accelerate the CI progression.

An important role in the RPD development is played by the stroke in strategic areas crucial for cognitive functions, which include the thalamus, angular gyrus, caudate nucleus, limbic system, prefrontal cortex, and medial temporal lobes. Thus, when the thalamus is affected, depending on the circulatory disorders of a particular artery, as well as the side of the lesion, various disorders may be observed: decreased memory for current events (both auditory and visual), impaired orientation in time, aphasia, akinetic mutism, impaired counting, impaired regulatory functions, neglect syndrome, impaired constructive practice, neuropsychiatric symptoms, and other neurological symptoms (e.g., oculomotor disorders) [28].

Stroke treatment depends on its type and includes not only specific therapy, but also secondary stroke prevention (correction of the main risk factors) and rehabilitation measures. It should be noted that a possible complete or partial regression of symptoms will depend on the stroke severity, lesion location, adequate therapy, proper prevention of post-stroke complications, and rehabilitation of the patient [27, 28].

The syndrome of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy

(CADASIL) can also underlie the RPD development. CADASIL is the most common hereditary disease of small cerebral vessels characterized by non-atherosclerotic and non-amyloid diffuse angiopathy with predominant lesion of small and medium penetrating and leptomeningeal arteries [29]. Clinically, this arteriopathy is manifested by migraine with aura, recurrent subcortical ischemic strokes, and/or stroke-like episodes, affective disorders, and CI. Cognitive dysfunction in this disease is detected in about 50% of patients. It is a progressive subcortical type of dementia. Moderate CI, including impairment of executive functions, memory, and attention, occur in patients long before the onset of subcortical ischemic events [29, 30].

Magnetic resonance imaging (MRI) of the brain in patients with CADASIL usually reveals bilateral symmetrical diffuse leukoareosis, accompanied by multiple lacunar infarcts in the subcortical and periventricular white matter, basal ganglia on both sides. A characteristic manifestation of CADASIL is hyperintensivity in the T2/FLAIR mode in the pole of the temporal lobe, the outer capsule and the corpus callosum [30]. Currently, genetic testing is considered the leading method of identification and diagnosis of CADASIL. Mutations of the *NOTCH3* gene, as well as, in more rare cases, the *Arg332Cys* gene, cause the disease development [30].

Currently, no treatment with proven efficacy has been developed; thus, symptomatic therapy and strategies for managing vascular risk factors are used. In some studies [31], executive function improvements were noted when taking donepezil; however, the clinical significance of these results remains unclear, requiring further confirmation.

In primary CNS vasculitis, most patients exhibit the signs of acute CNS, which progress rapidly during the period from two weeks to 12 months. These signs often include headache, motor deficiency (hemiparesis), speech disorders (aphasia), seizures, visual disturbances, and symptoms associated with spinal cord injury. Strokes, if they develop, are usually multiple and bilateral. Depending on the clinical picture, it is possible to indirectly judge which vessels are affected. In the case of damage to large-caliber vessels, symptoms similar to stroke and focal neurological symptoms prevail. In the case of damage to small blood vessels, symptoms associated with impaired cognitive functions and epileptic seizures are more common. In vasculitis-caused dementia, cephalgic syndrome is somewhat more common than in other types of dementia. Another feature is the faster progression of cognitive impairments (RPD): not years, as in dementias of primary degenerative origin, but months or even weeks. At the same time, the nature of neurological disorders depends on the area that is affected, and signs of systemic disease may be absent [32].

The differential diagnosis of primary vasculitis should exclude infectious, malignant or systemic inflammatory diseases, as well as reversible cerebral vasoconstriction syndrome. Typical MR signs of the disease are multifocal bilateral foci in the T2 or FLAIR mode in the cortical and subcortical regions, as well as in deep white and gray matter (basal ganglia). CT or MR angiography is used to refine the imaging picture. This method allows detecting changes mainly in large blood vessels in the form of thickening of the walls and intrahepatic post-contrast edema as a sign of active vasculitis. When the lesion is localized mainly in the distal parts (small vessels) and in the posterior cerebral artery system, cerebral angiography is a more sensitive method. At the same time, the gold standard diagnostics comprises a brain biopsy with histological verification [33].

The remission possibility in primary vasculitis underscores the importance of differential diagnostics of RPD. The corresponding treatment includes corticosteroids and/or cytostatics (usually cyclophosphamide) with the therapy being continued for 6–12 months after achieving remission [34, 35].

Infectious diseases as etiological factors in RPD development

Infectious diseases are among the most common causes of RPD, with their prevalence over other causes of rapidly progressive cognitive deficits being confirmed [7]. This observation was likely related to the inclusion of younger patients in these studies, among whom infectious and inflammatory diseases are much more common than neurodegenerative pathologies. Bacterial, viral, fungal, and protozoan brain infections leading to the development of dementia are well known; the relevant data are presented in the Table. In most cases, infections with CNS damage are characterized by an acute onset; however, there are forms with a subacute and chronic course [36].

Establishing the diagnosis of encephalitis caused by an infectious disease in the acute period can be difficult, since the clinical symptoms are nonspecific and may either include various neurological manifestations or not include them at all (in the abortive form). It should be noted that patients with infectious diseases are characterized not only by neurological symptoms (meningeal cerebral and focal neurological symptoms), but also by a general infectious syndrome (hypertemia, changes in peripheral blood, skin rashes, tachycardia, tachypnea and other manifestations) [37]. Other organs and systems may also be affected. For example, Whipple's disease, a rare infectious disease, which can cause the development of RPD, is associated with damage to several systems: gastrointestinal, respiratory, cardiovascular, nervous, as well as eyes and joints. The main neurological manifestations are dementia, supranuclear ophthalmoplegia, and myoclonus [37].

It should be noted that an infectious disease as the primary cause of dementia is usually considered as a diagnosis of exclusion. The cognitive decline in infectious diseases is not the sole neurological symptom, which may progress rapidly. This makes it possible to suspect an infectious lesion of the central nervous system and require a lumbar puncture followed by cerebrospinal fluid (CSF) examination and the exclusion of a potentially reversible infectious process. However, in the early stages of infectious diseases,

CSF analysis alone is insufficient for diagnosis, which requires additional research methods, such as brain MRI, electroencephalography (EEG), electroneuromyography (ENMG), etc. [36].

Treatment for RPD in such cases depends on the etiology of the infectious disease and may include antiviral, antibacterial, and other medications directed against infectious agents. It is important to emphasize the probability of a favorable outcome of the disease with a regression of symptoms, including CI, given that the infectious origin of RPD is confirmed and adequate treatment is initiated in a timely manner.

Inflammatory and autoimmune diseases as a cause of RPD development

Although RPD was first mentioned in patients with multiple sclerosis (MS), demyelinating inflammatory diseases such as MS and acute multiple encephalomyelitis rarely trigger RPD. In this group, autoimmune encephalitis (AE) is the most common cause of RPD [38].

AE is a heterogeneous group of immune-mediated paraneoplastic and nonparaneoplastic (idiopathic) encephalitis, which leads to the development of encephalopathy. Given the multifocal brain lesion, the clinic picture may be diverse. Cognitive, emotional, and mental disorders such as behavioral disorders (aggression, irritability), depressive symptoms, anxiety, obsessive-compulsive symptoms, hallucinations, subacute dementia, anterograde amnesia and others, which result from the damage to the structures of the limbic system (limbic encephalitis), also list cerebellar degeneration, Bickerstaff's disease, and epileptic seizures. Chorea involving the facial muscles or atypical Parkinsonism syndrome may also occur. Anti-N-methyl-D-aspartate (NMDA)-receptor encephalitis is the most studied and widespread among AE, which plays an important role in the development of RPD [38].

Anti-NMDA receptor encephalitis affects mainly young people (95% of patients under 45 years of age); the disease is more common among females (80% of women). The relationship of AE development with the presence of ovarian teratoma is revealed in more than half of female patients [39]. Anti-NMDA receptor encephalitis is characterized by a nonspecific flu-like prodrome, after which neuropsychiatric symptoms develop acutely in some cases. The most common symptoms of mental disorders are emotional lability, anxiety, fears, insomnia, manic state, delusions, and hallucinations. In this regard, about 60% of patients are initially admitted to psychiatric clinics. The majority of patients also experience rapidly progressive CI, in which episodic memory and regulatory functions are affected. Somewhat later, extrapyramidal disorders (dystonia, chorea, or stereotypy), catatonia, and autonomic dysfunction join. However, in clinical practice, the appearance of extrapyramidal disorders and catatonia is often regarded as the consequences of antipsychotic therapy, which is prescribed to patients taking into account the presence of neuropsychiatric disorders. This may lead to diagnostic errors. Almost 85% of patients develop epileptic seizures, which frequently remain unrecognized due to extrapyramidal symptoms, psychomotor agitation, or the need to maintain drug sedation. Overall, the clinical picture demonstrates the complexity of overlapping psychiatric and neurological symptoms and highlights the need for an interdisciplinary approach to diagnosis and treatment [40, 41].

To confirm the diagnosis, it is necessary to determine the titer of antibodies to NMDA receptors in biological fluids such as blood or CSF. Only about 50% of patients exhibit brain MRI changes, such as T2/FLAIR hyperintensivity areas in the hippocampus, cerebral cortex, cerebellar hemispheres, insula, fronto-basal region, basal ganglia, and brain stem. In the event that the brain MRI reveals no changes, patients undergo an additional study by positron emission tomography (PET) with ^{18}F-fluorodeoxyglucose (FDG). This study determines the frontal-temporal-occipital gradient of glucose metabolism, which correlates with the activity of the disease. The EEG may reveal a specific pattern of extreme delta brushes, i.e., a rhythmic δ-activity with a frequency of 1–3 Hz with bursts of rhythmic β-activity superimposed on each δ-wave, resembling δ-brushes in premature infants. This phenomenon occurs in almost 30% of cases of anti-NMDA receptor encephalitis [11, 40].

It should be noted that in about 70% of patients, timely treatment of anti-NMDA receptor encephalitis results in complete or almost complete regression of symptoms. There are three lines of therapy:

1. Pulse therapy with glucocorticosteroids and/or immunoglobulin intravenously, plasmapheresis;
2. Rituximab or cyclophosphamide or a combination thereof;
3. Other cytostatic immunosuppressants.

In case of ineffectiveness of first-line therapy, the drugs of the following lines are prescribed. Together with the therapy, a diagnostic search for oncological diseases is carried out and, if necessary, antitumor therapy is performed [9, 11].

Hashimoto's encephalopathy (HE) may be another cause of RPD development in this group of diseases. HE is a rare autoimmune disease known as steroid-reactive encephalopathy associated with autoimmune thyroiditis. HE is more common among women (70–85% of cases). At the same time, the clinical picture of HE includes various neurological and psychiatric symptoms, the variety of which complicates its timely diagnosis. Among the neurological symptoms, the most common are extrapyramidal symptoms, ataxia, epileptic seizures, transient aphasia, rapidly progressive cognitive decline to dementia, and confusion. There may even be stroke-like episodes. Patients also experience behavioral disorders and visual hallucinations. A rapid increase in the symptoms of the disease and a fluctuating course are characteristic [42].

In order to confirm the HE diagnosis, the necessary criterion involves the detection of a high titer of antithyroid antibodies (antibodies to thyroglobulin and antibodies to thyroperoxidase) in the blood and the absence of other causes of brain damage that could better explain the clinical picture. Patients with HE may have hypothyroidism,

hyperthyroidism, or euthyroidism; therefore, assessment of thyroid hormone levels does not have a diagnostic significance in HE detection. The results of CSF analysis and brain MRI scans do not reveal any specific changes. Thus, in patients with HE, there is only an increase in the level of protein in the CSF, and according to brain MRI, half of the patients have nonspecific changes in subcortical white matter and cerebral atrophy. Intermittent slow-wave activity and three-phase waves are most often observed in the EEG during HE. HE is a curable disease. In most cases, the symptoms regress after the use of immunosuppressive therapy. The first-line drugs are glucocorticosteroids. With timely and accurate diagnosis and well-selected therapy, the prognosis after treatment of the disease is favorable [43].

CONCLUSION

Although RPD accounts for only about 3–4% of dementia cases, it is a disproportionately large clinical problem due to the need for a broad differential diagnosis, a variety of possible diagnostic tests, and the need to complete the assessment at a pace consistent with the rate of cognitive decline. The existing extensive list of pathologies associated with the onset of rapidly progressive cognitive deficits and the presence of potentially curable diseases highlights the importance of further research in this direction.

Given the presence of such a wide range of diseases, the approach to the differential diagnosis of rapidly progressing AD should include a thorough medical history and physical examination, mandatory neuropsychological testing, and high-quality laboratory and instrumental diagnostics. PET scans of the brain and the study of AD biomarkers in the CSF are of the greatest importance in the differential diagnosis of rapidly progressing AD.

The study of other causes of RPD that are not related to neurodegenerative processes will make it possible to identify curable diseases. This indicates the need to increase the knowledge of medical professionals about the differential diagnosis of RPD.

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IDENTIFICATION OF NEGLECT SYNDROME IN CEREBRAL STROKE PATIENTS USING STANDARD TESTS AND EYE TRACKING METHOD

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Introduction. About 30–40% of patients who have suffered from acute cerebrovascular accident (ACVA) experience a syndrome of unilateral spatial neglect. Neuropsychological testing (NT) is a routine diagnostic technique, while the method eye tracking offers prospects for an objective assessment of visual attention.
Objective. Evaluation of the diagnostic capabilities of classical neuropsychological techniques and eye tracking to detect the neglect syndrome in stroke patients.
Materials and methods. The study involved 38 stroke patients (25 men, 13 women; mean age 59.7 ± 12.7 years). The Bells test (BT), Albert's test (AT), Line bisection test (LBS), the computer version of the Apple test (ApT), and the eye tracking method (a search task for recording visual activity) were used to diagnose the neglect syndrome.
Results. The LBS test data demonstrated the greatest sensitivity in the detection of neglect syndrome. Significant correlations ($p < 0.01$) were obtained between the results of BT, AT, LBS, and ApT and the results of eye tracker visual search ($p = 0.025$), indicating the detection of a similar degree of observed deficiency by different methods. The latency of finding stimuli in the left half-field when performing a search task on an eye tracker is significantly higher than in the right side ($p < 0.001$). Ischemic stroke patients performed AT worse ($p = 0.009$) than hemorrhagic stroke patients, and they were more mistaken in LBS ($p = 0.043$). The more pronounced severity of the patients' neglect, the worse the AT ($p = 0.004$), LBS ($p = 0.05$), and Aptego ($p = 0.036$) were performed. The visual impairment factor had a significant effect in LBS testing ($p = 0.02$).
Conclusions. The combination of neuropsychological tests and eye tracking provides objective data for the diagnosis of neglect syndrome. The LBS test demonstrated the greatest sensitivity in detecting the neglect syndrome. The results of eye tracking were found to be comparable with those of pencil-and-paper tests, which increases the accuracy of the diagnosis of neglect syndrome. The following factors influencing the performance of diagnostic tests were identified: stroke type, neglect severity, and visual impairment.

Keywords: neglect; unilateral spatial neglect syndrome; stroke diagnosis; neuropsychological tests; pencil-and-paper tests; eye tracking; stroke

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ВЫЯВЛЕНИЕ СИНДРОМА НЕГЛЕКТА У ЛИЦ, ПЕРЕНЕСШИХ ЦЕРЕБРАЛЬНЫЙ ИНСУЛЬТ, С ИСПОЛЬЗОВАНИЕМ ТРАДИЦИОННЫХ ТЕСТОВ И МЕТОДА АЙТРЕКИНГА

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Введение. У 30–40% пациентов, перенесших острое нарушение мозгового кровообращения (ОНМК), наблюдается синдром одностороннего пространственного игнорирования (неглект). Традиционные методы диагностики включают нейропсихологические тесты (НТ), а метод айтрекинга предлагает перспективы для объективной оценки зрительного внимания.
Цель. Оценка диагностической возможности классических нейропсихологических методик и метода айтрекинга для выявления синдрома неглекта у пациентов, перенесших инсульт.
Материалы и методы. В исследовании приняли участие 38 пациентов (25 мужчин, 13 женщин; средний возраст 59,7 ± 12,7 года), перенесших ОНМК. Для диагностики синдрома неглекта использовали Bells test (BT), Albert's test (AT), Line bisection test (LBS) и компьютерную версию Apple test (ApT), метод айтрекинга (поисковая задача для регистрации зрительной активности).
Результаты. Данные выполнения LBS теста продемонстрировали наибольшую чувствительность к выявлению синдрома неглекта. Получены достоверные корреляции ($p < 0,01$) между результатами BT, AT, LBS и ApT и результатами зрительного поиска на айтрекере ($p = 0,025$), указывающие на определение схожей степени наблюдаемого дефицита разными методами. Латентность нахождения стимулов в левом полуполе при выполнении поисковой задачи на айтрекере достоверно выше, чем в правом ($p < 0,001$). Пациенты с ишемическим инсультом хуже, чем пациенты с геморрагическим, выполняли AT ($p = 0,009$), чаще ошибались в LBS ($p = 0,043$). Чем сильнее была выражена у пациента тяжесть неглекта, тем хуже были выполнены AT ($p = 0,004$), LBS ($p = 0,05$), ApTego- ($p = 0,036$). Фактор снижения зрения оказывал значимое влияние при тестировании LBS ($p = 0,02$).

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Выводы. Объединение НТ и метода айтрекинга предоставляет объективные данные для диагностики синдрома неглекта. Тест LBS продемонстрировал наибольшую чувствительность к выявлению синдрома неглекта, а результаты метода айтрекинга сопоставимы с данными бланковых тестов, что повышает точность диагностики синдрома неглекта. Были выявлены факторы, влияющие на выполнение диагностических тестов: тип инсульта, степень выраженности неглекта и фактор снижения зрения.

Ключевые слова: неглект; синдром одностороннего пространственного игнорирования; диагностика инсульта; нейропсихологические тесты; бланковые методы; айтрекинг; инсульт

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Соответствие принципам этики: исследование одобрено локальным этическим комитетом ФГБУ «ФЦМН» ФМБА России (протокол № 10/25-04-22 от 25.04.2022). Все пациенты подписали информированное согласие на обследование.

Потенциальный конфликт интересов: авторы заявляют об отсутствии конфликта интересов.

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INTRODUCTION

About 30–40% of patients who have suffered from acute cerebrovascular accident (ACVA) develop sensory inattention, which refers to the manifestation of visuospatial gnostic disorders [1, 2]. In the middle of the last century, ophthalmologist S. Duke Elder coined the term of unilateral spatial agnosia [2, 3].

Unilateral spatial neglect is most often defined as the inability to perceive stimuli of various modalities or to respond to these stimuli from the side contralateral to the lesion. In addition, this syndrome includes the absence of voluntary movement of the contralateral side of the body or limb [4], while the physical ability to perform an action remains [5]. A systematic review of neglect syndrome cases after stroke [6] identified this syndrome in 18% of patients with left hemisphere stroke and in 38% with right hemisphere stroke, persisting in 20% of patients in the chronic phase.

It is customary to distinguish different types of neglect based on the disorder modality, the specifics of the lesion of the spatial component of perception and orientation to the stimulus, object or subject of perception. Thus, perceptual, visual, tactile, and auditory ignoring can be distinguished [5]. In addition, the phenomenon of representational and motor disregard, disorder of the voluntary movement of the opposite limb/sides of the body should be mentioned [5].

Depending on the spatial component, personal neglect (ignoring new and familiar stimuli on the body surface), peripersonal neglect (ignoring at arm's length), and extrapersonal neglect (ignoring stimuli at a distance far from the subject) are distinguished [5]. In determining the ignoring syndrome in the clinical picture of post-stroke disorders, special attention should be paid to the orientation of ignoring. In case of egocentric neglect, the patient ignores all stimuli on the left relative to themselves. In case of allocentric disregard, the patient will ignore the left side of the perceived objects, regardless of their location in space [7].

Due to the variety of ignoring manifestations, the similarity of the disorder pattern with visual disorders, as well as the high frequency of the phenomenon, methods for the diagnosis of neglect are acquiring particular importance. In 2021, the World Federation for Neurorehabilitation issued clinical guidelines outlining the main methods for diagnosing neglect [1].

The most commonly used methods used in neuropsychological practice are aimed at finding and labeling objects. These are so-called pencil-and-paper tests, including Albert's test, Bells test, Apple test, etc. Pencil-and-paper tests are considered suitable to diagnose and determine neglect due to the possibility of quantifying the performance of tests, as well as their high constructive validity [8].

Line bisection tests are also frequently used, with the most popular option being the Schenkerberg line bisection test (LBS). Using this test, the researcher is capable of estimating the percentage of displacement of the subjective visual midline. In clinical practice, tests for spontaneous drawing, writing, copying, and reproducing objects are also applied. These tests determine the level of disorder of visuospatial and representative representations. In addition, they may reflect the patient's ignoring of the left side of the presented stimuli [9, 10]. Despite the variety of pencil-and-paper tests, they are less sensitive in the assessment of attention and perception in the extrapersonal space, failing to solve the problem of differentiating the motor or visual type of ignoring [2].

In the diagnostics of ignoring syndrome, a strategy for scanning the patient's space should be selected. From this point of view, the most informative research methods are oculography and eye tracking. The latter approach can be used to track the movement of the subject's gaze, the number of gaze fixations, the duration of fixations, etc., thus providing objective quantitative data for analysis. A number of studies indicated the high diagnostic significance of the method, as well as its greater sensitivity to the manifestations of the syndrome in comparison with cancellation tests [11]. The important advantage of the eye-tracking technique consists in the absence of the need to involve a motor component in the testing process, which makes it possible to differentiate between motor and visual ignoring, visual-motor delay. However, although the eye-tracking

method shows high potential in solving various diagnostic tasks, it does not provide the opportunity to assess attention and perception in the peripersonal space and is ineffective in patients with visual impairment [2].

The World Federation for Neurorehabilitation has outlined an approach to the diagnosis of neglect, which consists in using more than two different types of diagnostic tests. Nevertheless, the current literature lacks studies aimed at assessing the diagnostic significance of an integrated approach based on the use of cancellation tests and hardware methods [1].

In this research, we set out to evaluate the diagnostic capabilities of classical neuropsychological tests and eye tracking to detect the neglect syndrome in stroke patients.

MATERIALS AND METHODS

Study participants

The conducted research was an observational, cross-sectional, and prospective study aimed at an in-depth diagnostic examination of neglect syndrome signs in patients undergoing rehabilitation at the Federal Center of Brain Research and Neurotechnologies.

Initially, the sample included 49 patients who had suffered a cerebral stroke. The inclusion criteria were a first history of stroke, less than 12 months after the stroke, visual acuity from –3 to +2D, the presence or suspicion of neglect syndrome (by neuropsychologist conclusion), understanding of instructions, ≥3 points on the rehabilitation routing scale (RRS). The exclusion criteria were repeated stroke, more than 12 months after stroke, aphasia, multiple hospitalizations for rehabilitation, right-sided hemianopsia, and other neurological lesions.

After excluding patients according to the criteria, the final sample consisted of 38 patients (25 males; 13 females; mean age 59.7 ± 12.7 years). The characteristics of the sample are presented in Table 1. The neglect syndrome and its severity were diagnosed by a neuropsychologist during a classical neuropsychological examination. According to the neuropsychologist conclusion regarding the patients without a neglect syndrome (in six out of 38 patients, the syndrome severity had not been detected), an assumption was made about its presence or the presence of other visuospatial disorders. Based on the calculated proportion of patients with the diagnosed neglect syndrome, all the examined patients were divided into two groups: those with a neglect syndrome H+ (32 people) and those without a neglect syndrome H– (six people). Decreased vision was determined according to objective ophthalmological examination data and/or subjective complaints of the patients.

RESEARCH DESIGN

A number of conventional neuropsychological tests were used to diagnose neglect syndrome, as well as a search task using the eye tracking method. For performing pencil-and-paper tests (Albert's test, Balls test, Line Bisection test), pencil and paper were used, while the patient was seated at a table, and the middle line of his body correlated with the middle line of the sheet.

Albert's test (AT) — the test of crossing out short lines, the maximum score is 41 [12]. The neglect syndrome is diagnosed with more than 70% of the left missed lines from the total number of missed lines.

Bells test (BT) — the maximum score in this test is 35; less than 29 points indicate the presence of neglect syndrome [13]. The sum of the crossed-out points in the left, center, and right parts of the form is estimated using a template.

Line bisection test (LBS) — the test divides straight lines in half; the author of the test recommended considering dividing only the left lines [14].

The test results were calculated using the following formula:

Percentage of deviation = (patient's mark is the mark of the true center) / (mark of the true center) × 100%.

The percentage of deviation from the true "0" on the left side will have a negative sign, on the right — a positive sign. If the value averaged over all left lines is >7%, the neglect syndrome is diagnosed (according to the norm established in the domestic neurotypical sample: *N* = 38 people, average age 49.8 ± 12.1 years; the calculated threshold for determining neglect is 7%). The higher the percentage, the more pronounced the neglect severity.

Apple test (ApT) — the test of crossing out closed (whole) circles [15]. In this study, a computerized version was used, containing 90 circles, 30 of each type (closed, unclosed on the left, unclosed on the right) The test allows the researcher to identify the neglect syndrome and determine its type: allocentric (allo) or egocentric (ego). The neglect syndrome is confirmed in the presence of three or more common errors (according to W.H. Jang, with modifications) [16]. To determine the egocentric type of neglect (ignoring one side of the space relative to one's own body), the difference between the right and left half of the screen in uncrossed whole circles was taken into account, which should be at least one unit. For an allocentric neglect (ignoring one side of objects, regardless of their location in space relative to the body), the difference between crossed-out

Table 1. Integral characteristics of the final sample of patients

Sign	Sign gradation	Patient number, *N*
Stroke type	ischemic hemorrhagic	28 (74%) 10 (28%)
Stroke focus localization	RMCA RH VBS	23 (61%) 13 (34%) 2 (5%)
Neglect syndrome	present absent	32 (84%) 6 (16%)
Neglect severity	Unknown mild moderate moderate-severe severe	6 (16%) 8 (21%) 15 (39%) 5 (13%) 4 (11%)
Decreased visual acuity	present absent	18 (47%) 20 (3%)

Table prepared by the authors using their own data

Note: RMCA — right middle cerebral artery; RH — right hemisphere; VBS — vertebrobasilar system.

non-closed circles on the left and on the right was also taken into account, as well as at least one unit. During the test, the patient's head was fixed in the frontal-chin support, which was performed using a computer mouse.

The visual search task was performed by a C-EyePro device, AssisTechSp. (z.o.o., Poland) [17] using the eye tracking technology. Eye tracking is a method for video recording of eye movements using video in infrared light to detect pupil position; the method determines patterns of gaze fixation when viewing a visual scene, as well as calculate quantitative characteristics, including the frequency of fixations and saccades, duration of fixations, amplitude of saccades, etc. The task was to detect a target object (brightness; realistic objects of various shapes, such as a soccer ball, a light bulb, a felt-tip pen, etc.) located in the left or right half-field of vision among a variety of distractors; each of the six samples began with a fixation stimulus from the center. The latency of finding the target object in each sample was recorded. If the patient did not find the object in more than 30 seconds, this sample was not counted.

Statistical data analysis was performed using the JASP 0.18.3 package (JASP Team, the Netherlands). Taking into account the small sample size, nonparametric statistical methods were used for data analysis. The Mann–Whitney nonparametric criterion was used for comparative intergroup analysis, the Spearman criterion for correlation analysis, and the Kruskal–Wallis criterion for analyzing the influence of clinical factors. Differences with a statistical significance level of less than 0.05 were considered significant.

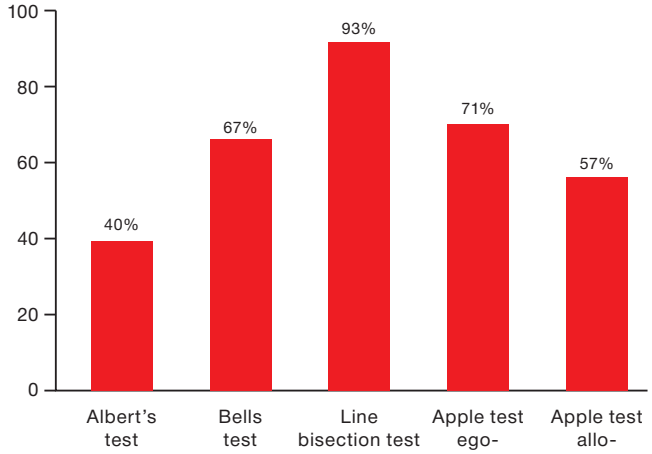

Figure prepared by the authors

Fig. 1. Proportion of patients with diagnosed neglect syndrome according to the threshold value of each neuropsychological test

RESULTS

Neglect syndrome diagnostics by neuropsychological tests

The study determined the proportion of patients who showed the signs of neglect syndrome according to the thresholds of each neuropsychological test; the corresponding data are shown in Fig. 1. Thus, 35 (93%) patients were diagnosed with neglect syndrome based on the LBS test results, compared to the AT technique — 15 (40%) patients, the Bells test method — 25 (67%) patients, the Apple test ego (ApTego) — 27 (71%) patients, and Apple test allo (ApTallo) — 22 (57%) patients.

According to the calculated proportions, groups of patients with and without neglect syndrome were formed, and intergroup comparisons of the results of each neuropsychological test were conducted (Table 2, Fig. 2), with the exception of LBS, due to the small number of patients without neglect.

When analyzing the differences between the two groups relative to the thresholds in patients with (H+), a statistically significant deterioration in test performance was found in the score: AT by 32%, BT by 56%, ApTego by 75%, ApTallo by 8.3%. At the same time, patients performed LBS test tasks less accurately by 33.5% compared to the results of patients in the (H–) group.

Intergroup comparisons show that patients with neglect syndrome perform statistically significantly worse on all tests (score lower in AT and BT, crossing out fewer objects; score higher in ApT, leaving more objects uncrossed) than patients without neglect.

Diagnosis of visual attention by eye tracking

To identify differences in the perception of stimuli in the left and right half-fields in all patients, an analysis of the latency of finding stimuli, averaged over all samples for each side, was performed. The results showed that patients found stimuli in the right half-field much faster, requiring an average of 1.43 s compared to 15.37 s to find a stimulus in the left half-field ($p < 0.001$).

Comparison of the results of neuropsychological tests and eye tracking diagnostics

To identify associations between the results obtained using neuropsychological tests and the average latency of finding the target stimulus in the search task on the

Table 2. Intergroup comparisons of patients without (H-) and patients with (H+) neglect on each of the neuropsychological tests

Parameter	Albert's test, scores		Bells test, scores		Line bisection test, %		Apple test, scores			
							ego-		allo-	
	H- (*n* = 23)	H+ (*n* = 15)	H- (*n* = 13)	H+ (*n* = 25)	H- (*n* = 3)	H+ (*n* = 35)	H- (*n* = 11)	H+ (*n* = 27)	H- (*n* = 16)	H+ (*n* = 22)
Me [Q1; Q3]	41 [41; 41]	28 [21; 33]	31 [30; 32]	11.5 [9; 22]	–9 [–11; –7]	42.5 [20; 55]	0 [0; 0]	7.5 [3; 20]	0 [0; 0]	2.5 [1; 7]
p Mann-Whitney	<0.001		<0.001		–		<0.001		<0.001	

Table prepared by the authors using their own data

Note: the data is presented as the median *Me* [*Q*1; *Q*3]; "–" could not be performed due to the small number of patients without neglect.

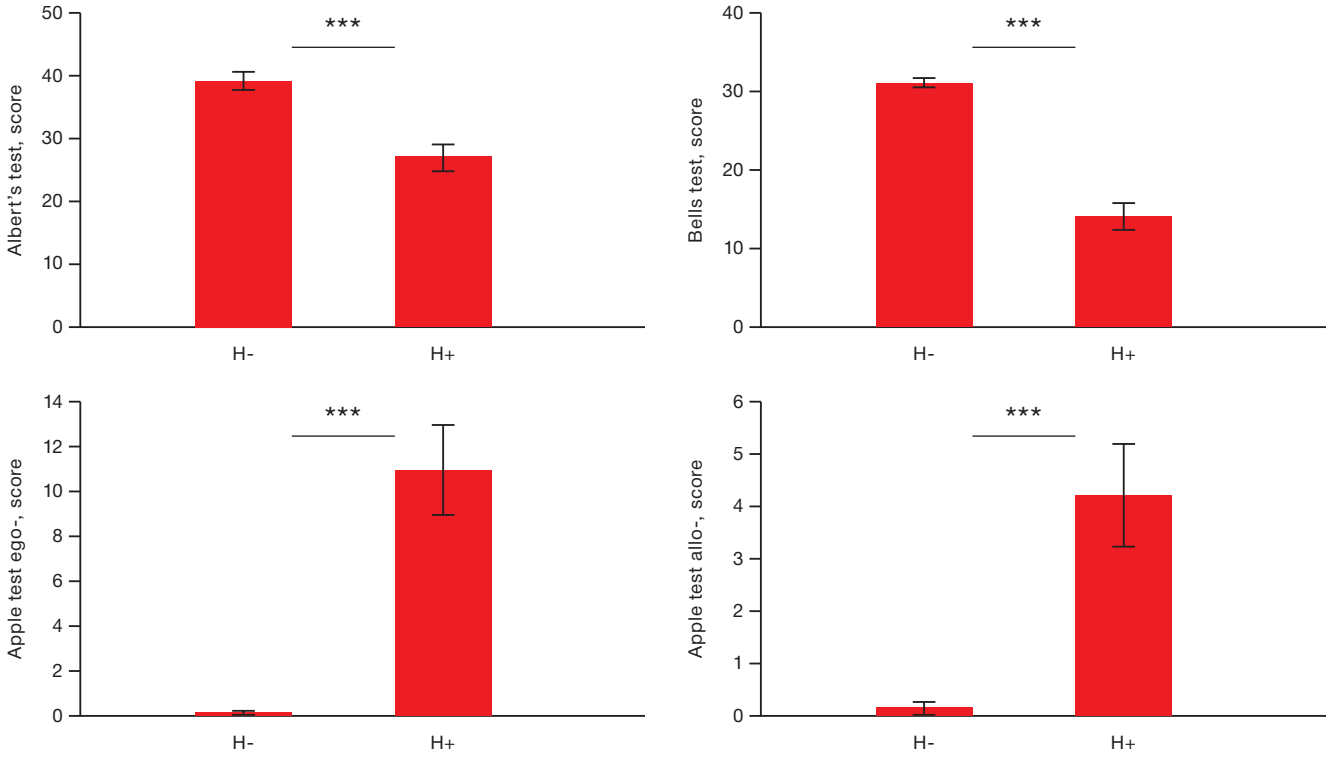

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Fig. 2. Intergroup comparisons of patients without (H–) and patients with (H+) neglect on each of the neuropsychological tests
Note: the data is presented as the average value and the error of the average value $M \pm m$; *** — $p < 0.001$ is the level of statistical significance according to the Mann–Whitney test.

tracker, Spearman's rank correlation (r_s) analysis was performed; the corresponding data are presented in Table 3.

Significant correlations were found between the results obtained on the tracker, BT, and AT, Thus, the worse patients coped with these tests, the slower they managed to find objects on the left on the eye tracker. Interestingly, the results of performing ApT with the detection of an allocentric defect have a weak correlation with the tendency to reliability only with the results of eye tracker diagnostics ($r_s = 0.370$; $p = 0.090$).

Influence of clinical factors on the results of neuropsychological tests and eye tracking

It was found that patients with ischemic stroke performed worse than those with hemorrhagic stroke AT ($H_{(1.37)} = 6.82$, $p = 0.009$), and made more mistakes when performing LBS ($H_{(1.27)} = 4.61$, $p = 0.043$); in other tests, the influence of the factor was not detected.

The more pronounced the severity of the defect in the patient, the worse the AT ($H_{(4.31)} = 7.27$, $p = 0.004$), LBS ($H_{(4.27)} = 2.79$, $p = 0.05$), ApTego- ($H_{(4.27)} = 3.09$, $p = 0.036$) were performed. In BT, a similar dynamics was observed,

Table 3. Correlation analysis of the results of neuropsychological tests and eye tracking diagnostics

Tests name	Albert's test	Bells test	Line bisection test	Apple test ego-	Apple test allo-
Albert's test					
Bells test	0.735 $p < 0.001$				
Line bisection test	–0.701 $p < 0.001$	–0.537 $p = 0.004$			
Apple test ego-	–0.526 $p = 0.004$	–0.647 $p < 0.001$	0.538 $p = 0.010$		
Apple test allo-	–0.003 $p = 0.986$	0.114 $p = 0.564$	0.131 $p = 0.563$	0.024 $p = 0.904$	
Visual search on the eye tracker	–0.422 $p = 0.025$	–0.613 $p < 0.001$	0.313 $p = 0.166$	0.370 $p = 0.090$	0.392 $p = 0.072$

Table prepared by the authors using their own data

Note: Spearman correlation coefficients (r_s) are presented; p is the level of statistical significance.

although insignificant ($H_{(4.31)}$ = 4.25, p = 0.333); the corresponding data are shown in Fig. 3.

The visual impairment factor had a significant effect when performing LBS tasks ($H_{(1.27)}$ = 6.11, p = 0.02), at the level of statistical significance trends when performing AT ($H_{(1.37)}$ = 3.86, p = 0.057) and the eye tracker search task ($H_{(1.28)}$ = 3.33, p = 0.07). In other words, patients with a decreased vision performed worse on these tests.

The time elapsed after the stroke had no effect on the results of any tests.

DISCUSSION

In this study, we evaluated the diagnostic capabilities of classical neuropsychological tests (AT, BT, ApT, and LBS) and the eye tracking method for detecting neglect syndrome in stroke patients. A number of studies showed that detection of neglect syndrome should rely on the use of several different tests (crossing out, dividing lines in half, hardware and computerized methods) [1], which relates to the heterogeneity of the manifestation of this syndrome [18]. We determined a different level of sensitivity of these tests: thus, AT detected neglect syndrome only in 40% of patients in our sample, while the detection sensitivity of LBS was 93%.

Our results demonstrate that the determination of the neglect syndrome according to the established thresholds of each of the neuropsychological tests allows, including from a statistical point of view, the groups of patients with and without neglect to be reliably differentiated.

One of the most interesting results to consider turned out the highest sensitivity of LBS in detecting neglect syndrome in our sample. The LBS form was presented vertically, while all other cancellation tests were presented horizontally. The patients needed to assess the middle of the line and put a vertical mark. A classic sign of neglect syndrome is considered to be a disorder of spatial perception along the lateral (left-right) axis, and deletion tests are aimed at identifying this spatial disregard. However, a number of studies are aimed at studying the perception of the vertical axis based on visual, postural, and tactile information in patients with neglect syndrome [9, 19, 20]. A stroke can affect two separate but adjacent neural networks, one of which encodes spatial information for the horizontal axis, and the other — for the vertical [19].

The systematic review reported in [21] showed that patients with neglect syndrome exhibit more pronounced vertical deviations from the standard and have an unstable body position in an upright position in the first 3–6 months after the stroke compared with patients without this syndrome. The majority of the papers included in this review reported that, in participants with neglect, the ratio of the direction of inclination of the vertical line relative to the reference mark was opposite to the side of the brain lesion (i.e., when assessing the true visual vertical, the patient's mark was tilted to the left with injuries in the right hemisphere).

When performing the bisection test, patients rely on visual-vestibular information about the vertical position of the cancellation tests and the vertical mark of the middle of the line. As shown in the studies described above, their subjective sense of vertical is shifted to the left; therefore, they perceive the task form positioned vertically to be shifted to the left relative to the real vertical. As a result, the right part of the space expands in front of patients, and they perceive

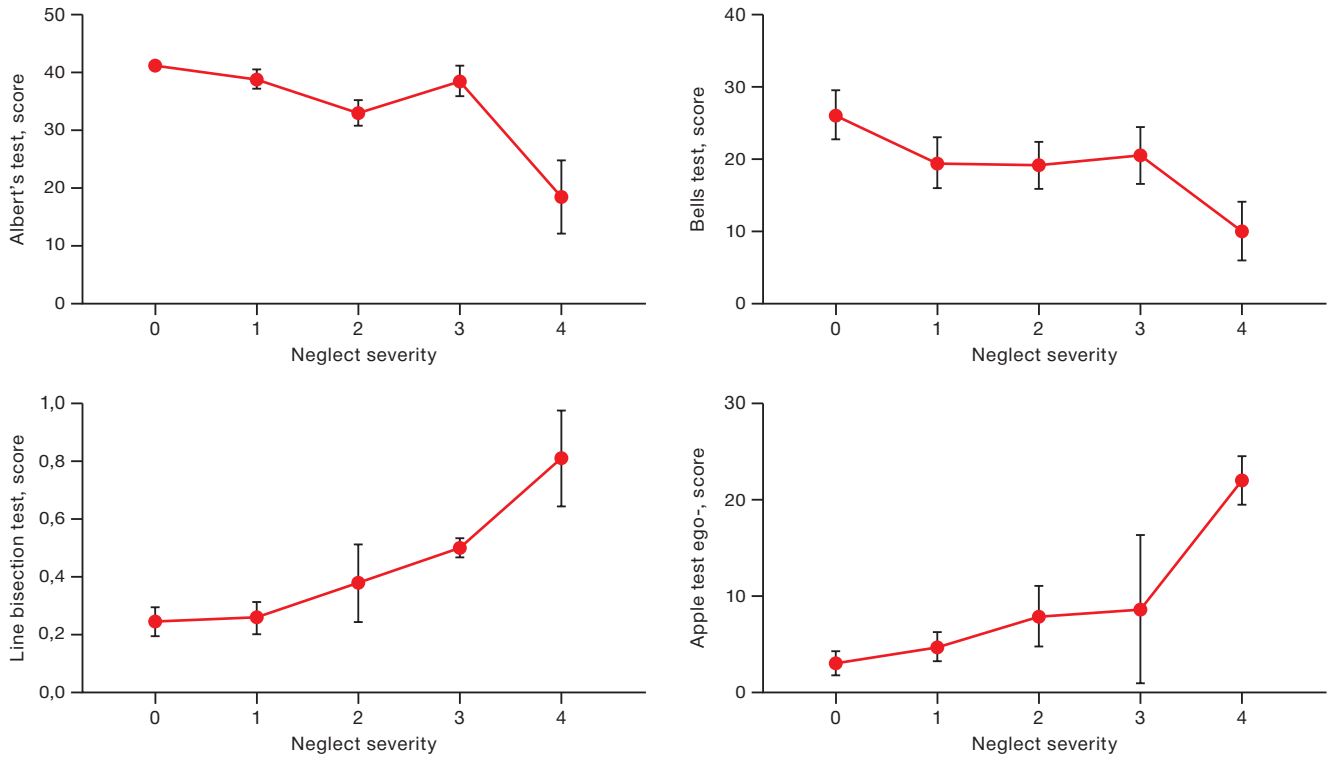

Figure prepared by the authors using their own data

Fig. 3. Dependence of the results of neuropsychological tests on the neglect severity
Note: the data are presented in the form of an average value and an error of the average value $M \pm m$; the abscissa scale is the degree of severity of the defect: 0 — absent; 1 — mild, 2 — moderate, 3 — moderate-severe, 4 — severe.

the right part of the lines, ignoring the left part. On this basis, patients mark the center to the right of the true center.

An important aspect of the high sensitivity of LBS is the absence of distractors and features of the stimulus: size, orientation, and quantity on the sheet.

Despite the relatively low sensitivity of the strikeout tests in our study, they revealed the difficulties of patients in finding objects on the left. This correlates with the results of visual search using the eye tracking technique, where BT and AT demonstrated significant correlations with the search task. Patients with a severe attention deficit on the left, found in cancellation tests, spend more time searching for stimuli on the tracker. The high reliable latency of searching for objects on the left side compared to the right, revealed on the eye tracker, confirms the presence of pronounced oculomotor scanning anomalies noted in previous studies [11].

Allocentric neglect is one of the manifestations of neglect syndrome, in which the patient does not perceive the contralateral side relative to the midline of the perceived object [7]. In our study, the results of patients in AT, demonstrating the presence of an allocentric neglect at a level close to statistically significant, correlate with the time spent searching for objects in a visual search task using an eye tracker. This may indicate that due to the established allocentric neglect, it took patients longer to analyze the stimulus space when searching for a target object, since they saw only half of each object, which made the process of identification more difficult.

The nature of the stroke is a factor influencing the performance of neuropsychological tests and the search task on the tracker. According to [22], the proportion of patients with ischemic stroke (IS) and hemorrhagic stroke (HS) is about 70–75% and 15%, respectively. In our sample, IS accounted for 73.6% of the cases. IS is characterized by localization of the lesion in the area of blood supply to the SMA, which is responsible for the blood supply to 2/3 of the outer surface of the hemispheres: the main part of the cortex of the frontal, parietal, and temporal lobes. These areas are part of the system related to human orientation to external stimuli (dorsal and ventral attention networks) [23]. Indeed, the neglect syndrome in IS is more frequent and more pronounced than in HS, which is explained by IS affecting the key brain structures responsible for attention and perception of space.

During the analysis of the data, we noted a significant influence of the factor of vision loss on the performance of LBS and the search task on the eye tracker, while its influence on the rest of the cancellation tests was recorded at the trend level. The finding requires a more detailed study due to the variability of the causes of visual impairment, taking into account age and clinical characteristics. When analyzing blank, computerized, and hardware methods, it is worth considering not only the influence of cortical disorders, but also analyzer systems. The study [24] established an association of neglect syndrome with age, the presence of concomitant diseases and deterioration of health before stroke. However, there are no large-scale studies that could offer a reliable understanding of neglect syndrome in clinical practice [24].

In the neuropsychological clinical practice, the following severity degrees of neglect syndrome are distinguished: severe, moderate, and mild. Objective data for differentiating the severity include the latency of searching for numbers in Schulte tables, the ability to notice object images, reading and writing. The level of criticism of the patient's actions also plays an important role in differentiating the neglect severity. The clinical picture of neglect often includes anosognosia syndrome, as a result of which patients are not critical and do not realize their defect, thereby not attempting to explore the contralateral lesion space [25]. Therefore, we compared the neuropsychologist conclusions with sensitized tests for neglect. As a result of the data analysis, a significant relationship was observed between the performance of AT, ApT, and LBS (not significant for BT, but direct) and the neglect severity: the worse the patients performed the tests, the more pronounced the neglect severity was according to objective neuropsychological examination data. These findings form the basis for studying the sensitivity of ApT and LBS in neglect syndrome of various severity and establishing their quantitative thresholds in larger cohorts of patients.

CONCLUSION

Neglect syndrome is a common visuospatial disorder in stroke patients, which has a negative impact on the rehabilitation process, subsequent recovery, and return to normal life. Our study has shown the importance of combining standard neuropsychological tests with the eye-tracking method for diagnosing neglect syndrome in stroke patients. Eye tracking has shown promise for determining the presence of neglect syndrome, which warrants further studies.

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THE ROLE OF THE FIBRINOLYTIC SYSTEM CHANGES IN THE DEVELOPMENT OF POST-STROKE COGNITIVE IMPAIRMENT

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Introduction. Post-stroke cognitive impairment (PSCI) is an important medical and social problem. Its prevalence after a stroke reaches 74%.

Objective. To study the relationship between integral parameters characterizing the processes of coagulation and fibrinolysis, assessed using dynamic thrombophotometry, and PSCI development to evaluate the possibility of predicting PSCI and unfavorable disease outcomes.

Materials and methods. The study included 35 patients who had suffered an ischemic stroke within 24 hours: 20 (57.1%) women and 15 (42.9%) men; the median age was 66.5 [62.3–73.3] years. The comparison group consisted of 45 conditionally healthy volunteers. Assessment of the state of the hemostasis system was carried out upon admission to the hospital, on days 6–8 and 13–15. Integral parameters evaluating the coagulation, coagulation and fibrinolysis systems and hemostasis in general were calculated using the "Fibrinodynamics" method. Cognitive functions were assessed on days 10–14 using to the Montreal Cognitive Assessment (MoCA) scale. The functional outcome of the disease was evaluated using the Modified Rankin Scale (mRS) on day 28. The SPSS 27.0 software (IBM, USA) was used for statistical analysis. Associations between continuous data were evaluated using the Spearman correlation coefficient, univariate and multivariate linear regression models. The difference at the level of $p < 0.05$ was considered statistically significant.

Results. An inverse correlation was found between the average brightness of the clot during the integral modeling of hemostasis processes at admission and the level of cognitive dysfunction on the MoCA scale ($r_s = -0.409$; $p = 0.02$); higher baseline HB values were associated with severe post-stroke cognitive impairment. On the contrary, there was a direct relationship between the initial fibrinolysis of the resulting clot (FB) and cognitive impairment on the MoCA scale ($r = 0.512$, $p = 0.003$); higher values of FB corresponded to a higher score on the MoCA scale and a higher level of cognitive functions.

Conclusions. In predictive multivariate linear regression models that included age and baseline stroke severity, it was found that every 11.5 arbitrary units increase in baseline HB or 9.8 arbitrary units decrease in baseline FB corresponded to a -1 point deterioration in cognitive status when assessed by MoCA. Patients with high baseline values of FB had more favorable functional outcomes of the disease assessed using the mRS. The use of extended dynamic thrombophotometry makes it possible to comprehensively assess changes in the hemostasis system in patients with ischemic stroke. Higher HB values and lower FB values make it possible to predict an unfavorable outcome of the disease and more severe PSCI in the early stages, while the hypoactivation of the fibrinolytic system is associated with a greater severity of PSCI and a less favorable functional outcome.

Keywords: ischemic stroke; cerebrovascular diseases; hemostasis; fibrinolytic system; cognitive impairment; dynamic thrombophotometry; fibrinodynamics

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РОЛЬ ИЗМЕНЕНИЙ СИСТЕМЫ ФИБРИНОЛИЗА В РАЗВИТИИ ПОСТИНСУЛЬТНЫХ КОГНИТИВНЫХ НАРУШЕНИЙ

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Введение. Постинсультные когнитивные нарушения (ПИКН) представляют собой важную медицинскую и социальную проблему. Их распространенность после перенесенного инсульта достигает 74%.

Цель. Изучение взаимосвязи между интегральными параметрами, характеризующими процессы коагуляции и фибринолиза и определяемыми при помощи динамической тромбофотометрии, и формированием ПИКН для оценки возможности прогнозирования ПИКН и неблагоприятных исходов заболевания.

Материалы и методы. В исследование были включены 35 пациентов, перенесших ишемический инсульт в срок до 24 ч от начала заболевания: 20 (57,1%) женщин и 15 (42,9%) мужчин; медианный возраст 66,5 [62,3–73,3] года. Группу сравнения составили 45 условно здоровых добровольцев. Оценка состояния системы гемостаза проведена при поступлении в стационар, на 6–8 и 13–15-е сут. Исследовали интегральные показатели, оценивающие системы коагуляции, фибринолиза и гемостаза в целом с помощью метода «Фибринодинамика». Когнитивные функции оценивали на 10–14-е сут по Монреальской шкале оценки когнитивных функций (МоСА). Функциональный исход заболевания определяли по Модифицированной шкале Рэнкина (МШР) на 28-е сут. Для статистического анализа использовали программное обеспечение SPSS 27.0 (IBM, США). Ассоциации между непрерывными данными оценивали при помощи коэффициента корреляции Спирмена, одномерных и многомерных линейных регрессионных моделей. Статистически значимыми считали различие при уровне $p \leq 0,05$.

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Результаты. Выявлена обратная корреляционная связь средней яркости сгустка при интегральном моделировании (НВ) процессов гемостаза при поступлении и уровнем когнитивной дисфункции по шкале МоСА (r_s = -0,409; p = 0,02); более высокие исходные значения НВ были ассоциированы с выраженными постинсультными когнитивными нарушениями. Напротив, отмечалась прямая связь исходного процесса фибринолиза образующегося сгустка (FB) и когнитивных нарушений по шкале МоСА (r = 0,512, p = 0,003); более высоким значениям FB соответствовала большая оценка по шкале МоСА и более высокий уровень когнитивных функций.

Выводы. В прогностических многомерных линейных регрессионных моделях, включавших возраст и исходную тяжесть инсульта, установлено, что каждые 11,5 усл. ед. увеличения исходной НВ или 9,8 усл. ед. снижения исходной FB соответствовали ухудшению когнитивного статуса при его оценке по МоСА на 1 балл. Пациенты с высокими исходными значениями FB имели более благоприятные функциональные исходы заболевания по МШР. Применение расширенной динамической тромбофотометрии позволяет комплексно оценивать сдвиги системы гемостаза у пациентов с ишемическим инсультом. Более высокие значения НВ и более низкие значения FB дают возможность прогнозировать неблагоприятный исход заболевания и более тяжелые ПИКН на ранних этапах, в то время как гипоактивация фибринолитической системы ассоциирована с большей тяжестью ПИКН и менее благоприятным функциональным исходом.

Ключевые слова: ишемический инсульт; цереброваскулярные болезни; гемостаз; система фибринолиза; когнитивные нарушения; динамическая тромбофотометрия; фибринодинамика

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INTRODUCTION

Stroke is the leading cause of disability worldwide [1]. The modern development of reperfusion technologies in the treatment of ischemic stroke has significantly improved the outcomes of the disease, at the same time, post-stroke cognitive impairment (PSCI) and especially post-stroke dementia (PSD) are still significant conditions that significantly worsen the quality of life of both patients and their relatives [2]. The PSCI prevalence, according to various sources, ranges 4.4–73% [3]. It should be noted that PSCI is an unfavorable outcome of not only ischemic, but also hemorrhagic stroke [4]. As a rule, the development of a persistent symptom complex peaks in the early recovery period, i.e. 3–6 months after a stroke. In addition to the characteristic disorders of higher cortical functions (aphasic disorders, memory disorders, agnosia, and apraxia) directly associated with damage to certain areas of the brain, patients with PSCI develop symptoms typical of vascular cognitive impairment (CI): impaired attention and regulatory functions [3].

Acute cerebral ischemia is a complex cascade consisting of many pathophysiological links, including prothrombotic and proinflammatory activation at both systemic and local levels. On the one hand, thrombosis of large and small cerebral vessels leads to varying degrees of ischemic damage to brain matter, on the other hand, in parallel with prothrombotic processes, excessive release of proinflammatory cytokines and leukocyte infiltration of ischemic brain areas occur [5]. The relationship between thrombosis and inflammation is commonly referred to as thromboinflammation, and, according to some researchers, is one of the causes of persistent post-stroke disorders, including PSCI [6, 7].

Dynamic thrombophotometry is a new method for assessing the state of the hemostasis system, which makes it possible to simulate and register in vitro processes of fibrin clot induced by tissue growth factor under conditions close to physiological conditions. Of particular interest is the modification of the method with the possibility of simultaneous modeling of the processes of fibrin clot growth and lysis, followed by the calculation of the values of a number of integral parameters, “Fibrinodynamics” (FD) [8]. This method allows us to evaluate not only the activation of coagulation and fibrinolysis systems, but also the shift in the balance between them towards thrombosis or hypocoagulation. In addition, this method allows us to determine the indirect contribution of additional factors, such as inflammation and endothelial dysfunction, to the overall balance of the hemostatic system within the framework of the thromboinflammation-immunothrombosis concept [9].

As part of the previous stage of our research, we studied the state of the coagulation link of hemostasis in patients with ischemic stroke (IS) using dynamic thrombophotometry. It was noted that the increase in the optical density of the fibrin clot and its high values can be considered as a possible prognostically significant biomarker of the early development of PSCI [10]. Currently, the study of promising areas for the use of dynamic thrombophotometry, in particular, its modification of FD in predicting the early manifestations of PSCI, is continuing.

The aim of the study was to study the relationship between the integral parameters characterizing the processes of coagulation and fibrinolysis, calculated using dynamic

thrombophotometry, and the PSCI development to assess the possibility of predicting PSCI and unfavorable disease outcomes.

MATERIALS AND METHODS

A non-interventional study of the integral parameters of coagulation and fibrinolysis systems and hemostasis in general was conducted at the clinical facilities of the Department of Neurology, Neurosurgery, and Medical Genetics (Faculty of General Medicine, Pirogov Russian National Research Medical University). The main group consisted of 35 patients who suffered an ischemic stroke within 24 h: 20 (57.1%) women and 15 (42.9%) men. The median age was 66.5 [62.3–73.3] years.

As a comparison group, a database of laboratory test results was formed for 45 conditionally healthy volunteers: 20 (44.4%) men and 25 (55.6%) women, with a median age of 31.0 [23.5–44.5] years. The comparison group was formed because of the pilot nature of this study, and of the need to compare the results obtained with conditionally physiologically normal values in people from the general population who do not suffer from chronic diseases. The inclusion of volunteer patients in the database was carried out in accordance with the principles of good clinical practice and the signing of informed voluntary consent to take venous blood samples and conduct laboratory tests.

The criteria for inclusion in the main group were: a diagnosis of ischemic stroke confirmed in accordance with the requirements of the Russian ischemic stroke guidelines[1], admission to the hospital within 24 h of the onset of the disease, age ≥40 years (for patients aged 40–59 years, the presence of at least one confirmed risk factor for ischemic stroke in the anamnesis), score <10 on the National Institutes of Health Stroke Scale (NIHSS), which is used to assess the neurological status, localization of stroke, differential diagnosis and treatment outcomes, planning thrombolytic therapy and monitoring its effectiveness. The NIHSS, translated in the current Russian ischemic stroke guidelines[2], includes a number of parameters reflecting the levels of impairment due to acute cerebrovascular disease: the level of consciousness — the level of wakefulness, eyeballs movements, visual fields examination, determination of the functional state of the facial nerve, assessment of the motor function of the upper extremities, assessment of movements coordination, sensitivity testing, identification of speech disorders, detection of perception disorders — hemi-ignoring or neglect, as well as an approximate prognosis of the disease. The criteria for inclusion in the study were the absence of speech and motor disorders that prevent the objectification of the cognitive status of patients, consent to participate in the study, and the absence of anamnestic signs of pre-stroke disorders.

The criteria for non-inclusion were as follows: admission to the hospital later then 24 h of the onset of the disease, regression of neurological symptoms with a confirmed transient ischemic attack, ischemic stroke of other specified etiology (migrainous, hemodynamic, etc.), the presence of oncological, terminal, somatic or other diseases that cause significant changes in hemostasis (including thrombophilia, hemophilia, Disseminated intravascular coagulation (DIC) syndrome, sepsis, etc.), pregnancy, refusal to participate in research work.

Venous blood samples obtained in accordance with the regulations established by the Russian ischemic stroke guidelines were used to study laboratory parameters[3].

Hemostasis parameters were assessed in citrated peripheral venous blood samples in a ratio of 1:10. Two-stage centrifugation was performed to obtain platelet-free plasma (15 min at a relative centrifugal acceleration of 1,500 g and 5 minutes at 10,000 g) using CM-6M centrifuge (ELMI, Latvia) and Microspin 12 centrifuge (Biosan, Latvia). To assess the state of the hemostasis system, the Thrombodynamics T-2 analyser system (Hemacore, Russia) was used. An extended technique was used with simultaneous modeling of coagulation and fibrinolysis processes [8]. Based on the results of the study, the parameters characterizing the functioning of coagulation, fibrinolysis, and hemostasis systems were calculated using the formula:

$$FB = CB - HB, \quad (1)$$

CB (coagulation brightness, arbitrary units) — the average brightness of a clot when modeling the coagulation process;
HB (hemostasis brightness, arbitrary units) — the average brightness of a clot in the integrated modeling of hemostasis processes;
FB (fibrinolysis brightness, arbitrary units) is a parameter that characterizes the activity of the fibrinolysis process of the growing clot.

In the main group, the hemostasis system was assessed upon admission to the hospital, on days 6–8 and days 13–15. In addition, a standard clinical, instrumental, and laboratory examination was performed at the indicated time intervals, and the severity of neurological deficit was assessed on the NIHSS. The patients' cognitive functions were assessed on days 10–14 using the Montreal Cognitive Assessment (MoCA) scale [11]. The functional outcome was evaluated on day 28 using a Modified Rankine Scale (mRS), which allows a complex assessment of both post-stroke disability and death [12].

The baseline characteristics of the patients in the main group are presented in the previous part of the work [10]. Arterial hypertension (any stage) was observed in 27 (77.1%) patients, type 2 diabetes mellitus — in 11 (31.4%), atrial fibrillation (any form) — in 9 (25.7%), stenosis of the affected artery over 50% of the lumen — in 14 (40.0%). The NIHSS score upon admission was 5 points [4-8]. The characteristics of the patients, as well as the distribution of ischemic stroke subtypes, were comparable to domestic and foreign literature data.

In the comparison group, the laboratory test was performed once.

[1] Clinical guidelines «Ischemic stroke and transient ischemic attack in adults». Ministry of Health of the Russian Federation; 2021
[2] Ibid.
[3] Ibid.

The SPSS 27.0 software (IBM, USA) was used for statistical analysis. The final sample size was calculated based on the results of an interim statistical analysis of the data (statistical power of 0.95, α value of 0.05). The data normality was tested using the Shapiro-Wilk test. Extreme data values, if any, were excluded from the calculation. The numerical data in the paper is presented as a median with upper and lower quartiles. The Mann-Whitney test in independent samples and the Wilcoxon test in dependent samples were used to compare variables that did not follow a normal distribution. Associations between continuous data were evaluated using the Spearman correlation coefficient, univariate and multivariate linear regression models.

RESULTS AND DISCUSSION

The results of the study of integral parameters of coagulation and fibrinolysis at different time intervals in patients from the main group were compared with the data of conditionally healthy volunteers; the corresponding parameters are shown in the table. Conclusions about the absence of concomitant hematological diseases causing a significant shift in coagulological indications were drawn based on the absence of deviations in the admission of standard coagulography parameters.

In the main group, upon admission, the values of the integral parameters CB and HB were comparable with the group of conditionally healthy volunteers, which confirmed the similar activity of the coagulation system and the similar functional state of the fibrinolysis system in both groups (Table). At the same time, patients with ischemic stroke showed a tendency to hypofibrinolysis (lower values of the FB parameter) of 18.3 [10.0–31.9] versus 25.3 [21.7–29.9] arbitrary units upon admission (p = 0.068) when comparing the results of volunteers from the comparison group. This characterized the general prothrombogenic state in ischemic stroke with greater resistance of fibrin clots to the fibrinolysis process. The statistical significance of the differences in these parameters was not achieved due to the presence of several conditionally healthy volunteers with extremely low FB values in the comparison group.

By the end of 1st week, patients with ischemic stroke showed a dramatic statistically significant drop in CB, HB, and FB values to 1.3%, 1.2%, and 1.6% of baseline values, respectively, with a shift towards hypocoagulation relative to the comparison group, which may be due to the initiation of antithrombotic therapy after admission. Overall, the shift in all parameters was possibly due to the following explanation. The method used in this work studies the growth of a fibrin clot from a surface covered with a tissue factor, and therefore active antithrombotic therapy led to a corresponding shift in all parameters. By days 13–15, the main group of patients showed a gradual recovery in the overall activity of the coagulation system, but the values remained lower than in the comparison group due to continued antithrombotic therapy. Taking into account the corresponding bias, the parameters of patients from the main group on days 6–8 and 13–15 were not compared with patients from the comparison group.

In the main group of patients, the study did not reveal any associations between the CB index at admission and cognitive status.

In patients with ischemic stroke, the relationship of HB at admission with a score on the MoCA scale was assessed: higher baseline HB values corresponded to a more pronounced post-stroke cognitive decline (r_s = -0.409; p = 0.02); these data are presented in Figure 1. To quantify this association, a simple linear regression model was computed (regression coefficient β = -0.106; 95% CI 0.188–0.024; p = 0.022).

To account for the influence of additional factors (age and initial stroke severity on the NIHSS), a multivariate linear regression model was constructed (regression coefficient β = -0.087; 95% CI from -0.159 to -0.015; p = 0.019). It was found that every 11.5 arbitrary units of increase in the initial HB corresponded to a subsequent deterioration in cognitive status according to MoCA (attention and concentration, control functions, memory, language functions, visual-constructive skills, abstract thinking, counting and orientation) by -1 point. By days 6–8 and 13–15, this association of variables was similar, but did not reach statistical significance.

The association of the initial activity of the fibrinolysis process and cognitive function on the MoCA scale was also evaluated. It was revealed that higher values of FB correspond to a higher score on the MoCA scale and, consequently, a higher level of cognitive functions (r_s = 0.512; p = 0.003); the corresponding data are presented in Figure 2.

To quantify the predictive significance of this relationship in detail, a simple linear regression model was constructed (regression coefficient β = 0.121; 95% CI 0.018–0.223; p = 0.022).

In order to account the influence of age and the initial severity of focal neurological symptoms on the development of cognitive impairment subsequently, a multivariate model was computed (regression coefficient β = 0.102; 95% CI 0.014–0.190; p = 0.025). It was found that every 9.8

Table. Dynamics of parameters of the hemostasis system in the main group and the comparison group

Parameter	Main group (n = 35)			Comparison group (n = 45)
	On admission	Days 6–8	Days 13–15	
CB, arbitrary units	64.0 [42.4–72.8]	0.8 [0.1–7.5]*	14.9 [9.1–22.7]*	63.3 [53.7–73.6]
HB, arbitrary units	33.9 [24.8–51.4]	0.4 [0.1–3.0]*	8.4 [6.3–7.6]*	37.4 [31.0–49.4]
FB, arbitrary units	18.3 [10.0–31.9]	0.3 [0–3.6]*	5.6 [3.4–7.6]*	25.3 [21.7–29.9]

The table is prepared by the authors using their own data

Note: * — p < 0.001 the level of statistical significance compared to the values at admission (Wilcoxon criterion).

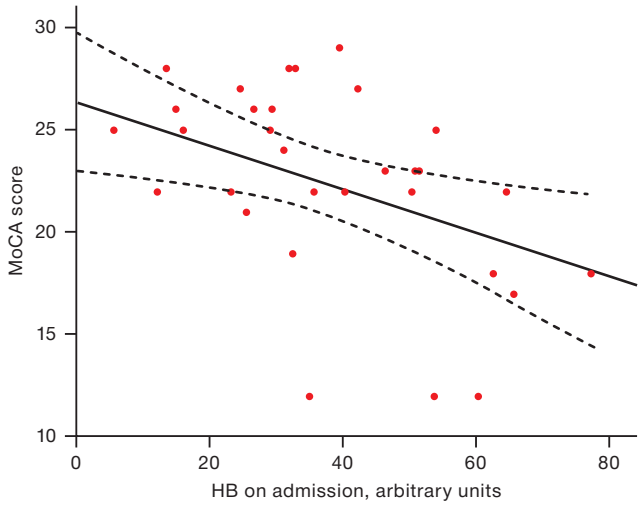

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Fig. 1. Association between the HB (integral assessment of hemostasis system activity) and the MoCA score

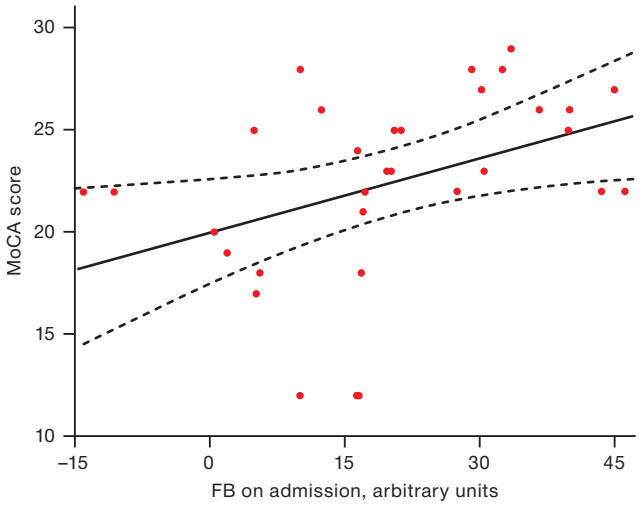

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Fig. 2. Association between FB (fibrinolysis integral assessment) and cognitive status evaluated by the MoCA scale

arbitrary units of the initial FB shift towards hypofibrinolysis corresponded to a -1 point deterioration in cognitive status when assessed by MoCA. This relationship persisted by days 6–8 and 13–15 of follow-up, but did not reach statistical significance.

Notably, when comparing participants with mRS scores of 0–1 and participants with mRS scores of 2–6, ischemic stroke patients (n = 17) with excessive FB values, indicative of elevated fibrinolytic system activity, had more favorable functional outcomes at discharge (21.2 [16.6–35.0] and 16.2 [1.9–27.4] arbitrary units, respectively; p = 0.033) (Fig. 3).

On the first day in patients with ischemic stroke, there were no significant signs of a major shift in the activity of the coagulation and fibrinolytic processes of the hemostasis system beyond the “hematological norm”. At the same time, there was a tendency to lower FB values relative to the comparison group, which may indicate a relative hypofibrinolytic state in acute ischemic stroke [13, 14]. It was found that higher values of the patient’s HB on the first day of stroke are potentially associated with a greater severity of cognitive impairment when assessed using the MoCA scale. On the contrary, higher baseline values of the patients’ FB, characteristic of relative hypofibrinolysis, are associated with a smaller cognitive defect later, as well as with a more favorable functional outcome classified using the modified Rankine scale. Comparable results indicating the importance of hypercoagulation conditions in acute vascular diseases are also presented in coronary heart disease [15].

Considering the above, it can be assumed that an important contribution to the development of PSCI is made not only by damage to a certain volume of brain matter as a result of critical ischemia associated with occlusion of the vessel supplying the corresponding zone [16], but also by changes at the level of the microvasculature [17], such as ongoing microthrombosis of small vessels in the hypoperfused region surrounding the infarct core [18, 19].

Patients with more active fibrinolysis had more favorable outcomes. The data obtained in this work correlate with the results of the first part of our study, which

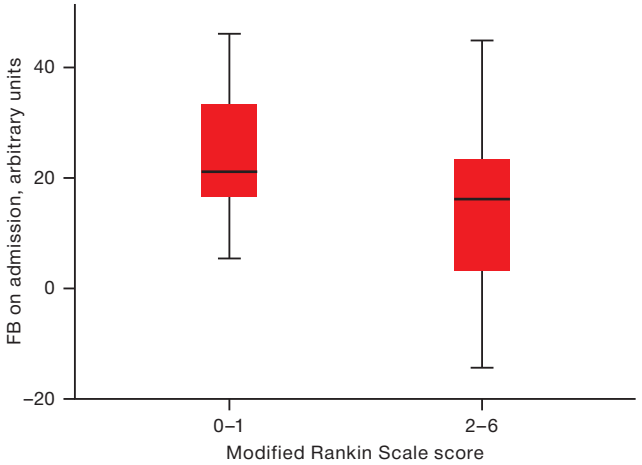

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Fig. 3. Integral assessment of fibrinolysis in different functional outcomes of the disease

showed that a higher optical density of a fibrin clot is associated with lower MoCA scores in such patients [10]. It should be noted that recently a number of works have appeared that show the role of fibrin in triggering thromboinflammation processes. In particular, there is evidence that in coronavirus infection, fibrin binds to viral proteins and forms blood clots that activate a systemic inflammatory response that potentiates further thrombotic complications [20].

CONCLUSION

Post-stroke cognitive impairment, including PSD, is an important medical and social problem. The study of various predictors of the PSCI development in the acute period of stroke is extremely relevant, as this may potentially allow the development of new therapeutic and rehabilitative approaches that may improve the functional outcome in patients with cute cerebrovascular accidents.

A combined laboratory assessment of hypercoagulation and hypofibrinolysis development mechanisms using dynamic thrombophotometry and its extended version of

FD allows for a comprehensive measurement of hemostatic shifts in stroke patients and evaluation of systemic and local effects (i.e., thromboinflammation) cumulative contribution to hemostasis activity.

In this research paper, we outlined fibrin clot optical density changes when modeling fibrinolysis and estimating the HB integral parameter. It was found that an increase in the optical density index of a fibrin clot, which characterizes coagulation activity, is associated with a low score on the MoCA scale.

In predictive multivariate linear regression models that included age and baseline stroke severity, it was shown that every 11.5 arbitrary units increase in baseline HB corresponds to a –1 point deterioration in cognitive status when assessed by MoCA. A 9.8% shift in the initial FB values towards hypofibrinolysis was associated with a deterioration in cognitive status and a -1 shift in the MoCA score. The combination of HB and FB parameters made it possible to predict more severe PSCI.

Thus, the study of the hemostasis system using dynamic thrombophotometry and its extended version of FD seems to be extremely relevant and promising in patients with various vascular diseases. This method makes it possible to better predict the probability of unfavorable stroke outcomes. In addition, changes in fibrinolytic activity and the state of the coagulation system are directly related to the activation of systemic inflammation processes, the assessment of which, in conjunction with the study of dynamic thrombophotometry, is a promising but understudied scientific field.

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HYBRID IMAGING TECHNIQUES IN THE ASSESSMENT OF EPILEPTIC FOCI: A CLINICAL CASE

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Introduction. Localization of an epileptic focus (EF) in patients with pharmacoresistant focal epilepsy can be a challenging task, and the detection of multiple EFs limits the possibilities of surgical treatment. A more accurate description of the structure and metabolism of EFs may provide additional information for managing such patients.

Clinical case description. A 35-year-old patient suffered from pharmacoresistant focal epilepsy, which debuted at the age of 24 with grand mal seizures followed by focal episodes with fading and tonic tension of the left arm. The MRI revealed bilateral mesial temporal sclerosis with more pronounced changes on the left; however, the EEG revealed epileptiform activity in the right temporal region, which created diagnostic difficulties in determining the primary epileptogenic zone. To clarify the location of the lesion, a comprehensive examination was performed, including PET/MRI, which revealed pronounced hypometabolism in the right temporal lobe (with a difference of up to 31% compared with the contralateral side) while maintaining symmetrical accumulation of RPh in the hippocampus. Additionally, diffusion kurtosis imaging (DKI) was performed, which showed a significant decrease in radial and median kurtosis, as well as axonal water fraction in the right temporal lobe (up to 1.33 SD from normal). Invasive EEG monitoring confirmed the bilateral nature of epileptogenicity with a predominance of right-hemisphere seizure initiation.

Conclusions. The combined use of PET/MRI and DKI increases the accuracy of detection of epileptogenic foci in pharmacoresistant epilepsy. Hybrid imaging allows for a comprehensive assessment of metabolic and microstructural changes, which is important for planning surgical treatment. Data integration helps differentiate primary and secondary foci, thus optimizing patient management tactics. The introduction of automated analysis for standardization of diagnostics is promising.

Keywords: focal epilepsy; PET/MRI; diffusion kurtosis imaging; DKI; epileptogenic zone; epileptic focus

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Compliance with ethical principles: the patient signed a voluntary informed consent for the study and its publication in a scientific journal.

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ГИБРИДНЫЕ МЕТОДЫ ЛУЧЕВОЙ ДИАГНОСТИКИ В ОЦЕНКЕ ЭПИЛЕПТОГЕННЫХ ОЧАГОВ: РАЗБОР ДИАГНОСТИЧЕСКОГО СЛУЧАЯ

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Введение. Локализация эпилептогенного очага (ЭО) у пациентов с фармакорезистентной фокальной эпилепсией нередко бывает затруднительной, а выявление множественных ЭО ограничивает возможности хирургического лечения. Более точная характеристика структуры и метаболизма ЭО может дать дополнительную информацию в рамках ведения таких пациентов.

Описание клинического случая. Пациент 35 лет с фармакорезистентной фокальной эпилепсией, дебютировавшей в 24 года генерализованными тонико-клоническими приступами, а затем фокальными эпизодами с замиранием и тоническим напряжением левой руки. На МРТ выявлен двусторонний мезиальный темпоральный склероз с более выраженными изменениями слева, однако ЭЭГ показала эпилептиформную активность в правой височной области, что создало диагностические сложности в определении первичной эпилептогенной зоны. Для уточнения локализации очага было проведено комплексное обследование, включавшее ПЭТ/МРТ, выявившую выраженный гипометаболизм в правой височной доле (с разницей до 31% по сравнению с контралатеральной стороной) при сохранении симметричного накопления РФП в гиппокампах. Дополнительно выполнена диффузионно-куртозисная МРТ, которая показала значимое снижение показателей радиального и среднего куртозиса, а также аксональной фракции воды в правой височной доле (до 1,33 SD от нормы). Инвазивный ЭЭГ-мониторинг подтвердил билатеральный характер эпилептогенности с преобладанием правополушарной инициации приступов.

Выводы. Комбинированное применение ПЭТ/МРТ и диффузионно-куртозисной МРТ повышает точность выявления эпилептогенных очагов при фармакорезистентной эпилепсии. Методы позволяют комплексно оценивать метаболические и микроструктурные изменения, что важно для планирования хирургического лечения. Интеграция данных способствует дифференциации первичных и вторичных очагов, оптимизируя тактику ведения пациентов. Перспективно внедрение автоматизированного анализа для стандартизации диагностики.

Ключевые слова: фокальная эпилепсия; ПЭТ/МРТ; диффузионно-куртозисная МРТ; эпилептогенная зона; эпилептогенный очаг

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INTRODUCTION

Structural focal epilepsy shows pharmacoresistance in 30–40% of cases [1]. This course of the disease forces specialists to resort to surgical methods of treatment [2]. The modern concept describing the pathophysiological basis of structural focal epilepsy implies the presence of an epileptogenic zone (EZ) in the brain substance, which, in turn, includes not only the focus of morphological changes, but also areas of functional changes [16, 17]. In this regard, complete resection of the epileptogenic zone is one of the most effective surgical methods to achieve a long-term seizure-free period [3]. Up to 57% of patients who undergo resection of a suspected epileptic focus (EF) become completely seizure-free (according to Engel's classification — Ia) [4, 5], and 87% of patients cease to have severe epileptic seizures (according to Engel's classification — Ib) [5]. In this regard, it is critically important to identify the epileptogenic zone and determine the resection boundaries at the stage of preoperative planning.

The conventional set of diagnostic methods, which involves analysis of the clinical picture, noninvasive scalp electroencephalography (EEG), and magnetic resonance imaging (MRI) of the brain according to the epileptic protocol, does not always allow lateralization and localization of the EF. In turn, invasive EEG monitoring with the installation of depth brain electrodes, although exhibiting high sensitivity and being the gold standard in localization and assessment of the prevalence of EF [6], is associated with certain difficulties and risks of various complications [7]. The current research directions in studying epilepsy are aimed at a continued search for more accurate and safe diagnostic methods. In this connection, the presented clinical case demonstrates the potential of combining hybrid imaging techniques of PET/MRI with ^{18}F-fluorodeoxyglucose (FDG) and a promising method of diffusion kurtosis imaging (DKI) [8] in assessing epileptogenic foci in a patient with structural focal epilepsy.

CLINICAL CASE DESCRIPTION

Patient A., 35 years old, was admitted to the neurological department of the Federal Center of Brain Research and Neurotechnologies with complaints of absence seizures, sometimes in combination with tonic tension of the left arm, followed by amnesia, as well as grand mal epilepsy. The onset of the disease at the age of 24 began with a grand mal seizure that developed during sleep, accompanied by wheezing, cyanosis, and post-seizure confusion. The patient turned to a neurologist only one year later, after a second seizure attack. According to the results of the examination, epilepsy of unspecified etiology with bilateral tonic-clonic seizures was diagnosed. An MRI scan of the brain revealed bihemispheric cortical atrophy, suggesting a possible sclerotic change in the right hippocampus. EEG analysis recorded an interictal epileptiform activity in the right frontotemporal region. The combination therapy with antiepileptic drugs resolved generalized seizures (grand mal epilepsy); however, focal nonmotor seizures of the absence type appeared with an average rate of once per month and persisted despite repeated changes in the treatment regimen. Therefore, the patient was admitted to the Federal Center of Brain Research and Neurotechnologies to undergo an in-depth examination in order to localize the epileptogenic zone and consider the possibility of surgical treatment of epilepsy.

The patient underwent MRI of the brain according to the epileptic protocol (isotropic T1 and T2 FLAIR pulse sequence 1×1×1 mm, T2 pulse sequence in the hippocampus plane, T2 and T2 FLAIR pulse sequence perpendicular to the hippocampus plane, T2 pulse sequence in the plane of the anterior and posterior commissures) and daily video EEG monitoring. The analysis of MR images revealed a decrease in the volume of both hippocampuses with a disorder of their structure, which was regarded as bilateral mesial temporal sclerosis, with the changes being noticeably more pronounced in the left hippocampal-amygdalar complex (Fig. 1). No other structural changes characteristic of the potential epileptogenic zone were found.

According to the MRI data shown in Fig. 1, the patient had a bilateral decrease in hippocampal volume with signs of their structural changes in the form of a hyperintensive signal, which were convincing signs of bilateral mesial temporal sclerosis. Daily video EEG monitoring recorded epileptiform activity, represented by acute-slow wave complexes in the right temporal region, occasionally extending to the right frontal-central region. No epileptic seizures were observed.

Given the incomplete correspondence of EEG and MRI data, which made it difficult to lateralize the potential primary epileptogenic zone, it was decided to perform PET/MRI of the brain with ^{18}F-FDG using an integrated SIGNA PET/MR system (GE Healthcare, USA) (Fig. 2). A visual and semi-quantitative analysis of PET/MRI images was performed with the calculation of the standardized uptake volume (SUV) of radiopharmaceutical (RPh) in the areas of interest and in the contralateral areas. The study established a pronounced hyperfixation of the RPh by the substance of the anterior sections of the right temporal lobe in comparison with the contralateral side and a difference of up to 31%. The accumulation of ^{18}F-FDG in the hippocampus and amygdala remained symmetrical.

The data presented in Fig. 2 shows a marked decrease in the ^{18}F-FDG accumulation in the right temporal lobe, which indicates hypometabolic activity in the substance of the right temporal lobe.

In addition to brain PET/MRI, the patient underwent diffusion kurtosis imaging (DKI) as part of the same study. DKI was performed on the same SIGNA PET/MR tomograph with a 3T magnetic field using a 24-channel head coil according to the protocol with three b-factors (0, 1000, 2500) and with gradients in 60 directions, an isotropic voxel size of 3×3×3 mm and a matrix of 80×80 voxels. Postprocessing was performed in the Explore DTI and SPM12 software (MATLAB R2023a). It included correction of the movement of 4D diffusion volumes, correction of Gibbs outliers and Gaussian smoothing. In total, 10 parametric maps were obtained, which were subsequently combined with the anatomical T1 FSPGR series. The areas of interest (both temporal lobes) were semi-automatically segmented based on an individual anatomical atlas,

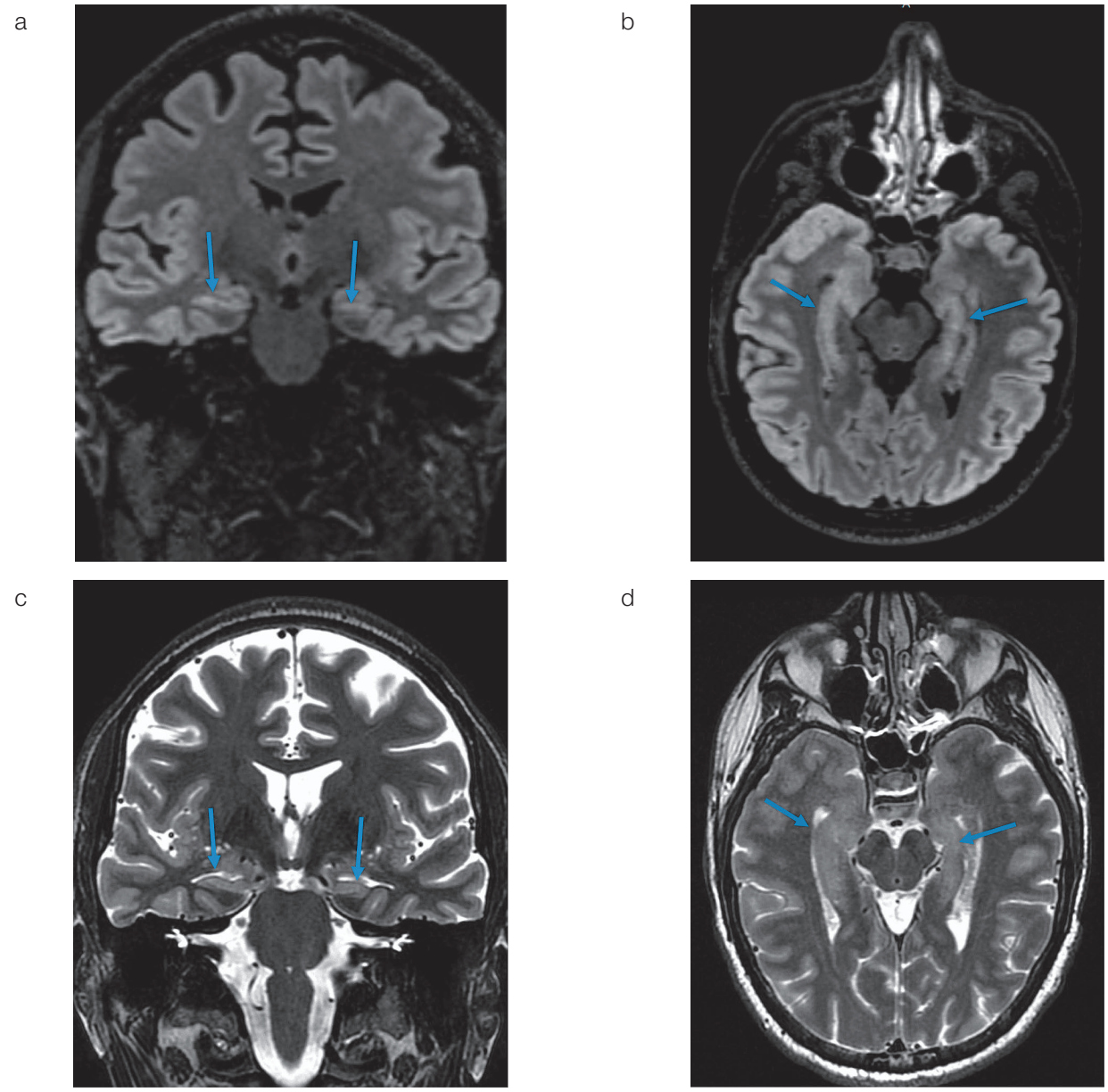

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Fig. 1. MRI of the brain according to the epileptic protocol in a patient with bilateral mesial temporal sclerosis
Note: a — T2 FLAIR image in the coronal plane, aligned along the long axis of the hippocampus; b — T2 FLAIR image in the axial plane aligned along the long axis of the hippocampus; c — T2-weighted image in the coronal plane aligned along the long axis of the hippocampus; d — T2-weighted image in an axial plane aligned along the long axis of the hippocampus. The arrows indicate the hippocampus.

pre-generated using FastSurferCNN using T1 FSPGR series (Desikan–Killiany atlas) [19]. The maps of the mean (MK) and radial (RK) kurtosis, as well as the map of the axonal water fraction (AWF), presented particular interest (Fig. 3). These DKI parameters in the right temporal lobe demonstrated the greatest decrease compared to the relatively small-size norm database previously recruited from healthy volunteers (n = 15) in another study [8]. A decrease of more than one standard deviation (up to 1.33 SD) from the average value according to the norm base was recorded. In addition, there was a visual and quantitative decrease in MK, RK, and AWF values in the substance of the anterior sections of the right temporal lobe relative to the contralateral side.

The maps of radial (Fig. 3a), median (Fig. 3b) kurtosis, and axonal water fraction (Fig. 3b) demonstrate a visual asymmetry of signal intensity between the temporal lobes (D < S), which is confirmed by quantitative comparison with the norm database (the largest decrease by 1.33 SD was observed in the radial kurtosis map).

In order to confirm or refute the assumption of the presence of the primary epileptogenic zone in the right temporal lobe, it was decided to conduct invasive EEG monitoring. Four intracranial electrodes were installed: one in the hippocampus and the corpus amygdaloideum on both sides; monitoring was carried out for nine days. According to the results of the study, a high-index interictal epileptiform activity was recorded under all electrodes with its greater representation on the right, ictal activity in the form of electrographic seizures, also more often with initiation on the right. During electrical stimulation, focal autonomic seizures were recorded with the same

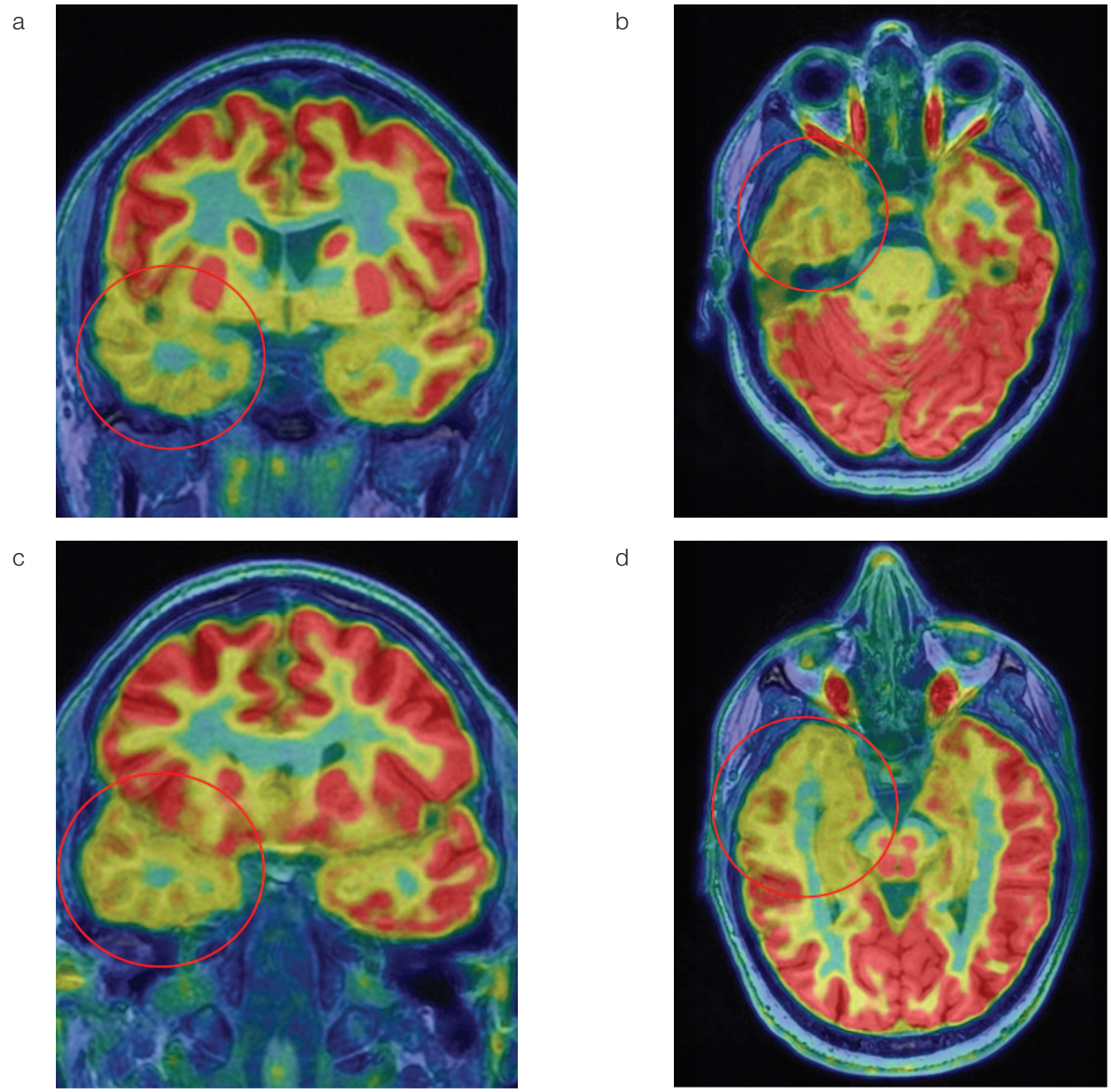

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Fig. 2. PET/MRI of the brain in a patient with bilateral mesial temporal sclerosis
Note: a — combined PET and T1-weighted images in the coronal plane; b — combined PET and T1-weighted images in the axial plane; c — combined PET and T1-weighted images in the oblique coronal plane; d — combined PET and T1 weighted images in the oblique axial plane. The mark indicates the area of hypometabolism ^{18}F-FDG in the right temporal lobe.

frequency when both the left and right corpus amygdaloideum were stimulated (Fig. 4, 5).

Figure 4 shows an electrographic attack with an initiation zone in the left hippocampus: low-amplitude fast-wave activity in the area of the left hippocampus (electrode No. 4, contacts 1–2) extending to 2–4 contacts of the hippocampus and to the corpus amygdaloideum (electrode No. 95 contacts 1–3) with evolution in frequency and amplitude.

Figure 5 shows an electrographic attack with an initiation zone in the right hippocampus: rhythmic fast-wave activity in the area of the right hippocampus (electrode No. 64, contacts 2–3) extending to 1–2 contacts of the hippocampus and to the corpus amygdaloideum (electrode No. 4, 2–3 contacts → evolution of ictal activity in frequency and amplitude).

CLINICAL CASE DISCUSSION

Localization of an EF is a challenging task in 30% of patients with pharmacoresistant focal epilepsy [9]. This is related to the capacity of brain MRI based on the epileptic protocol to detect macrostructural, rather than functional, changes. Nevertheless, MRI analysis may reveal structural changes in multiple locations, including in different hemispheres [10]. Such structural changes may manifest secondary epileptogenesis, which is frequently observed in the contralateral hemisphere [11]. Secondary EFs also have epileptogenic activity; therefore, resection surgery in some of such patients seems ineffective and impractical [12]. However, the presented clinical case shows that the latest non-invasive imaging techniques in the form of PET/MRI and DKI can provide additional information about the nature of changes in potential

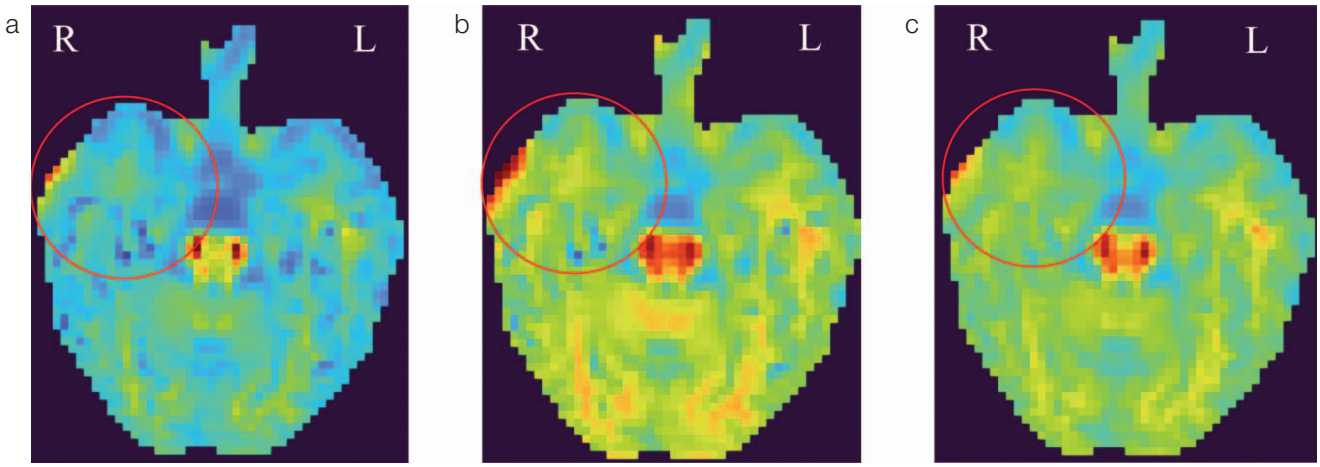

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Fig. 3. Parametric DKI maps in a patient with focal epilepsy and bilateral mesial temporal sclerosis
Note: a — parametric map of radial kurtosis; b — parametric map of average kurtosis; c– parametric map of axonal water fraction. The mark indicates the zone of decrease in DKI parameters in the right temporal lobe; R — right hemisphere; L — left hemisphere.

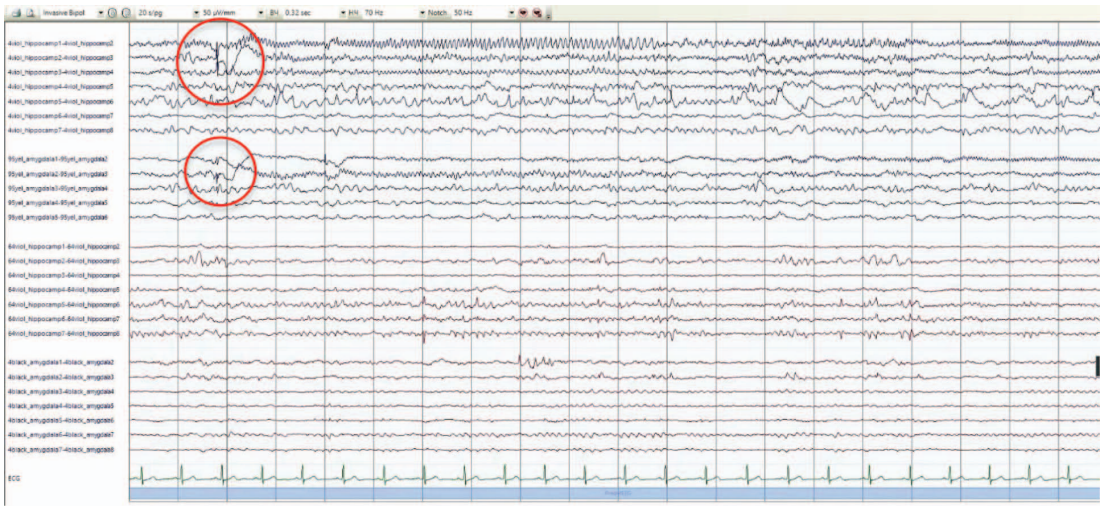

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Fig. 4. Results of invasive EEG monitoring
Note: the markers show the zones of the onset of an electrographic attack.

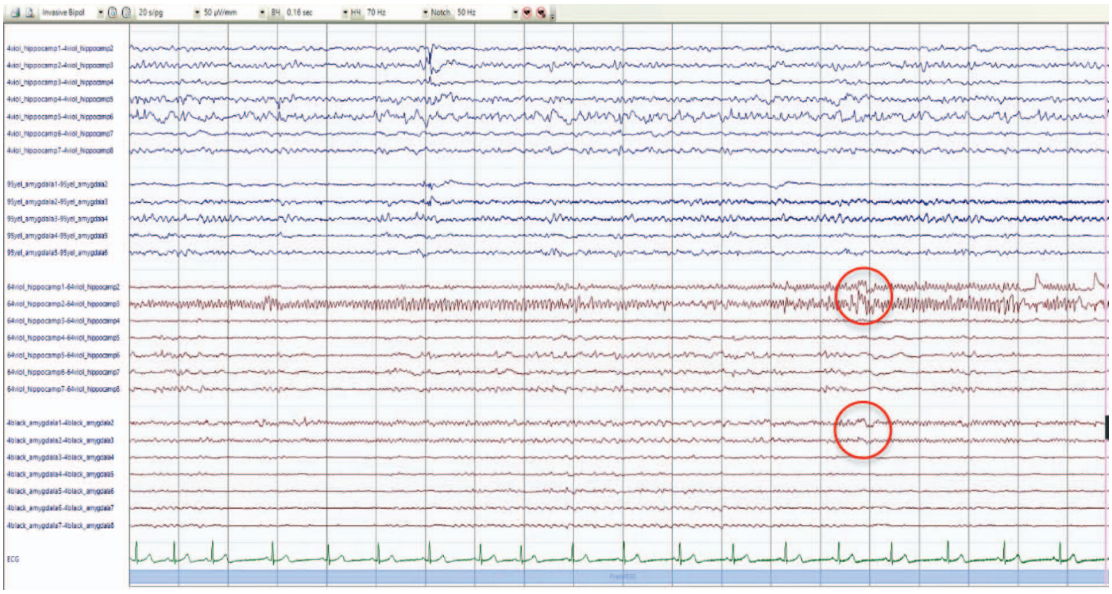

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Fig. 5. Results of invasive EEG monitoring
Note: the markers show the zones of the onset of an electrographic attack.

EFs, as well as their contribution to the course of epilepsy in a particular patient. This combined approach may be effective in patients with discordant results of continued EEG monitoring and conventional brain MRI according to the epileptic protocol, which was noted in the described patient. The DKI parameters make it possible to assess microstructural changes in the brain matter that cannot be reflected on conventional MRI sequences [13, 14]. In turn, functional and metabolic changes caused by microstructural damage can be assessed by PET/MRI [15]. According to literature sources, the PET/MRI method demonstrates a higher sensitivity in the diagnosis of structural focal epilepsy compared to PET, PET/CT, and MRI scans [18].

In the presented case, the revealed changes indicate a more pronounced microstructural damage to the substance of the right temporal lobe, which can be interpreted as potential markers of more active EF or primary, longer-existing EF. The patient was discharged with recommendations for continued therapy and observation at his place of residence. In the future, hospitalization at the Federal Center of Brain Research and Neurotechnologies is planned for subsequent monitoring and determination of treatment tactics.

CONCLUSION

The combined use of PET/MRI and DKI extends the possibilities of pre-surgical diagnostics of patients with focal epilepsy, particularly in cases with potential multiple epileptogenic foci. These techniques provide a multimodal assessment of pathological changes, combining data on metabolic disorders (PET) and microstructural features of tissue (DKI), thus allowing identification of even subclinical or morphologically undetectable epileptogenic zones. The integration of the as-obtained results contributes to differentiation of primary and secondary foci, determination of their functional activity, and optimization of treatment tactics. This is especially important when planning a surgical intervention. In addition, identification of the most active epileptogenic sites can also influence the management of such patients. Thus, PET/MRI and DKI in combination with conventional methods increase the accuracy of topical diagnostics and contribute to personalized treatment of pharmacoresistant epilepsy. Another promising research direction is the development of algorithms for automated data analysis to standardize the diagnostic process.

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ROLE OF REGULATORY T LYMPHOCYTES IN THE FORMATION OF IMMUNOSUPPRESSIVE MICROENVIRONMENT IN GLIOBLASTOMA

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Introduction. Being the most common tumor of the central nervous system with an extremely unfavorable prognosis, glioblastoma remain to be a major health issue. Conventional neuro-oncological strategies demonstrate insufficient effectiveness, which requires the development of improved approaches.
Objective. Analysis of the mechanisms of functioning of regulatory T lymphocytes (Treg) in the tumor microenvironment as a potential target for therapy, as well as identification of promising therapeutic methods to reduce the suppressive effect of regulatory T lymphocytes in glioblastoma.
Discussion. The resistance of glioblastoma against antitumor immunity and the low effectiveness of some types of treatment is largely related to the immunosuppressive microenvironment of the tumor, the key components of which are Treg. Tregs suppress the antitumor response through the secretion of anti-inflammatory cytokines, perforins, and granzymes, as well as the expression of inhibitory molecules. Drugs that selectively affect the metabolic pathways of activation, differentiation, and migration of regulatory T cells can reduce their activity and total number in the microenvironment.
Conclusions. Tregs can act as a target for therapy aimed at suppressing the immunosuppressive microenvironment of the tumor, reducing the activity and progression of glioblastoma. New targeted therapeutical approaches may supplement the existing standards of glioblastoma treatment.

Keywords: regulatory T lymphocytes; glioblastoma; glioma; tumor microenvironment; immunosuppression; immunotherapy; solid tumor

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РОЛЬ РЕГУЛЯТОРНЫХ Т-ЛИМФОЦИТОВ В ФОРМИРОВАНИИ ИММУНОСУПРЕССИВНОГО МИКРООКРУЖЕНИЯ ПРИ ГЛИОБЛАСТОМЕ

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Введение. Глиобластома является серьезнейшей проблемой для современной нейроонкологии, поскольку представляет собой наиболее частую опухоль центральной нервной системы с крайне неблагоприятным прогнозом для пациентов. Применяемая в настоящий момент стандартная терапия недостаточно эффективна, поэтому существует необходимость в создании новых подходов.
Цель. Анализ механизмов функционирования регуляторных Т-лимфоцитов (Treg) в микроокружении опухоли как потенциальной мишени для терапии, а также выявление перспективных терапевтических методов, используемых для снижения супрессорного действия регуляторных Т-лимфоцитов при глиобластоме.
Обсуждение. Устойчивость глиобластомы к противоопухолевому иммунитету и низкая эффективность некоторых видов лечения во многом обусловлена иммуносупрессивным микроокружением опухоли, одним из ключевых компонентов которого являются Treg. Они подавляют противоопухолевый ответ посредством секреции противовоспалительных цитокинов, перфоринов и гранзимов, а также экспрессии ингибиторных молекул. Препараты, селективно воздействующие на метаболические пути активации, дифференцировки и миграции регуляторных Т-клеток, способны снижать их активность и общее число в микроокружении.
Выводы. Treg могут выступать в качестве мишени для терапии, направленной на подавление иммуносупрессивного опухолевого микроокружения, снижение активности и прогрессирования глиобластомы. Новые подходы таргетной терапии могут внести изменения в существующие стандарты лечения глиобластомы.

Ключевые слова: регуляторные Т-лимфоциты; глиобластома; глиома; опухолевое микроокружение; иммуносупрессия; иммунотерапия; солидная опухоль

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INTRODUCTION

Glioblastoma is the most common and aggressive brain tumor characterized by an extremely high mortality rate with the median survival rate of about 13.5 months and the overall five-year survival rate of about 5.8% [1].

According to the WHO classification of 2021, glioblastoma is categorized as diffuse glioma grade IV. The primary form of glioblastoma, which occurs *de novo*, is differentiated from the secondary form, which develops as a result of progression of gliomas of a lower grade of malignancy (Grade II and III). At the same time, the primary type characterized by high invasiveness and rapid development is more common (up to 90% of the total number of cases) [2].

Factors that can trigger the development of malignant brain gliomas include genetic aberrations, viral infections (cytomegalovirus, herpes, etc.), and ionizing radiation, as well as a history of Turcot syndrome, neurofibromatosis type 1 and 2, or tuberous sclerosis [3]. At the same time, the risk of disease development increases with age due to a decrease in the effectiveness of DNA repair processes and the weakening of the immune response [4].

Surgical resection of the tumor, radiotherapy, and chemotherapy with temozolomide are used as treatment standards in glioblastoma patients. Surgical intervention is complicated by the invasive growth of glioblastoma, which prevents complete excision of the pathological tissue and subsequently leads to relapses of the disease. The prognosis for each individual patient is different, depending on various factors (neoplasm location, tumor subtype, diagnosis time, therapy initiation, etc.). In many cases, chemo- and radiotherapy is accompanied by the formation of resistance [5]. Overall, the standard therapy currently used shows low effectiveness, leading to negative side effects and relapses [6]. Moreover, in case of recurrence, the tumor runs a more aggressive course and shows increased therapeutic resistance [7].

The relatively high resistance of glioblastoma against various therapeutical strategies is caused by the tumor heterogeneity and the immunosuppressive microenvironment [8]. Therefore, new treatment methods that account for the characteristic features of glioblastoma are required. In this regard, immunotherapy has great potential [9] due to the possibility of modulating — either directly or indirectly — the immune response, stimulating the patient's natural antitumor immunity and reducing pronounced immunosuppression in the glioma focus to increase the effectiveness of other types of treatment as part of combination therapy.

In this study, we aim to analyze the mechanisms of functioning of regulatory T lymphocytes (Treg) in the tumor microenvironment as a potential target for therapy, as well as to identify promising therapeutic methods for reducing the suppressive effect of regulatory T lymphocytes in glioblastoma.

MATERIALS AND METHODS

The literature search was conducted through the PubMed, Google Academy, and eLibrary databases using the following query keywords: glioblastoma, glioma, regulatory T lymphocytes, immunosuppression, microenvironment, and immunotherapy. The search depth was five years.

RESULTS AND DISCUSSION

Immunosuppressive microenvironment in glioblastoma

The development of glioblastoma is associated with the development of a tumor microenvironment (TME), which plays an important role in neovascularization initiating, progression, invasion, and metastasis of glioma [10]. As a result of this process, a complex heterogeneous system is formed, consisting of the tumor cells themselves, as well as the extracellular matrix, fibroblasts, endotheliocytes, pericytes, immune cells, and signaling molecules secreted by these cells [11]. According to [12], the TME components interact with one another and tumor cells through intercellular contacts and the secretion of various cytokines, chemokines, and growth factors.

The research team [13] noted that glioblastoma significantly affects immune cells and models their phenotype by secreting a range of biologically active molecules. In turn, the immune cells of the microenvironment maintain a high level of immunosuppression in the glioma microenvironment, which contributes to tumor progression.

The focus of glioblastoma contains immune cells whose function is inflammation and antitumor response: cytotoxic T lymphocytes (CTL), natural killers, T-helpers, dendritic cells, B lymphocytes, neutrophils, monocytes, and M-1 polarized macrophages. Tumor infiltration by effector cells has a positive prognostic value in glioblastoma [14]. However, the cells present in TME either show reduced antitumor activity or acquire a pro-tumor phenotype under the influence of glioma.

The TME contributes to the successful escape of the tumor from immunological surveillance, leading to suppression of activation and proliferation of cytotoxic T lymphocytes and NK cells, B lymphocytes, disruption of the tumor antigen presentation on the major histocompatibility complex (MHC) of dendritic cells, and the involvement of regulatory T cells in the microenvironment [15]. The lack of a sufficient level of antigen presentation associated with glioblastoma naturally leads to a low effectiveness of the adaptive immune response.

The cells that mainly provide immunosuppression in the tumor microenvironment are tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), and regulatory T cells (Treg) [16, 17]. Tumor-associated macrophages, including brain microglia and macrophages of peripheral origin, are the most numerous non-tumor populations in the TME in glioblastoma. The macrophage population exhibits plasticity: cells are capable of polarizing into both pro-inflammatory and anti-inflammatory phenotypes [18].

Suppressor cells of myeloid origin are a heterogeneous population of myeloid progenitor cells at different stages of differentiation, which cause inhibition of the activity of cytotoxic T lymphocytes, suppression of the function of NK cells, macrophages, and dendritic cells,

as well as induction of regulatory T and B lymphocytes in the TME [19].

Regulatory T lymphocytes are the main cell population, on the one hand, supporting the immune system homeostasis, and, on the other, playing a key role in avoiding glioblastoma from the immune response. Thus, although regulatory T cells are of interest as a target for the treatment of malignant gliomas, the non-selective effects on the Treg population are associated with numerous side effects.

In the glioblastoma microenvironment, MDSC, TAM, and Treg enter synergy, complementing and enhancing the pro-tumor effects of one another. Regulatory T lymphocytes stimulate the polarization of TAMs, which in turn support the Treg suppressive activity [20]. Tregs also enhance the expansion and inhibitory function of suppressor cells of myeloid origin; MDSCs promote proliferation and induction of regulatory T cells [21].

The researchers in [22] investigated the role of regulatory B lymphocytes and regulatory NK cells as components of the immunosuppressive microenvironment. Regulatory B cells perform the immunoregulation functions through cytokine secretion and intercellular contacts. In the tumor microenvironment, regulatory B cells inhibit effector T lymphocytes, induce Treg activation, and affect other TME-infiltrating cells such as MDSCs, NK cells, and macrophages [23]. NK cells in the tumor microenvironment can perform a regulatory function, influencing the maturation of dendritic cells and leading to a decrease in CTL activation [24].

Population of regulatory T lymphocytes

Regulatory T cells are a subpopulation of $CD4^+$ T lymphocytes, which control the duration of immune response and maintain dominant immunological tolerance to their own antigens. Disruption of the normal Treg functioning plays an important role in the pathogenesis of the graft-versus-host reaction, i.e., in autoimmune, allergic, and oncological diseases [25].

Regulatory T lymphocytes have a fairly wide repertoire of T cell receptor (TCR) specificity, predominantly recognizing their own peptides. Most Tregs are formed in the thymus as functionally mature T lymphocytes (natural Tregs), while a smaller part is induced from naive T cells after antigen-dependent differentiation in the periphery (adaptive Tregs) [26]. The population of natural regulatory T lymphocytes provides tolerance to autoantigens, while adaptive Tregs limit inflammation during infection and suppress the pathological immune response associated with transplantation and allergic conditions.

The Treg population is highly heterogeneous. Thus, the expression of many membrane and intracellular markers of these cells, including FOXP3 and CD25, varies significantly depending on a number of factors, including the functional state of the cells, tissue localization, the presence of pathology or cytokines in the environment [27].

The CD3, CD4, CD25, CD127, and FOXP3 markers are the main markers for the identification of human Treg cells. Staining on Ki67 and CD45RA provides additional information about the Treg activation status. Each of the markers of regulatory T lymphocytes has its own functional significance for the correct functioning of cells:

- The CD3 multiprotein complex is the main T cell receptor co-receptor, expressed on the membrane surface of all T lymphocyte subpopulations;
- Transmembrane glycoprotein of the CD4 immunoglobulins superfamily plays an αβ-TCR co-receptor role, participating in the recognition of antigen presented by antigen-presenting cells;
- CD25 protein is an alpha subunit of the low-affinity receptor for the anti-inflammatory cytokine IL-2, found on Treg, as well as on activated B cells, NK cells, myeloid progenitors, and oligodendrocytes;
- Transcription factor forkhead box protein P3, or scurfin (FOXP3), is a specific protein for activated $CD4^+$ $CD25^+$ Treg. Stable expression of FOXP3 is necessary for the regulation of differentiation and functions of regulatory T lymphocytes. Defects in the *FOXP3* gene lead to a deficiency or absence of normally functioning Tregs. However, FOXP3 is also important for the functioning of Treg in the tumor microenvironment [28];
- CD127 is an alpha chain of the IL-7 receptor. For regulatory T cells, its expression was found to be inversely proportional to the expression of FOXP3; therefore, CD127 is used as a negative Treg marker.

Among $CD4^+CD25^+$ lymphocytes, cells with a stable and unstable expression of the transcription factor FOXP3 are distinguished. At the same time, cells that do not express FOXP3 do not exhibit suppressive properties. It was noted [29] that a certain percentage of the total Treg population exhibit the capacity of the Treg/Tconv transformation, i.e., cells with suppressive effects and non-regulatory T-helper cells.

Role of regulatory T cells in the tumor microenvironment

Regulatory T lymphocytes, whose functioning is necessary to maintain an adequate immune response, are also an important component of the tumor microenvironment. Tregs exhibit significant plasticity and functional diversity in various tumors within the microenvironment [30].

Although the brain was considered an organ isolated from the peripheral immune system for a long time, now it is increasingly recognized as being involved in the structure of systemic immunity. The integration and interaction of the brain with the components of peripheral immunity require strict control and fine regulation. The key population providing additional mechanisms of immunoregulation in the brain are regulatory T lymphocytes [31]. However, in the case of malignant neoplasms, Tregs can contribute to the development of the tumor and its evasion from immune surveillance. In the late stages of the high-grade glioma development, damage to the blood-brain barrier often occurs, which additionally promotes the migration of Treg and other immune cells into the peri-tumoral space [32].

Tumors, including gliomas in particular, maintain a high level of immunosuppression in the microenvironment due to infiltration by regulatory cells. It was noted in [33] that in IDH-mutant glioma, the infiltration of TME Treg is less

pronounced compared to the more aggressive IDH-wild type glioblastoma. A large amount of Treg accumulates in the tumor focus through selective chemokine-mediated recruitment of peripheral T lymphocytes. Tregs in patients with glioblastoma were shown to have a significantly higher level of CCR2 CCR4 receptor expression than Tregs in healthy people [34]. In addition to attracting peripheral regulatory T cells, tumors stimulate acquisition of a regulatory phenotype by naive CD4⁺ T cells [35]. It was found that the conditioned environment of glioblastoma can promote the expansion of Treg in vitro, which indicates the direct influence of factors produced by tumor cells on regulatory T lymphocytes [36].

Regulatory T lymphocytes exert an immunosuppressive effect in the tumor microenvironment due to several basic mechanisms (Fig. 1).

Regulatory T cells produce granzyme B and perforin, acting on effector cells and stimulating their apoptosis.

- Tregs secrete TGF-β, IL-10, and IL-35 inhibitory cytokines, which inhibit CTL activity by binding to receptors on the surface of CD8⁺ cells.
- Cytotoxic T-lymphocytic protein 4 (CTLA-4) on the membrane of regulatory T lymphocytes competes with CD80/CD86 on the surface of T-killers, which leads to suppression of their activity and promotes the secretion of indolamine-2,3-dioxygenase (IDO); IDO activates signaling pathways of apoptosis of effector T cells. The interaction of CTLA-4 and LAG-3 with CD80/CD86 and MHC-II on the surface of dendritic cells also leads to suppression of their maturation and a decrease in the effective presentation of antigens.
- ICOS (inducible T cell costimulator) on the surface of Treg binds to ICOSL on the membrane of effector cells, stimulating the production of the anti-inflammatory IL-10 cytokine.
- The CD39/CD73 ectonucleotidase on the Treg membrane converts ATP into adenosine, which binds to CTL receptors and leads to a decrease in their functional activity.

Regulatory T cells ensure the escape of glioblastoma from antitumor immunity mainly by inhibiting CD8⁺ cytotoxic lymphocytes and reducing the functional activity of NK cells through intercellular interactions and secretion of soluble factors [38]. In addition, due to the production of TGF-β and IDO and a decrease in the secretion of IL-2 and IFN-γ, regulatory T lymphocytes can suppress

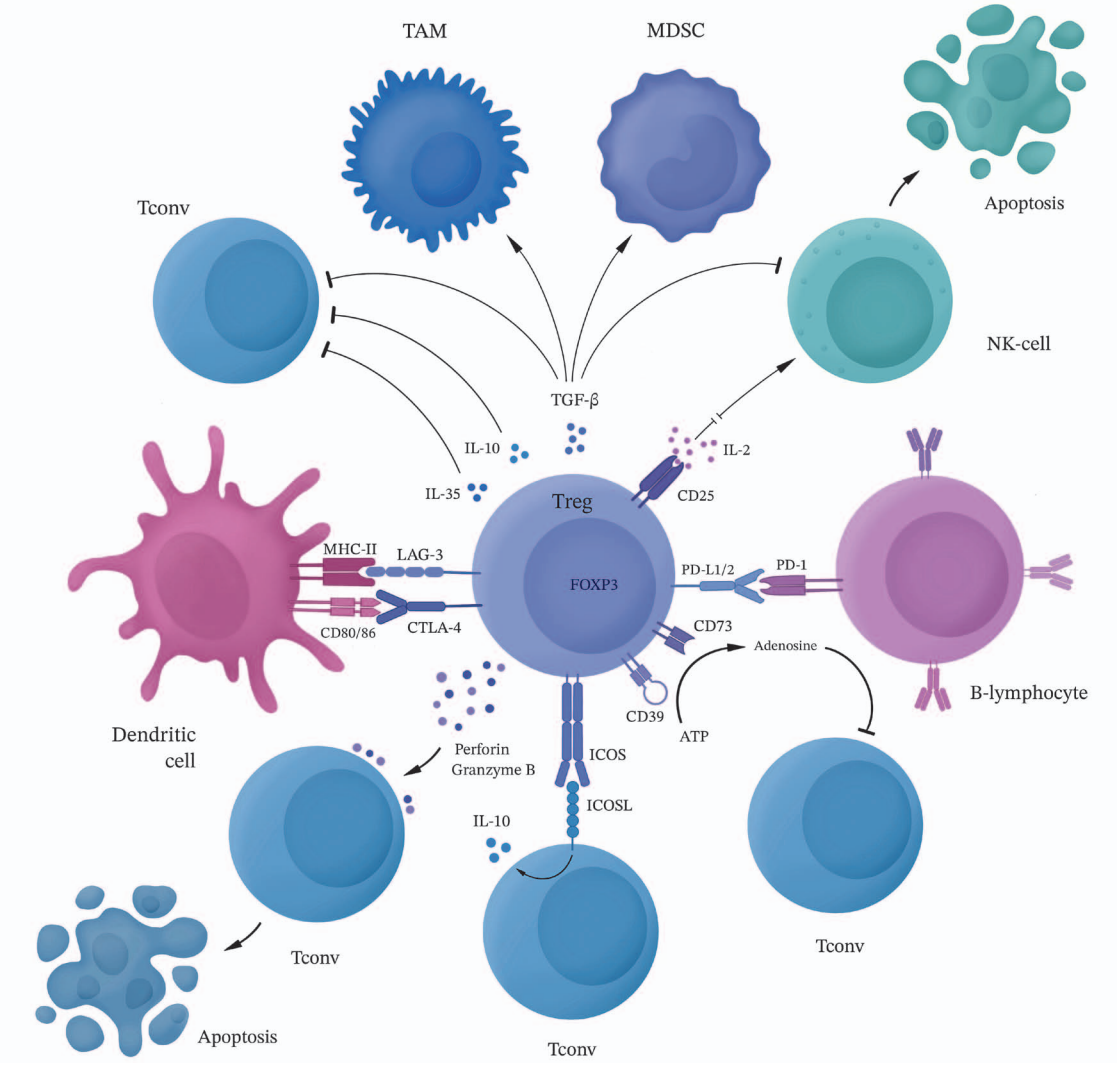

Figure prepared by the authors using data from the reference [37]

Fig. 1. Mechanisms of immunosuppressive action of regulatory T lymphocytes in the tumor microenvironment

antigen-presenting cells and increase the activity of TAM and MDSC, which contributes to the maintenance of the immunosuppressive microenvironment in the glioma focus. It was also noted that the FOXP3 transcription factor can induce the expression of heme oxygenase HO-1, which leads to the expansion and increased survival of the Treg population, as well as to a decrease in the expression of proinflammatory cytokines and suppression of the proliferation of effector T lymphocytes [39]. In addition, regulatory T cells can cause replicative aging and death of effector $CD4^+$ T lymphocytes, CTL, B lymphocytes, and NK cells both *in vitro* and *in vivo* [40].

The TGF-β secreted by regulatory T lymphocytes is not only involved in the process of immunosuppression maintenance, but also acts on tumor cells by inducing the expression of the main genes associated with glioma stem cells (CD133, SOX2, NESTIN, MUSASHI1, and ALDH1A), as well as the NF-kB-IL6-STAT3 signaling pathway, which enhances the carcinogenic potential and glioblastoma stemming [41].

The multitude of mechanisms of the immunosuppressive action of regulatory T lymphocytes in the glioblastoma microenvironment can serve as the basis for the development of targeted drugs for certain metabolic pathways and Treg effects. However, such a diversity creates difficulties in selecting the necessary foci of action for therapy.

Targets of regulatory T lymphocytes for targeted therapy

Regulatory T lymphocytes make a significant contribution to tumor progression, invasion, and therapeutic resistance; therefore, they can act as a target for the treatment of patients with glioblastoma [42]. Currently, drugs aimed at various types of targets and metabolic processes of Treg are being developed and undergoing preclinical and clinical studies for targeted therapy [43].

Although systemic depletion of regulatory T cells can lead to increased antitumor immunity, this process is accompanied by severe autoimmune reactions. Therefore, numerous studies have attempted to selectively deplete regulatory T cells only in tumors, without affecting Tregs in healthy tissues. A decrease in the activity and proliferation of effector T cells (including due to exposure to Treg) leads to low effectiveness of, e.g., CAR-T therapy. At the same time, it was found that a combination with the therapy aimed at depletion of the total number of T cells increases the effectiveness of not only CAR-T [44], but also radioimmunotherapy treatment [45].

One possible approach to selectively affect Treg consists in the use of drugs targeting receptors for certain interleukins essential for the functioning of regulatory T lymphocytes. These include, e.g., drugs against the alpha chain of the IL-2 and CD25 receptors [46]. A member of the tumor necrosis factor (TNF) OX40 (CD134) receptor superfamily is mainly expressed by $CD4^+$ and $CD8^+$ T cells, while tumor-infiltrating Tregs exhibit a higher OX40 expression than peripheral Tregs. After TCR activation on TILs, OX40 is temporarily expressed to transmit a powerful costimulatory signal when it is bound to OX40L. Thus, OX40 agonists can enhance antitumor immunity [47]. The TNF CD27 receptor, as well as its CD70 ligand, can also act as a target [48].

The metabolic pathways of activation and inhibition of regulatory T cells, as well as transcription factors and various costimulating molecules can act as targets for targeted drugs [49]. Drugs of this type include, e.g., checkpoint inhibitors (CTLA-4, IDO, programmed cell death protein 1 — PD-1, T cell immunoglobulin 3 — Tim-3, STAT3 signaling pathway, etc.), which are successfully used in some malignancies [50]. The CTLA-4 receptor is constitutively expressed on naive Tregs and other T-lymphocyte populations; however, its expression is most pronounced in tumor-infiltrating Tregs. Monoclonal antibodies against CTLA-4 can deplete Treg cells in the tumor microenvironment through the mechanism of antibody-dependent cell-mediated cytotoxicity, thereby enhancing antitumor immunity [51]. Although ICB treatment (including the most widely used PD-1 blockers [52]) has not so far shown sufficient effectiveness in glioblastoma patients, some drugs of a new generation of inhibitors may be more effective [53].

The phenomenon of mutual transformation of activated Treg and unregulatory T lymphocytes not expressing FOXP3 (Treg/Tconv) can potentially be used for glioblastoma therapy. Indeed, shifting the balance in favor of inactive regulatory T lymphocytes may reduce the immunosuppression severity in the microenvironment, which in turn will lead to greater effectiveness of the patient's own immune response and other types of therapy [54].

The current evidence shows that Tregs do not play such an unambiguous role in the tumor focus as previously thought. A number of studies confirm the antitumor activity of Treg and their correlation with improved prognosis in certain types of malignant neoplasms (stomach cancer, squamous cell carcinoma of the head and neck, colorectal cancer, etc.) [55]. Regulatory T cells, on the one hand, suppress inflammatory reactions that contribute to the progression of certain types of tumors; on the other, some Treg subpopulations can enhance antitumor immunity. For example, targeting the glucocorticoid-induced TNFR-related protein (GITR) of regulatory T cells with an agonist antibody (αGITR) promotes $CD4^+$ differentiation Treg in effector T cells. Reprogrammed regulatory T lymphocytes express genes characteristic of Th1, produce IFN-γ, and acquire cytotoxic activity against glioma cells, while losing their suppressive function. In turn, αGITR and αPD1 combined with standard treatment of newly diagnosed glioblastoma increased recovery rates in experimental models [56].

In addition to affecting regulatory T lymphocytes directly, the attraction of Treg from peripheral blood into the glioblastoma microenvironment is also possible [57]. Modulation of the interaction of chemokines and their receptors can be used to develop immunotherapeutic drugs for the treatment of malignant gliomas.

CONCLUSION

Pronounced immunosuppression and a high cellular heterogeneity in the glioblastoma focus prevent the development of a natural antitumor response, thus reducing the effectiveness of standard treatment methods.

Regulatory T lymphocytes play an ambiguous role. On the one hand, Tregs are necessary for maintaining immune homeostasis in the body. On the other, regulatory T cells in the glioblastoma microenvironment ensure the escape of the tumor from immunological surveillance. Due to intercellular contacts and secretion of anti-inflammatory cytokines, perforins, granzymes, and other biologically active molecules, regulatory T lymphocytes suppress the activity and proliferation of effector cells in the microenvironment, contributing to the growth and progression of glioblastoma.

Currently, the development of effective and highly selective therapy for malignant gliomas remains an urgent task for researchers. Trials of new therapeutic drugs and modified treatment regimens are necessary to improve the quality of life and the overall survival of patients with glioblastoma, while reducing the incidence of side effects and disease relapses. Regulatory T cells make a significant contribution to the suppression of antitumor immunity and can act as a target for cancer therapy. To reduce the activity and total number of Tregs, drugs acting on interleukin receptors, chemokines, costimulating molecules, metabolic pathways of regulatory T cells, etc., can be used.

In addition to the treatment aimed at regulatory T lymphocytes, other approaches are currently undergoing preclinical and clinical trials: CAR-T therapy, dendritic vaccines, microRNAs, mRNAs, oncolytic virus therapy, etc. It is worth noting that the effectiveness of treatment of glioblastoma patients (in particular, immunotherapy and oncolytic virus therapy) largely depends on the level of local and systemic suppression of the immune response, which requires attention when selecting personalized therapeutical approaches.

An improved understanding of the functional diversity and metabolic features of regulatory T lymphocytes as a key component of tumor immunosuppression, as well as the study of their interaction with other cells in the microenvironment, may offer new possibilities for the treatment of malignant gliomas. In the future, the use of various therapeutic methods as part of a combination treatment, including with targeted Treg drugs, may demonstrate greater effectiveness in comparison with standard approaches.

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PROSPECTS OF APPLICATION OF TEAR FLUID ANALYSIS IN AEROSPACE MEDICINE

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Introduction. The improvement of methods for remote health monitoring of astronauts, as well as the search for new noninvasive biomarkers of metabolic adaptation to microgravity conditions, are priority directions in the field of aerospace medicine.
Objective. To assess the possibility of using individual indicators of tear fluid in aerospace medicine.
Discussion. A number of prospects for the application of human tear biomarkers to determine disorders occurring under the influence of spaceflight factors or during their imitation were identified. The use of filter paper is a priority method for collecting lachrymal fluid in spaceflight conditions due to its relative noninvasiveness and simplicity of sample preparation for assay. It was found that the unstimulated tear fluid contains proteins with an antibacterial activity: lysozyme, lipocalin, and secretory immunoglobulin A. The concentration of lysozyme in the tear fluid shows a marked increase relative to pre- and post-flight values. Changes in the concentration of natriuretic peptide, angiotensin II, dopamine, and α2-macroglobulin under conditions of real and simulated microgravity are described. A high diagnostic potential of determining the level of D-dimer in tear fluid under the influence of extreme factors of space flight was established.
Conclusions. The conducted literature review emphasizes the significant theoretical potential for the quantitative determination of natriuretic peptide, D-dimer, and individual components of the dopamine and renin-angiotensin-aldosterone systems in tear fluid for noninvasive diagnostics of pathological processes associated with spaceflight factors.

Keywords: tear fluid; tear fluid collection; tear fluid metabolism; spaceflight; microgravity

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ПЕРСПЕКТИВЫ ПРИМЕНЕНИЯ АНАЛИЗА СЛЕЗНОЙ ЖИДКОСТИ В КОСМИЧЕСКОЙ МЕДИЦИНЕ

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Введение. Усовершенствование методов дистанционного контроля состояния здоровья космонавтов, а также поиск новых неинвазивных биомаркеров метаболической адаптации к условиям микрогравитации являются приоритетными задачами космической медицины.
Цель. Оценка возможности использования анализа отдельных показателей слезной жидкости в космической медицине.
Обсуждение. Выявлен ряд перспектив применения анализа состава слезы человека для определения биомаркеров различных нарушений организма, происходящих в условиях действия факторов космического полета и при их имитации. Приоритетным методом забора слезной жидкости в условиях космического полета является использование фильтровальной бумаги ввиду относительной атравматичности, простоты метода, более легкой пробоподготовки биообразцов для анализа. Установлено, что в нестимулированной слезной жидкости содержатся белки, обладающие антибактериальной активностью: лизоцим, липокалин и секреторный иммуноглобулин А, причем отмечено выраженное повышение концентрации лизоцима в слезной жидкости относительно до- и послеполетных величин. Описаны изменения концентраций натрийуретического пептида, ангиотензина-II, дофамина и α2-макроглобулина в условиях истинной и моделируемой микрогравитации. Обнаружен высокий диагностический потенциал определения уровня D-димера в слезной жидкости при воздействии экстремальных факторов космического полета.
Выводы. На основании анализа данных литературы подчеркивается существенный теоретический потенциал применения количественного определения натрийуретического пептида, D-димера и отдельных компонентов дофаминовой и ренин-ангиотензин-альдостероновой систем в слезной жидкости для неинвазивной диагностики ассоциированных с факторами космического полета патологических процессов.

Ключевые слова: слезная жидкость; сбор слезной жидкости; метаболизм слезной жидкости; космический полет; микрогравитация

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INTRODUCTION

The habitability of space environments is associated with numerous medical and biological risks. Since the beginning of manned spaceflights, astronauts have shown significant adaptive shifts in water-salt metabolism due to changes in the parameters of the cardiovascular system and neurohormonal regulation. These shifts were shown to be determined by a volume regulatory reflex, manifested by clinically insignificant changes in the concentrations of osmotically active substances in the blood. At the same time, tight correlations between the initial vestibular vegetative stability of astronauts and the specifics of their neurohormonal changes under the influence of spaceflight factors were found [1].

The initial period of weightlessness, due to blood redistribution toward the cranial direction, is associated with jumps in central and renal hemodynamics and is characterized by a decrease in the secretion of hormones of the renin-angiotensin-aldosterone system (RAAS) and antidiuretic hormone during the formation of a new fluid and electrolyte homeostasis with a negative balance of sodium and calcium [2]. The observed hypohydration and hypovolemia, associated with primary adaptive hormonal mechanisms, contribute to an increase in the production of volume and osmoregulatory hormones [3]. These reactions can lead to the development of pathological changes in cardiovascular and hemostatic systems, increase the risk of urolithiasis, and cause demineralization of bone tissue [1].

Monitoring the metabolic adaptation of astronauts to microgravity is an essential aspect in the implementation of space missions. High-precision monitoring of physiological parameters of astronauts using molecular biomarkers is carried out by various methods. However, their analytical performance remains to be problematic, requiring modernization of current diagnostic procedures to minimize errors and facilitate the interpretation of the data obtained. Under conditions of spaceflight, selection of the most noninvasive biomaterial sampling techniques, which allow an informative qualitative and quantitative analysis, is of particular importance.

Tear fluid (TF) is one of the most accessible biological fluids for analysis, characterized by noninvasiveness of sampling and a widely studied composition equivalent to blood plasma [4, 5, 6]. Tear production is regulated by the autonomic nervous system, which allows the glands to rapidly adapt to changing environmental conditions and homeostasis disorders during the development of pathological processes [7].

In this research, our aim was to evaluate the possibility of individual indicators of tear fluid as biomarkers in aerospace medicine.

MATERIALS AND METHODS

The literature search and review was performed using electronic bibliographic databases in the Russian (eLibrary, CyberLeninka) and English (Web of Science, Scopus, PubMed) languages. The search queries included the following key phrases: tear fluid, tear fluid collection, tear fluid metabolism, water-salt metabolism, homeostasis, osmoregulation, glomerular filtration, hormonal regulation, nonspecific protective factors, acute phase proteins, hemostasis system, dopamine system, biomarkers, hypokinesia, immersion, space flight, microgravity. The search depth was 10 years. The inclusion criteria were the availability of structured information on the methods of tear fluid collection, prognostic and diagnostic biomarkers of human body adaptation to the conditions of real spaceflight and during its simulation, qualitative and quantitative methods for their determination.

RESULTS AND DISCUSSION

Methods of collecting tear fluid

The TF sampling method may affect the TF composition. To date, tear collection for biochemical analysis is carried out by two principal methods: using microcapillary tubes with minimal irritation of the conjunctiva and by absorption using an absorbent material (filter paper, polyvinyl acetate sponges) [8].

Sampling with microcapillary tubes without touching the eye provides optimal, unstimulated TF with minimal accompanying components for further research [9]. However, due to the high risk of mechanical injury, this method requires the participation of specially trained personnel, which limits its use in routine practice [4].

The advantage of filter paper in TF sampling consists in its relative noninvasiveness, implementation simplicity, and easier sample preparation [10]. In addition, this method is applicable under the conditions of deficiency of the aqueous component of the tear film observed in microgravity [11, 12].

Antibacterial proteins and natriuretic peptides

Unstimulated TF contains about 20 g/L of proteins, most of which exhibit antibacterial activity, including lysozyme, lipocalin, and secretory immunoglobulin A [7, 13]. Lysozyme, being the leading factor of nonspecific protection of TF, quantitatively prevails over other biological components [14]. Thus, according to [15], crewmembers of the International Space Station (ISS) during spaceflight showed a marked increase in the lysozyme concentration relative to pre- and post-flight values.

Natriuretic peptides (NP_S) are a group of low molecular weight proteins, the main source of which in physiological conditions is atrial tissue [16]. Currently, three types of NP_S and their organic proteolysis products used in clinical diagnostic practice have been identified: atrial natriuretic peptides (ANP), brain natriuretic peptides (BNP), and type C natriuretic peptides (CNP) [17]. The latter are a local regulatory factor of blood vessels and bones, not secreted into the blood [18]. The main physiological effect of NP_S concerns a reduction of the load of hemodynamic factors on the myocardium [19]. In response to increased pressure on the cardiac wall, NP_S cause fluid redistribution to the extravascular sector at the level of the capillary bed, venodilation, and natriuresis stimulation due to increased glomerular filtration rate and RAAS depression [18, 20].

During the initial period of weightlessness and in simulated microgravity, a maximal increase in the NP level in

blood plasma under a decrease in sympathetic influence was observed [21, 22].

The researchers in [23] studied the level of the N-terminal prohormone of brain natriuretic peptide (NT-proBNP), secreted in an equimolar BNP ratio and more stable upon release. A high correlation between the concentration of NT-proBNP in blood serum and TF was observed both in normal conditions and during the development of an ophthalmic pathology [23, 24].

Components of the renin-angiotensin-aldosterone system

The RAAS state plays an important role in the adaptation of water-electrolyte metabolism to spaceflight conditions, which significantly affect the kidneys osmoregulatory function [25]. The main RAAS effector is the angiotensin-II (AT2) oligopeptide hormone [26]. AT2 possesses vasoconstrictor properties, stimulates the production of aldosterone and antidiuretic hormone, increasing sodium reabsorption and contributing to the development of hypervolemia [27].

During dry immersion experiments, a significant decrease in the plasma renin activity and serum AT2 concentration was revealed [28, 29].

At the end of the last century, a local renin-angiotensin system was discovered in the human visual system with components (prorenin, renin, angiotensin converting enzyme, AT2) in concentrations exceeding those in blood plasma [30].

At present, convincing data points to the diagnostic significance of AT2 determination in diabetic retinopathy (DR). Thus, according to the authors, in patients with DR, a significant increase in the concentration of AT2 in TF was recorded in close correlation with a similar indicator in the blood serum [31].

Components of the dopamine system

The negative effects of microgravity on the brain dopamine system have also been described. A decrease in the expression of tyrosine hydroxylase during dopamine synthesis in the substantia nigra and a decrease in the expression of the dopamine receptor of the 1st subtype in the hypothalamus were noted [32]. These changes may underlie motor disorders, dyskinesia, and Parkinsonism during and after space flight, which was shown in studies under the Bion-M1 program [33, 34].

The involvement of dopaminergic neurons in the regulation of tear production leads to a higher TF level of dopamine and its metabolites compared to blood plasma [35]. In [36], a more than threefold excess of dopamine levels in tears over its plasma levels was observed, indicating a high diagnostic potential of TF as a noninvasive source of biomarkers of Parkinson's disease (PD) and conditions accompanied by a decrease in the expression of dopamine system genes.

Acute phase proteins

The initial stage of the body's adaptation to microgravity, in addition to the above, is accompanied by an increase in humoral inflammatory factors and changes in the hepatocyte synthesis of acute phase proteins (APPs) induced by inflammatory cytokines (interleukin-1β, interleukin-6, and tumor necrosis factor α) through interaction with liver cells [37, 38].

In an experiment with seven-day dry immersion, the dynamics of APPs in blood plasma corresponding to the response acute phase was demonstrated. On the second day of exposure to simulated microgravity, statistically significant changes in plasma levels of haptoglobin, α1-antitrypsin, and α2-macroglobulin were observed [39, 40].

α2-macroglobulin is an acute phase protein, an inhibitor of proteolytic enzymes with a wide spectrum of action. Upon the development of inflammatory reactions, it reduces damage to structural proteins by proteases released from leukocytes [41]. Over the years, studies have repeatedly been conducted on the diagnostic significance of determining the activity of α2-macroglobulin in TF in ophthalmic and systemic pathologies [42].

It was found that in patients with an early-stage PD and when modeling the preclinical stage of Parkinsonism in mice, the activity of TF α2-macroglobulin significantly exceeded the clinical norm. In addition, the high specificity of α2-macroglobulin activity (>85%) was shown [43], which validates the study of this protein as a biomarker of certain neurodegenerative diseases and conditions of the acute period of body adaptation to spaceflight conditions or during its simulation.

Components of the hemostasis system

Conditions accompanied by shifts in the hemostasis system and coagulation balance also affect the TF composition. In the acute period of exposure to microgravity, as mentioned above, hemodynamic shifts are observed, which in turn lead to changes in the rheological properties of the blood [2, 44]. These changes, along with physical inactivity, may induce abdominal congestion, which increases the risk of developing thrombophilia in astronauts [45]. A striking example is the case of occlusive thrombosis in an ISS crewmember during a recent orbital space flight [46].

The determination of D-dimer (DD), which is a product of the proteolytic degradation of fibrin, is currently a widespread test for assessing the activity of fibrin formation and fibrinolysis processes [47]. A number of studies have described significant changes in blood plasma DD levels during the period of adaptation to gravity unloading, with a tendency to be more pronounced in individuals with signs of vascular endothelial damage [48, 49].

Tear fluid contains components of the hemostasis system. Thus, the researchers in [50] demonstrated a high diagnostic informative value of antithrombin III and plasminogen levels in tears in patients with complicated diabetes mellitus and hypertensive angiosclerosis.

According to the results of a number of studies on determining the DD level in the TF, a statistically significant increase in the DD concentration was revealed in patients with retinal vein occlusion compared to the control group with minor changes in blood plasma [51].

The risk of developing occlusive retinal lesions in real microgravity is probably due to the high level of the

homocysteine neurotoxin in blood plasma, recorded in astronauts with ophthalmic pathology before and during a spaceflight mission [52].

The above information justifies further investigation into the diagnostic potential of DD in tear fluid.

The adaptive restructuring of the water-electrolyte balance, recorded at an early stage of weightlessness exposure, may affect the mineral composition of tear fluid. Basal tear is characterized by high concentrations of potassium and sodium ions compared to blood serum, which ensures metabolic processes at the level of the ocular surface through Na, K-ATPase of the lacrimal glands [53]. An increased level of calcium ions in TF is observed during bacterial infections, cystitis, and dry eye syndrome [54, 55].

Pharmacological research

The acquisition of on-board first-aid kits remains relevant to the medical and biological support of manned space flights, taking into account the potential change in the pharmacological properties of individual drugs under the constant influence of extreme factors on astronauts. Pharmacokinetic studies of antiemetic, anti-inflammatory, and antibacterial agents, during flights demonstrated a significant decrease in the bioavailability of active substances relative to similar values on the Earth [56]. To date, the retrospective analysis is limited by the heterogeneity of the conducted experiments [57].

Despite the difficult-to-permeate blood-ocular barrier, a correlation between the concentrations of normothymic agents, macrolide, and beta-lactam antibiotics in blood plasma and TF was established [58, 59]. A deeper understanding of the entry patterns of drugs and their metabolites into tear fluid is a promising task, the solution of which will contribute to optimization and standardization of methods for studying the pharmacokinetic and pharmacodynamic properties of various compounds for more effective formation of medical packages on board spacecraft.

CONCLUSION

The conducted literature review allow us to draw a preliminary conclusion about the high diagnostic and prognostic potential of TF analysis in a wide range of health pathologies and disorders detected under the impact of adverse spaceflight factors. Noninvasive and effortless under appropriate conditions, the study of tear composition appears to be a promising method for monitoring the state of functional systems, as well as for monitoring the correction of deviations in physiological parameters induced during space missions. The practical application of such studies involves the search for specific changes in the TF components, which can be sensitive biomarkers of the body adaptation to microgravity and, subsequently, to other extreme factors of outer space. In the long run, such studies may contribute to successful implementation of interplanetary expeditions.

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NEW APPROACHES TO THE ORGANIZATION OF LOCOMOTOR TRAINING DURING LONG-TERM SPACEFLIGHT

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Introduction. In Russia, locomotor training is the key approach to mitigating the negative effects of weightlessness. Locomotor training is performed according to strictly defined protocols, without individualization and periodization of the training process.
Objective. To study the effect of periodization of locomotor training on the performance of crewmembers during long-term space missions.
Materials and methods. The study involved 12 cosmonauts, who were divided into two groups. The first group (BD, n = 6) included the participants who performed locomotor training in strict accordance with the standard on-board documentation system. The second group (ID, n = 6) included the participants who performed training using individual protocols and periodization of the training process. The assessment of physical performance was carried out according to the results of a regular stepwise locomotive test prior to a spaceflight (SF) mission and three times during SF. The test evaluated the achieved speeds at the most intensive stages of testing, the distance traveled during the test, and heart beats per distance (pulse value performance). Statistical processing was carried out in Statistica 10; nonparametric methods of descriptive statistics were used.
Results. In the second part of SF, cosmonauts in the ID group reached higher speeds at the stages of medium and fast running and covered a greater distance by 18.5–20.7% ($p < 0.05$) and 5–12% ($p < 0.05$) compared with the BD group and with the baseline testing, respectively. The beats per distance in the ID group was lower throughout the SF compared to both the baseline values and the BD group in the 2nd and 3rd flight testing sessions.
Conclusions. In the conditions of SF, locomotor training programs based on periodization and individualization demonstrate a greater preventive effectiveness compared to standard on-board training.

Keywords: space flight; prevention of the negative effects of weightlessness; physical performance; locomotor training; periodization; individual approach

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НОВЫЕ ПОДХОДЫ К ОРГАНИЗАЦИИ ЛОКОМОТОРНЫХ ТРЕНИРОВОК В ДЛИТЕЛЬНЫХ КОСМИЧЕСКИХ ПОЛЕТАХ

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Введение. Основным средством российской системы профилактики негативного влияния невесомости являются локомоторные тренировки, выполняющиеся по строго заданным протоколам, без индивидуального подхода и периодизации тренировочного процесса.
Цель. Изучение влияния периодизации локомоторных тренировок на работоспособность членов экипажей длительных космических миссий.
Материалы и методы. В исследовании приняли участие 12 космонавтов. Космонавты были разделены на две группы: группа БД (n = 6), участники которой выполняли локомоторные тренировки в строгом соответствии со стандартной системой бортовой документации; группа ИД (n = 6), в которой выполнялись тренировки с использованием индивидуальных протоколов и периодизацией тренировочного процесса. Оценка физической работоспособности проводилась до космического полета (КП) и 3 раза в КП по результатам штатного ступенчатого локомоторного теста. Оценивали достигнутые скорости на наиболее интенсивных ступенях тестирования, пройденное за тест расстояние, пульсовую стоимость тестирования. Статистическая обработка проведена в программе Statistica10, использовали непараметрические методы описательной статистики.
Результаты. Было показано, что во второй части КП космонавты группы ИД достигали бóльших скоростей на ступенях среднего и быстрого бега, а также преодолевали большее расстояние на 18,5–20,7% ($p \leq 0,05$) и 5–12% ($p \leq 0,05$) по сравнению с группой БД и с фоновым тестированием соответственно. Пульсовая стоимость нагрузки в группе ИД была ниже на протяжении всего КП по сравнению с фоновыми значениями и ниже по сравнению с группой БД во 2-й и 3-й полетной сессии.
Выводы. Использование периодизации и индивидуального подхода в условиях КП обладает большей профилактической эффективностью по сравнению со стандартными бортовыми тренировками.

Ключевые слова: космический полет; профилактика негативного влияния невесомости; физическая работоспособность; локомоторные тренировки; периодизация; индивидуальный подход

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Финансирование: работа выполнена в рамках базовой тематики РАН 65.1.

Соответствие принципам этики: исследование не требовало одобрения локального этического комитета в связи с тем, что работа проведена в рамках медицинского сопровождения космических полетов.

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INTRODUCTION

One of the main medical support systems for long-term spaceflights is that for mitigating the negative effects of weightlessness. Such a system is designed to maintain the physical performance of members of long-term space expeditions and to alleviate the symptoms of the microgravity adaptation syndrome through a range of measures to reduce negative effects or promote the restoration of altered functions [1].

In long-term SF, physical training aimed at maintaining the physical performance and basic physical qualities of crewmembers is of particular importance. In the Russian prevention system, physical training is conventionally divided into three stages:

(1) the adaptation to microgravity stage lasts approximately 30 days; at this initial stage, physical training is performed at 50% of the recommended load, followed by its gradual increase to the recommended level;

(2) the stabilization stage, lasting 110–130 days depending on the duration of the flight, assumes physical training according to the standard scheme; the load at this stage does not change significantly;

(3) the final stage, starting 30 days before boarding, assumes physical training with increased locomotor load; in addition, training with negative pressure on the lower body is applied [1, 2].

It should be noted that SF conditions, onboard of the ISS in particular, limit the performance of physical training to the equipment available on board. These include a treadmill as the main means, a bicycle ergometer, a power loader, an Advanced Resistive Exercise Device (ARED) strength simulator of the American segment of the ISS, and resistance bands. In our study, only locomotor training modes will be considered, taking into account the periodization of physical activity.

Periodization is the cyclical ordering of training workouts in accordance with the principles of volume and intensity specificity in order to achieve maximal performance during the most important stages of physical training. In relation to a space station, the stages of a manned expedition that place the greatest demands on the physical performance of a cosmonaut are extravehicular activities and return to Earth. Periodization of physical training is essential to managing physical performance by reducing the risk of fatigue and the decrease of fitness. Periodization includes long-term (macro- and mesocycles) and short-term (microcycles) planning of physical training [3]. Cyclical changes in the volume, intensity, and types of exercises in training cycles avoids the effect of overtraining, while contributing to achieving peak levels of physical performance at the appropriate stages of SF [4].

Currently, locomotor training is carried out according to on-board documentation using the BD-2 treadmill equipment in four-day microcycles. Each day of this microcycle is aimed at maintaining a certain physical quality, i.e., day 1 — speed, day 2 — strength, day 3 –endurance, and day 4 — active recovery. On day 4, cosmonauts either do not exercise at all or perform low-intensity workouts at their discretion [5, 6]. Longer periodization in meso- and macrocycles is rarely carried out, as a result of which this approach contradicts modern ideas about the organization of the training process [7].

In this article, we set out to investigate the effect of periodization of locomotor training on the performance of crewmembers during long-term space missions.

MATERIALS AND METHODS

The study involved 12 cosmonauts participating in long-term space expeditions (average age 42 ± 5 years). The participants were divided into two groups, depending on the selected approach to performing locomotor training during SF:

- BD group (on-board documentation) (n = 6), in which participants performed training sessions in strict accordance with the standard system defined by on-board documentation. This implied training using four-day microcycles without periodization of the training process, i.e., with constant load, except for the first month of SF;
- ID group (individual training) (n = 6), in which participants performed locomotor training using individual protocols developed by experts specializing in the prevention of hypogravity disorders and ensuring the periodization of the training process.

In the individual protocols of the ID group, an interval training method was used. The intensity of the intervals used was 70–80% of the maximum heart rate (HR) recorded in the MO-3 test (medical examination 3) [1] during preflight testing. In the ID group, the spaceflight itself was a macrocycle, conditionally divided into 5–6 mesocycles lasting 4–5 weeks, depending on the tasks and duration of the flight. In this group, a pyramidal approach to periodization was used, assuming a reduction in the volume and an increase in the intensity of the load [8, 9, 10]. In addition, in the ID group, in the middle of SF (2–3 mesocycles), a planned decrease in the intensity of physical activity and unloading microcycles were used during the transition to a new mesocycle.

Physical performance was assessed on the basis of the standard MO-3 medical test. This test was performed in the passive mode of operation of the treadmill (i.e., the treadmill was moved by the force of the cosmonaut's legs). The test had a strict time structure: 3 min of walking, 2 min of slow running, 2 min of medium running, 1 minute of fast running, and 3 min of walking; the speeds in this test were selected by the crewmembers subjectively according to their state of health.

Locomotor training, as well as MO-3 testing, were performed on a BD-2 treadmill (Institute of Biomedical Problems of the RAS, Russia). Prior to SF, testing was performed on a full analog of the BD-2 treadmill, but without a vibration isolation system and a training loading suit. Heart rate was recorded using a Polar heart rate monitor (Polar Electro Oy, Finland).

The research comprised the following stages:

- the preflight stage, during which all cosmonauts participating in the study completed an MO-3 test 60–30 days before SF (baseline testing);
- the flight stage, during which the cosmonauts performed tests in accordance with the specified flight days, i.e., flight session *1* — 40–50 days of SF; flight

session *2* — 80–100 day of SF; flight session *3* — 130–150 day of SF.

The test results were analyzed by the groups described above in terms of the parameters described below.

The distance traveled in the test was analyzed, as well as the maximum speeds of locomotion at the most intense stages: moderate and fast running. The moderate running stage (the 3rd pre-maximum load stage in the MO-3 test) was performed at a speed that cosmonauts defined personally as of medium intensity. In our study, the average speed of locomotion at this stage was 8 km/h. The fast-running stage (the 4th stage in the MO-3 test) was performed with maximum intensity; in our study, the average speed of locomotion at this stage was 9.5 km/h.

The number of heart beats per the distance traveled (pulse value performance[1]) was used as an integral indicator of performance. This indicator was calculated as the ratio of the total heart rate per test to the distance traveled.

$$PV = \frac{\Sigma HR}{S}, \quad (1)$$

PV — beats per distance traveled (hereinafter pulse value performance);
ΣHR — heart rate during the test (beats per min);
S — distance traveled during the test (m).

It should be noted that the magnitude of axial load during SF testing was significantly lower than that on Earth (60–70% of body weight). However, this parameter was not integrated into the pulse value formula, since its effect on the heart rate response is apparently nonlinear. Given the absence of statistically significant differences between the groups according to this parameter (Fig. 1), the groups are to be compared with each other.

In addition, we calculated the pulse debt as the difference between the sum of heart beats during a 5-min recovery period and the resting heart rate before the onset of testing.

Statistical processing was carried out in the Statistica10 software; nonparametric methods of descriptive statistics were used. When comparing indicators within the group, the Wilcoxon *t*-test with the Benjamini-Yekutieli procedure was used [11]; when comparing between the groups, the Mann–Whitney *U*-test was used. The median Me, interquartile range, and percentage of changes relative to the baseline were calculated. For a more complete description of the research cohort, outliers and extreme values are additionally presented. The former are the values that are highly different from those in the cohort; extremes are the border values in the cohort.

RESULTS

In order to determine the volume and intensity of the load in the BD and ID groups, we considered the average distances traveled during each workout and the distances traveled in the passive mode of operation of the treadmill. The number of training sessions for the cosmonauts of both groups was almost identical; thus, the average distance traveled per training session may indicate the amount of work performed.

Throughout SF, we did not detect any differences between the groups in terms of the average distance traveled during training (Fig. 2).

The average distance traveled during training in the ID group was 3326 m in the first month of SP, having increased by 6.6% (to 3547 m) by the second month of SP. During the third month of SP, this indicator decreased by

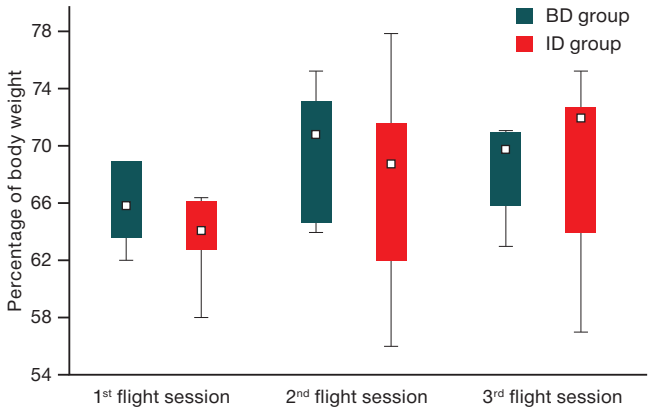

Figure prepared by the authors using their own data

Fig. 1. Axial load in groups when performing the MO-3 test

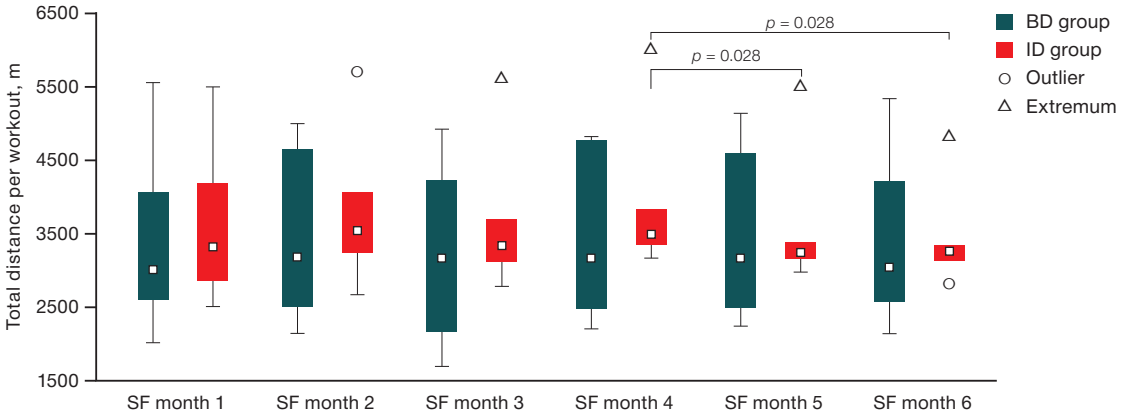

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Fig. 2. Total distance per workout
Note: data presented as the median (*Me*) of the values of the lower and upper quartiles *Q* [25–75%]; *p* — statistical significance level.

[1] Translator's note: In the Russian system, the indicator of heart beats per distance is referred to as "pulse value performance". These terms are used interchangeably throughout the text.

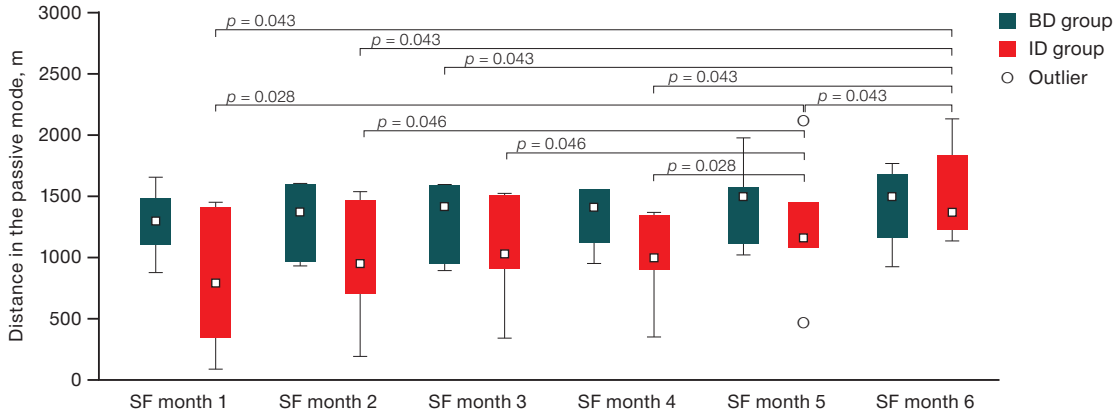

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Fig. 3. Distance traveled in the passive mode of operation of the treadmill
Note: data presented as the median (*Me*) of the values of the lower and upper quartiles *Q* [25%–75%]; *p* — statistical significance level.

5.8%, i.e., to 3341 m, compared to the second month of SP. During the fourth month of SP, in order to achieve the undulation of the training load, the distance traveled increased by 4.3% compared to the previous month, which corresponded to 3484 m. During the fifth month of SP, this indicator decreased by 7% to 3241 m, being significantly different from that in the fourth month ($p < 0.05$). In the sixth month of SF, the average distance traveled in training was 3261 m, being also significantly different from that in the fourth month ($p < 0.05$) (Fig. 2).

In the BD group, in the first month of SF, the average distance per workout was 3018 m. In the second month of SF, this indicator increased by 5.1% and amounted to 3172 m. Further (in the third, fourth, and fifth months of SF), the changes in distance traveled were less than 1%. During the sixth month of SF, this indicator decreased by 3.8% compared to the fifth month and amounted to 3055 m.

In order to assess the intensity of the load, the distance traveled in the passive mode of operation of the treadmill was used. This mode is more stressful than the active mode, since its implementation requires an additional force of 3.5 kgf [12]. Overall, the total training time remained unchanged, and the distance traveled in the passive mode of operation of the treadmill depended on the speed and time of execution of this mode. Taking into account the above, we believe that a change in the distance traveled in the passive mode of operation of the treadmill during a workout may indicate a change in the intensity of the load. Data on the average distance traveled in the active mode of operation of the treadmill were not considered, since this mode is less stressful and less indicative in terms of intensity.

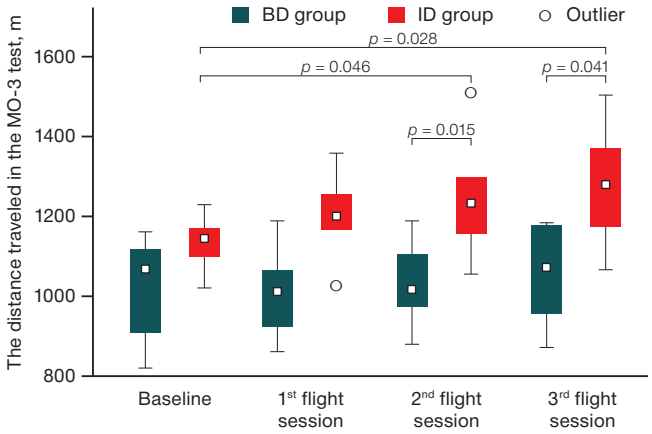

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Fig. 4. The distance traveled in the MO-3 test
Note: data presented as the median (*Me*) of the values of the lower and upper quartiles *Q* [25–75%]; *p* — statistical significance level.

There were no significant differences between the groups in terms of the average distance traveled in the passive mode throughout SF (Fig. 3).

In the ID group, from the first to the third month of SF, an increase in the distance traveled in the passive mode was observed (Fig. 3). During months 1, 2, and 3 of SF, this indicator was 787, 955, and 1024 m, respectively. During the fourth month of SF, in accordance with the periodization of the training process, the distance traveled in the passive mode was reduced to 1004 m. In the fifth month of SF, this indicator increased by 15.9% compared to the fourth month to 1167 m; this indicator was also significantly higher than that in all previous months of SP ($p < 0.05$). During the sixth month of SF, the distance traveled in the passive mode of operation of the treadmill increased by 17.7% relative to the fifth month and amounted to 1369 m. This indicator was also significantly higher ($p < 0.05$) than that in all previous months of the flight cycle (Fig. 3).

In the BD group, this indicator did not undergo such significant changes throughout the SF. During months 1, 2, 3, 4, 5, and 6, this value was 1297, 1367 (+5.5%), 1418 (+3.7%), 1413 (–0.3%), 1494 (+5.7%), and 1497 m (+0.2%), respectively.

The distance traveled before SF in the MO-3 test between the groups did not differ and amounted to 1144.5 m in the ID group and 1095 m in the BD group (Fig. 4).

In the first flight session, the ID group experienced a 6.9% increase in the distance traveled (1202.5 m) compared to the baseline. In the BD group, the distance traveled decreased by 7.3% (1040 m) compared to the preflight survey, while no significant differences were recorded between the groups. In the second flight session, the ID group experienced a further increase in the distance traveled (1234.5 m), which was significantly higher than in the baseline test ($p < 0.05$). In the BD group, the distance traveled practically did not change, while this indicator

was significantly lower than in the ID group ($p < 0.05$) and amounted to 1022 m. In the third flight session, there was an increase in the distance traveled in both groups: 1281 m in the ID group, being significantly higher compared to both the baseline values and the BD group ($p < 0.05$). In the BD group, this indicator was 1126 m, which effectively corresponded to the preflight value (Figure 4).

The speed of locomotion at the fast-running stage before SF in the ID and BD groups was 9.65 and 9.2 km/h, respectively. However, no significant differences were recorded between the groups (Fig. 5). In the first flight session, the ID group experienced an increase in this indicator by 5.2% (10.15 km/h) compared to the baseline, while the BD group showed a decrease in speed by 4.9% (8.7 km/h) compared to the baseline test.

In the second flight session, the ID group experienced a further increase in speed at the fast-running stage to 10.45 km/h ($p < 0.05$), which was 8.3% higher relative to the baseline value. In the BD group, this indicator was 8.9 km/h, while a significant difference in this indicator was recorded between the groups ($p < 0.05$). In the third flight session, the locomotion speed at the fast-running stage in the ID group was 10.35 km/h, being significantly higher than in the BD group, where this indicator was 8.95 km/h.

No significant differences between the groups were recorded at the stage of moderate-intensity running during the preflight testing (Fig. 6). At the same time, in the ID group, the speed of locomotion was increasing from session to session and comprised 8.2 km/h in the baseline testing, 8.45 km/h in the first flight session, 8.63 km/h in the second flight session, 8.78 km/h in the third flight session. Significant changes compared to the baseline ($p < 0.05$) were noted in the second and third flight sessions.

In the BD group, the moderate-running speed of locomotion throughout SF was reduced relative to the baseline by 4.7–8.7%, namely 7.55 km/h in the first flight session, 7.15 km/h in the second flight session, 7.55 km/h in the third flight session against the baseline values of 7.93 km/h. In addition, in the third flight session, the speed in the BD group was significantly lower compared to the ID group (Fig. 6).

The indicator of beats per distance (pulse value performance) in the preflight testing did not differ significantly between the groups. In the ID and BD groups, this indicator before SF was 1.48 and 1.54 beats/min/m, respectively (Fig. 7).

During SF, the ID group showed a significant decrease in the pulse value parameter in all flight sessions, compared to the baseline. Thus, its values were 1.35 beats/min/m (–8.8%) in the first flight session, 1.28 beats/min/m (–13.2%) in the second flight session, and 1.31 beats/min/m (–11.3%) in the third flight session. In the BD group, this indicator tended to decrease, although never reaching the level of significance in any flight sessions and comprised 1.44 beats/min/m in the first flight session (–6.7%), 1.48 beats/min/m in the second flight session (–4.2%), and 1.43 beats/min/m in the third flight session (–7.1%). In addition, in the second and third flight sessions, the beats per distance parameter in the ID group was significantly lower compared to the BD group (Fig. 7).

In terms of pulse debt, we found no statistically significant differences between or within the groups.

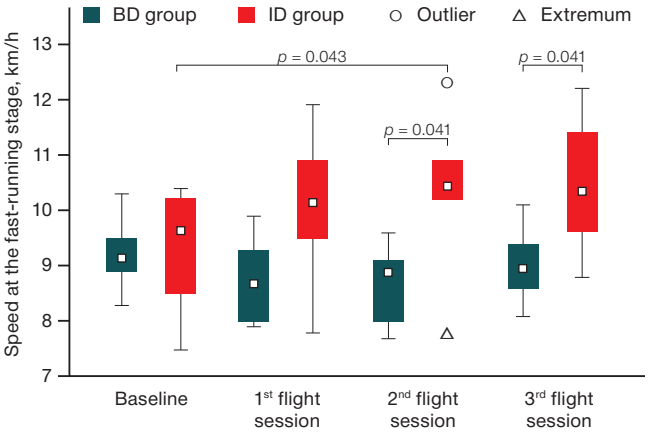

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Fig. 5. Locomotion speed at the fast-running stage in the MO-3 test
Note: Data presented as the median (*Me*) of the values of the lower and upper quartiles of *Q* [25–75%]; *p* — statistical significance level.

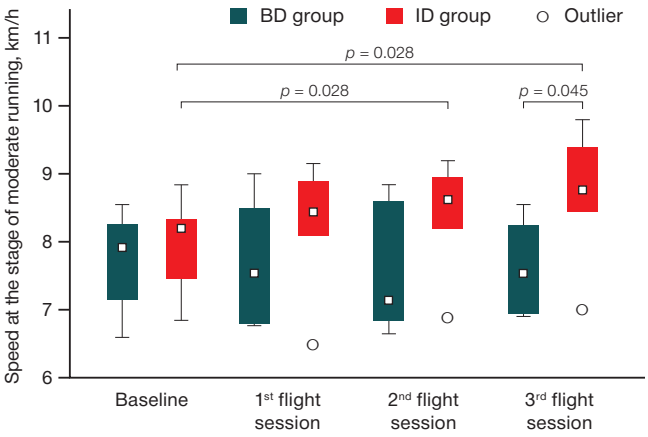

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Fig. 6. Locomotion speed at the stage of moderate running in the MO-3 test
Note: data presented as the median (*Me*) of the values of the lower and upper quartiles *Q* [25–75%]; *p* — statistical significance level.

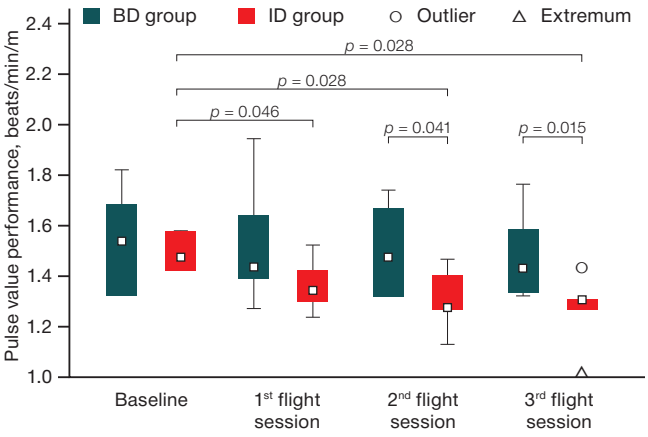

Figure prepared by the authors using their own data

Fig. 7. Beats per distance (pulse value performance) in the MO-3 test
Note: data presented as the median (*Me*) of the values of the lower and upper quartiles *Q* [25–75%]; *p* — statistical significance level.

DISCUSSION

Prolonged exposure to SF conditions leads to a decrease in the level of physical performance [13]. Physical training can mitigate the negative effects of SF factors, which makes both the equipment and organization of the training process, including its periodization, highly important. Experts of the Russian prevention system proposed the use of periodization of physical activity under conditions of prolonged SF, based on the principle of undulation [14]. In addition, the developers of this system noted the need for an individual approach, taking into account the selective attitude of crewmembers to the recommended means and methods of physical training [14]. It should be noted that our study considered only treadmill training; however, the use of other preventive measures also had an effect on the level of physical performance of crewmembers. At the same time, the results of training achieved using a bicycle ergometer and a strength simulator was similar in both groups. These workouts were performed every other day, while treadmill training was performed daily and, accordingly, had a greater effect on the physical performance of the crewmembers.

In our study, the BD group showed a decrease in the studied indicators (distance traveled, speed of locomotion at the stages of fast and moderate running) in the first flight session, despite the reduced axial load, thus indicating a decrease in physical performance. Subsequently, during the flight, these indicators demonstrated a slight increase, except for the speed at the moderate-intensity running stage, which indicated some recovery in performance. In the ID group, the distance traveled and the speed of locomotion increased in the first flight session compared to the baseline. The results obtained in the BD group are consistent with those reported by other researchers on a decreased performance of crewmembers during early flight stages, which gradually normalized at later stages. This observation was explained the implementation of preventive measures [15].

The MO-3 test can be represented as an analog of the Cooper test [16], where performance is assessed by the distance traveled. The step-by-step structure of this test makes it possible to identify the most significant stages in testing, i.e., moderate and fast running, indicating the maximum achievable power in testing. Testing in the passive mode requires an additional 3.5 kgf of effort from crewmembers to maintain the speed of the BD-2 treadmill [12], which is significantly complicated in SF conditions due to developing muscular atony and atrophy [17]. It should be noted that during SF, the use of the passive mode is also complicated by the specific features of the biomechanics of movement on the treadmill, which is operated by a vibration isolation system, as well as by the use of a training loading suit. Prior to SF, when performing testing, cosmonauts place their hands on special handrails located in front of them at the shoulder level, thereby facilitating pushing the treadmill belt back. In SF conditions, the use of these handrails is impossible due to the presence of a passive vibration isolation system. The additional hand support from the front shifts the center of mass of the treadmill belt forward, moving beyond the center of mass of the vibration isolation system. This significantly increases the pitch and leads to a downward tilt of the front of the belt, while the back of the belt rises and rests against the frame. In order to avoid this effect, cosmonauts use a training loading suit as a support, which requires additional body tilt to create a force pushing the belt back, while the step length is significantly reduced.

The observed changes in the beats per distance parameter (pulse value) during SF in our study are consistent with the results obtained by Moore et al. [15]. A gradual decrease in this indicator during SF indicated an increase in physical performance, with the changes being more pronounced in the ID group. The noted decrease in physical performance during the first month of SF may be due to various factors [18], including a decrease in plasma volume [19], a decrease in left ventricular mass [20, 21], and muscular atrophy [22, 23]. Significantly lower pulse value indicators in the ID group, starting from the second flight session, may indicate the efficiency of periodization of the training process in maintaining muscle strength and performance in SF conditions.

In other space agencies, the periodization of physical training is carried out in limited volumes. At the National Aeronautics and Space Administration (NASA), treadmill training involves a gradual increase in axial load from 60% to 80%; training protocols are created individually for each crewmember, or the astronaut performs training at his discretion. The ARED strength simulator uses a training periodization that includes two three-month macrocycles. Each macrocycle consists of four mesocycles of three weekly microcycles. The microcycle consists of six workouts performed with heavy (4 sets, 6 repetitions), light (4 sets, 12 repetitions), and medium (4 sets, 8 repetitions) loads sequentially, using three training protocols. In the mesocycle, workouts vary such that each protocol uses a hard, light, and medium day. In each microcycle, there is a gradual increase in the weight of the load by 5% from the repeated maximum. In the second three-month macrocycle, the weight of the load increases based on the values obtained in the last weeks of the first macrocycle [24].

In the European Space Agency, the entire flight period is conditionally divided into three stages, similar to the Russian prevention system. These include the initial (the first 20 days of SF), the main (about 130–150 days), and the final (15–30 days before landing) stages. At the initial stage of the flight, the load on the treadmill and bicycle ergometer is low and gradually increases at the discretion of the crew. On a strength simulator, the load is 50–60% of the repeated maximum. At the main stage, the load is increased gradually on all the tools used. At the final stage, the load is maintained at a high level, while the proportion of workouts performed on a strength simulator and a treadmill increases, and the number of workouts on a bicycle ergometer decreases [25].

Thus, in all the considered systems of prevention of the negative effects of weightlessness, the periodization of the training process is not implemented at its fullest. The main approach is to gradually increase the load (speed, axial load, weight of the load); however, none of

the considered systems takes into account differences between the adaptation phases, as well as the time difference between recovery and adaptation during the training process.

CONCLUSION

In our study, the use of locomotive training, taking into account the principles of periodization and individualization, allowed the crewmembers to perform testing with a greater power and a greater amount of work than the training recommended by on-board documentation.

The indicator of heart beats per distance (pulse value) in the group of cosmonauts performing individual training was lower, despite the greater volume of load and the developed power.

Thus, the use of the principles of periodization and individualization in the conditions of SF has a greater preventive effectiveness compared to standard on-board training.

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OXIDATIVE STRESS SIGNS IN BLOOD PROTEOME ANALYSIS OF FEMALE VOLUNTEERS IN FIVE-DAY DRY IMMERSION TEST

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Introduction. Dry immersion is a model for reproducing the physiological effects of weightlessness. Such tests allow assessment of changes in the functions of the cardiovascular, musculoskeletal, and other body systems. Mass spectrometry instruments can be used for blood proteome analysis in order to establish mechanisms of physiological adaptation to spaceflight factors simulated in dry immersion tests.

Objective. To clarify molecular participants in the acute period of adaptation of physiological systems to the conditions of simulated microgravity according to proteome analysis of dry blood spots of participants in a five-day dry immersion test.

Materials and methods. The study involved eight healthy female volunteers (average age 30 ± 4.8 years). Blood proteins were analyzed using the method of dried blood spots; capillary blood dried on a special paper filter (Perkin Elmer) was used. The enzyme cleavage of proteins was performed using trypsin (Thermo Scientific, USA). Mixtures of tryptic peptides were analyzed by liquid chromatography-mass spectrometry (LC–MS) on a Dionex Ultimate 3000 nano-HPLC combined with a TimsTOF Pro mass spectrometer. Mass spectrometric analysis was performed using the method of parallel accumulation with sequential fragmentation (PASEF). The obtained LC–MS/MS data were semi-quantitatively analyzed using DIA-NN 1.8.1. Statistical analysis was performed in the Statistica 12 software package.

Results. The molecular response to immersion conditions was found to lead to increased levels of antioxidant defense proteins, activation of catabolism processes and the pentose phosphate pathway. The levels of negative regulators of endopeptidases and iron homeostasis proteins decreased. The revealed elevated levels of NADPH oxidase activators indicate activation of NADPH oxidase under experimental conditions. These results may indicate the development of oxidative stress during immersion.

Conclusions. The identified molecular participants in the female body's response to immersion conditions can provide information about the signaling pathways and mechanisms involved in the response to hypokinesia, and in the future will contribute to the development of pharmacological measures to support the health of female astronauts. These results may also be useful for understanding the processes leading to adverse effects in people with low levels of physical activity.

Keywords: dry immersion; proteome; chromatography-mass spectrometry; dry blood spots; oxidative stress

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Compliance with the ethical principles: the research protocol was approved by the Bioethical Commission of the Institute of Biomedical Problems of the Russian Academy of Sciences. (Protocol No. 544 of 16.07.2020) in compliance with the principles of the 1964 Helsinki Declaration. All participants voluntarily signed an informed consent after having been explained the potential risks, benefits, and nature of the upcoming study.

Potential conflict of interest: the authors declare no conflict of interest.

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ПРИЗНАКИ РАЗВИТИЯ ОКИСЛИТЕЛЬНОГО СТРЕССА ПРИ АНАЛИЗЕ ПРОТЕОМА КРОВИ ЖЕНЩИН-ДОБРОВОЛЬЦЕВ В ЭКСПЕРИМЕНТЕ С 5-СУТОЧНОЙ «СУХОЙ» ИММЕРСИЕЙ

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Введение. «Сухая» иммерсия, являясь моделью для воспроизведения физиологических эффектов невесомости, позволяет оценить изменения функций сердечно-сосудистой, опорно-двигательной и других систем организма. Исследования протеома крови на основе масс-спектрометрии могут способствовать выявлению механизмов физиологической адаптации к факторам космического полета, моделируемых в «сухой» иммерсии.

Цель. Расширение научных представлений о молекулярных участниках острого периода адаптации физиологических систем к условиям моделируемой микрогравитации по данным протеомного анализа сухих пятен крови участниц 5-суточной «сухой» иммерсии.

Материалы и методы. В исследовании приняли участие 8 здоровых женщин-добровольцев (средний возраст 30,0 ± 4,8 года). Белки крови анализировали по методу «сухих пятен крови»; использовали капиллярную кровь, высушенную на специальном бумажном фильтре (Perkin Elmer). Ферментное расщепление белков проводили с использованием трипсина (Thermo Scientific, США). Смеси триптических пептидов анализировали методом жидкостной хромато-масс-спектрометрии на хроматографе нано-ВЭЖХ Dionex Ultimate3000, совмещенном с масс-спектрометром TimsTOF Pro. Масс-спектрометрический анализ проводили с использованием метода параллельного накопления при последовательной фрагментации (PASEF). Полученные данные LC-MS/MS были полуколичественно проанализированы с помощью DIA-NN 1.8.1. Статистический анализ проводили в программе Statistica 12.

Результаты. Обнаружено, что молекулярный ответ на условия иммерсии связан с повышением уровней белков антиоксидантной защиты, активацией процессов катаболизма и пентозофосфатного пути. Уменьшались уровни отрицательных регуляторов эндопептидаз, белков гомеостаза

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железа. Выявленные повышенные уровни активаторов НАДФН-оксидазы свидетельствуют об активации НАДФН-оксидазы в условиях эксперимента. Эти результаты могут указывать на развитие окислительного стресса во время иммерсии.

Выводы. Выявленные молекулярные участники ответа женского организма на условия иммерсии могут предоставить информацию о сигнальных путях и механизмах, задействованных в ответе на гипокинезию, и в последующем будут способствовать разработке фармакологических мер поддержки здоровья женщин-космонавтов. Эти результаты также могут быть полезны для понимания процессов, приводящих к неблагоприятным последствиям у людей с низким уровнем двигательной активности.

Ключевые слова: «сухая» иммерсия; протеом; хромато-масс-спектрометрия; сухие пятна крови; окислительный стресс

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Соответствие принципам этики: протокол исследования был одобрен биоэтической комиссией Института медико-биологических проблем РАН (протокол № 544 от 16.07.2020) и полностью соответствовал принципам Хельсинкской декларации 1964 г. Все участницы исследования добровольно подписали информированное согласие после объяснения им потенциальных рисков, преимуществ и характера предстоящего исследования.

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INTRODUCTION

Dry immersion (DI) is an effective ground-based model for assessing the impact of initial stages of space flight on the astronaut health. In comparision with other models, such as head-down bed rest (HDBR), DI shows a higher adequacy due to the possibility of simulating physiological changes similar to those in the initial period of space flight and their dynamic monitoring [1]. DI simulates a lack of support, immobilization, hypokinesia, and centralization of body fluids, i.e., phenomena observed during space flight [2]. This model can be used to evaluate microgravity-induced changes in the functions of the vestibular apparatus, cardiovascular, musculoskeletal, somatosensory, and other body systems.

The biological response of the human body to spaceflight conditions is manifested in pronounced oxidative stress, which is capable of causing damage to all cellular structures, including DNA. Oxidative stress occurs when the production of free radicals exceeds the natural antioxidant capacity of the cell [3]. Exposure to microgravity and cosmic radiation increases the production of reactive oxygen and nitrogen species (ROS and RNS), thus disrupting the functions of the cardiovascular system and bone tissue [4]. An increase in the level of 8-oxoguanosine (a product of DNA oxidation) in the urine of 59 astronauts was also shown [5]. At the physiological level, oxidative stress and redox imbalance contribute to disturbances in the regulation of metabolism of the cardiovascular, immune, and nervous systems associated with space flight [6].

During ground-based experiments, an increase in various parameters was also observed, indicating the development of oxidative stress [7, 8] as a result of either increased generation of ROS or dysfunction of antioxidant defense systems [9]. Thus, an increase in the marker of oxidative DNA damage — 8-OH-deoxyguanosine — was detected in HDBR, accompanied by an increase in the excretion of markers of bone resorption [N-telopeptide type I collagen (NTX), pyridine crosslinking, deoxypyridinoline] [8]. An increase in iron reserves during space flight is also associated with an increase in oxidative DNA damage and bone loss [10]. During experiments with hypokinesia, the growing ROS generation in muscles not only affects bone metabolism but also leads to changes in the activity of antioxidant systems [11].

The action of microgravity and hypokinesia on human physiological systems is actively studied, including using postgenomic methods. Mass spectrometry-based proteome studies aimed at analyzing dynamic changes in blood proteins, tissues, etc., extend the existing understanding of physiological adaptation mechanisms to spaceflight factors simulated in DI tests.

Thus, previous five-day dry immersion studies detected an increase in the level of free bilirubin and myoglobin in the blood serum, thereby indicating increased levels of hemolysis and myolysis. Increased levels of hepcidin, ferritin, and haptoglobin were also shown, which was attributed to increased levels of serum iron [12]. When comparing changes in iron metabolism in males and females who underwent a five-day DI test, an increase in the systemic availability of iron and serum hepcidin levels was revealed, which indicates an incorrect distribution of iron in these conditions, regardless of the gender [13].

At the same time, changes in the level of a number of proteins both after space flights and in ground-based experiments under conditions of 21-day HDBR and DI were revealed. These proteins include A1BG, A2M, SERPINA1, SERPINA3, SERPING1, SERPINC1, HP, CFB, and TF. This observation indicates changes in the processes affected by microgravity, i.e., hemostasis, platelet degranulation, and protein metabolism [14]. A three-day DI involving female volunteers found significant changes in the transcriptomic

profile in the human soleus muscle and a decrease in tissue respiration stimulated by adenosine diphosphate (ADP); however, no changes in the content of mitochondrial proteins/respiratory enzymes was observed. This indicates a regulation disorder of cellular respiration processes. Downregulated RNAs were closely related to mitochondrial function, as well as to lipid metabolism, glycolysis, insulin signaling, and various transporters [15]. In that experiment, the proteome analysis of dry blood spots revealed intracellular proteins with an increased level of expression, which are involved in the processes of pentose phosphate shunt (PGM2, TKT, BPGM). At the same time, the level of extracellular proteins decreased (ALBU, APOA4, AGT, LUM, HPX, SERPINA7) [16].

In the present work, we aim to study the molecular markers of the acute period of physiological adaptation of healthy females to the conditions of simulated microgravity in a five-day dry immersion test according to the proteome analysis of extracts of dry blood spots.

MATERIALS AND METHODS

Design of a five-day dry immersion experiment

Dry immersion is a model for ground-based reproduction of the physiological effects of weightlessness. In our research, testing was organized using the facilities of the Institute for Biomedical Problems of the RAS and conducted at the dry immersion bench base. Eight young healthy female volunteers (average age 30±4.8 years) participated in the study. Each study participant voluntarily signed an informed consent after having been explained the potential risks, benefits, and nature of the upcoming study. A board of medical experts confirmed that all the subjects were in good health and had a normal body mass index. During the period of dry immersion, the subjects were not subjected to any additional effects aimed at correcting adaptive changes in physiological systems. At the onset of the experiment, the female volunteers were synchronized according to the phase of the menstrual cycle (follicular phase) in order to avoid differences in hormonal effects on the studied parameters.

Collection of capillary blood specimens

For the blood proteome analysis, capillary blood dried on a special paper filter (Perkin Elmer) was used (dried blood spot, DBS). The advantages of this method lie in its simplicity and low invasiveness, which makes it possible to collect specimens with high accuracy. Capillary blood specimens were taken from volunteers two days before the onset of the experiment (baseline), on days 1, 3, and 5 during the DI experiment, and two days after the completion of immersion (post-DI). The specimens taken prior to immersion served as a control. Capillary blood was taken from the subjects from the terminal phalanx of the ring finger with an automatic scarifier. Blood in the amount of 20 μL was taken using an automatic pipette; a drop of blood was placed on a special filter paper, dried for 2 h, and stored at minus 20°C.

Dry blood spots were excised and placed in microcentrifuge tubes. Proteins were then extracted, reduced, alkylated, and precipitated as described in [16]. The enzyme cleavage of proteins was performed using trypsin (Thermo Scientific, USA). The obtained mixtures of tryptic peptides were analyzed by liquid chromatography–mass spectrometry (LC–MS) on a Dionex Ultimate 3000 nano-HPLC chromatograph (Thermo Fisher Scientific, USA) combined with a TimsTOF Pro mass spectrometer (Bruker Daltonics, USA). The peptides were separated by an emission packed column (C18, 25 cm×75 μm×1.6 μm; Ion Optics, Parkville, Australia) at a flow rate of 400 nL/min by gradient elution of 4–90% of phase B for 40 min. Mobile phase A consisted of 0.1% formic acid in water, and mobile phase B consisted of 0.1% formic acid in acetonitrile.

Mass spectrometric analysis was performed using the method of parallel accumulation with sequential fragmentation (PASEF). The electrospray ionization (ESI) source operated at a capillary voltage of 1500 V, an end plate offset of 500 V at a temperature of 180°C. The measurements were carried out in the m/z range 100–1700 Th. The ion mobility ranged 0.60–1.60 V s/cm^2. The total cycle time was 1.88 s, and the number of PASEO MS/MS scans was set to 10.

The obtained LC–MS/MS data were semi-quantitatively analyzed using DIA-NN 1.8.1. The specified restrictive parameters were as follows: the mass accuracy of the fragments was 1.5e-05 (MS2) and 2e-05 (MS1); the enzyme was trypsin; the maximum number of missing bonds was 3; the fixed modification was carbamidomethyl (C). The threshold for the frequency of false detections (FDR) was set at 0.01. A semi-quantitative analysis was performed using normalized peak intensities in the MS spectra, which reflects the relative levels of proteins in the samples.

Statistical analysis was performed in the Statistica 12 software using the nonparametric Mann–Whitney test (p-value < 0.05). The biological processes, in which the identified proteins are involved, were determined using the STRING web resource[1].

RESULTS

As a result of the proteome analysis of DBS samples of female volunteers, 829 different proteins were identified, with about 700 proteins being detected in each sample. When comparing the protein levels at each point of the experiment on day 1, 3, and 5 of DI and post-DI (on the 2nd day after the end) compared to baseline (2 days before the experiment), about 214 proteins were identified. The relative levels of such proteins significantly changed during different periods of the experiment. Upon lengthening of the experiment period, statistically significant differences in the number of proteins responsible for various pathophysiological processes in the body is observed.

On day 1 of immersion, the female participants showed an increase in the levels of four proteins and a decrease in the level of one protein compared to the baseline levels. On day 3, the levels of 18 proteins increased and the levels of 28 proteins decreased compared to the baseline. On day 5, the levels of 43 proteins increased, and 32 proteins decreased. At the same time, an unexpected result was variations in the protein level during the recovery period. Thus, 139 proteins underwent variation, out of which 59 and 80

[1] Search Tool for the Retrieval of Interacting Genes/Proteins — https://string-db.org

proteins showed elevated and reduced levels compared to the baseline, respectively.

For all proteins, whose relative levels increased during immersion, nine biological processes were identified using the Gene Ontology (GO) database (Fig. 1A). The most reliable process was the detoxification of cellular oxidants, which included proteins of the CAT, TXNDC17, PRDX1, TXN, TXNRD1, HBB, and HBD genes. Some of these proteins were involved in the process of hydrogen peroxide catabolism (CAT, PRDX1, HB, HBD). Other processes to note were catabolism and biosynthesis of glyceraldehyde-3-phosphate, which is a key intermediate of hexose metabolism in such biochemical processes as glycolysis, gluconeogenesis, pentose phosphate shunt, etc.

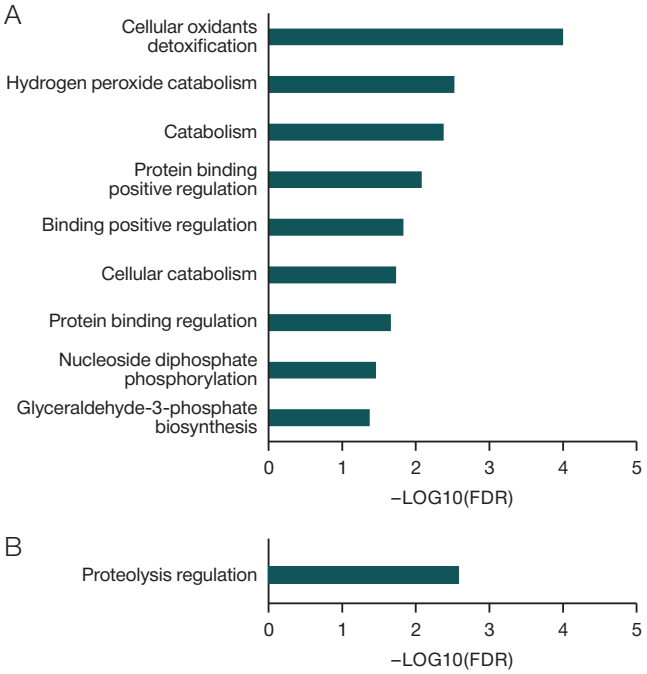

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Fig. 1. Statistically significant biological processes involving proteins with altered levels, according to the Gene Ontology database
Note: A — biological processes that include all proteins with significantly elevated levels during immersion; B — biological processes that include all proteins with significantly reduced levels during immersion.

Among the biological processes involving proteins with reduced levels during DI (HRG, F12, PEBP1, SERPINA10, AMBP, CLEC3B, USP9X, FN1, SERPINC1, GSN, PSMC2, SERPINA4), only one was identified — regulation of proteolysis (Fig. 1B). Among these proteins involved in the regulation of proteolysis, half of the proteins were protease inhibitors (histidine-rich glycoprotein (HRG); hippocampal cholinergic neurostimulating peptide (PEBP1); protein Z-dependent protease inhibitor (SERPINA10); inter-alpha-trypsin light chain inhibitor (AMBP); antithrombin-III (SERPINC1); callistatin (SERPINA4). A decrease in protease inhibitors is likely to enhance proteolysis under immersion conditions.

The biological process of cellular oxidants detoxification, which is part of antioxidant protection, demonstrated the most statistically significant change. This change was manifested in an increase in protein levels involved in this process. Table 1 lists the antioxidant protection proteins, the levels of which varied throughout the dry immersion period.

Figure 2 presents a scheme of involvement of antioxidant protection proteins in response to immersion conditions. On day 3 of DI, protein 17 containing the thioredoxin domain (TXNDC17), peroxiredoxin-1 (PRDX1), catalase (CAT), and the glutamate-cysteine ligase modifier (GCLM) subunits showed an increase. These subunits exhibit peroxidase activity and promote the removal of cellular hydrogen peroxide, an active form of oxygen capable of damaging cellular components. On day 5, thioredoxin (TRX) and thioredoxin reductase 1 (TXNRD1) were involved in the

Table 1. Dynamics of changes in levels of antioxidant protection proteins during and after dry immersion as a percentage compared to the baseline levels

Proteins	Genes	Changes in protein levels compared to baseline, %			
		DI period, days			
		1	3	5	post-DI
catalase	CAT	109.7	119.7*	125.5*	114.3
peroxiredoxin 1	PRDX1	113.1	119.2*	113.1*	101.5
glutamate-cysteine ligase regulatory subunit	GCLM	112.9	119.1*	124.0*	114.0
glutamate-cysteine ligase catalytic subunit	GCLC	108.6	112.6	111.9	125.8*
thioredoxin domain-containing protein 17	TXNDC17	101.4	130.7*	121.7*	118.5
thioredoxin reductase 1, cytoplasmic	TXNRD1	117.8	122.4	147.2*	128.6
thioredoxin	TXN	118.1	114.7	150.6*	123.8
hemoglobin subunit delta	HBD	118.2	109.5	126.6*	108.4
hemoglobin subunit beta	HBB	109.6	113.3	124.4*	118.1*
hemoglobin subunit theta 1	HBQ1	103.5	126.7	128.4	143.7*
superoxide dismutase	SOD1	112.6	118.5	115.2	134.2*

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Note: *significant changes compared to the baseline levels (p-value < 0.05).

response. Thioredoxin reduction is carried out by thioredoxin reductase, which uses a single NADPH molecule for this purpose. The levels of beta and delta subunits of hemoglobin (HB, HBD) also demonstrated a growing trend during this period.

The molecular response to the completion of immersion and return to habitual living conditions was also associated with an increase in proteins involved in this process. Thus, The levels of superoxide dismutase (SOD1), beta- and theta-1 subunits of hemoglobin (HB, HBQ1) increased, while those of PRDX1, CAT, TSN, TXNDC17, and TXNRD1 recovered to the pre-experimental values.

In addition, processes associated with antioxidant protection and oxidative stress, such as the pentose phosphate pathway, NADPH oxidase regulation, and iron homeostasis, were also identified. Thus, a significant increase (p-value<0.05) in the proteins of the pentose phosphate pathway (transketolase (TKT) and phosphoglucomutase-2 (PGM2) (Fig. 3A) was noted, presumably required for the formation of NADPH as one of the main components of the antioxidant defense system.

In addition, in our experiment, after the completion of immersion, a significant increase in the level of Ras-related C3 botulinum toxin substrate 1 (RAC1), which is a NADPH oxidase regulatory subunit that stimulates its activity, was detected compared to the baseline level before immersion (Fig. 3B). Another NADPH oxidase regulator is CDC42 [17], which, according to our data, was elevated on day 3 of DI and after the completion of immersion (Fig. 3B). In this chain of interactions, an important link is the adapter protein beta-parvin PARVB (the level was increased on day 3 of immersion). This protein plays a role in the transmission of integrin signals through the integrin-bound kinase ILK (the level was increased only after the completion of immersion) (Fig. 3B) and the activation of the aforementioned GTPases CDC42 and RAC1.

Attention should be paid to decreased levels of iron ion homeostasis proteins (V-type proton ATPase catalytic subunit A (ATP6V1A), BolA-like protein 2 (BOLA2)), which were observed during dry immersion (Fig. 3B). A steady decrease in the levels of these proteins, starting on day 3 of immersion, may indicate changes in iron metabolism caused by experimental conditions.

DISCUSSION

The molecular response to five-day immersion conditions is primarily associated with increased levels of antioxidant defense proteins and proteins of the pentose phosphate pathway, which is important for NADPH generation. Interestingly, our three-day immersion experiment conducted earlier also observed an increase in proteins of the pentose phosphate pathway, including the aforementioned PGM2 and TKT [18]. Since the material for the proteomic study was protein extracts of dry blood spots (which, in addition to plasma proteins, also contained cytosol proteins of destroyed blood cells), we believe red blood cells, as the most represented cells of the bloodstream, to make the main contribution to changes in these proteins. In erythrocytes, the pentose phosphate pathway of glucose oxidation provides anabolism processes, being not associated with energy production. In erythrocytes, only NADPH is formed as a product of the pentose phosphate pathway. In this case, pentose is not the final product, but turns into phosphohexose, which closes the cycle or passes into glycolysis, completing the shunt. NADPH is an important component of antioxidant protection. This component is necessary for the regeneration of glutathione, which destroys reactive oxygen species (ROS) together with glutathione peroxidase, as well as for the restoration of thioredoxin with the participation of thioredoxin. Since NADPH is formed in erythrocytes only in reactions of the pentose phosphate shunt, an increase in the concentration of pentose phosphate shunt proteins may be a response to oxidative stress. The results of studies of erythrocyte metabolism conducted before and after space flight showed changes in the metabolic status of cells, expressed in a decrease in the activity of the processes of the regenerative system (a decrease in reduced glutathione) [19], which confirms our assumption.

It has been repeatedly shown that the lack of physical activity increases ROS generation in muscles [11], activates the glutathione system [11, 20], and affects the activity of antioxidant systems [11]. Glutathione is a component of one of the main antioxidant systems stimulated both in muscles and at the systemic level (in liver and red blood cells) during activation of oxidative processes [21]. Therefore, it is

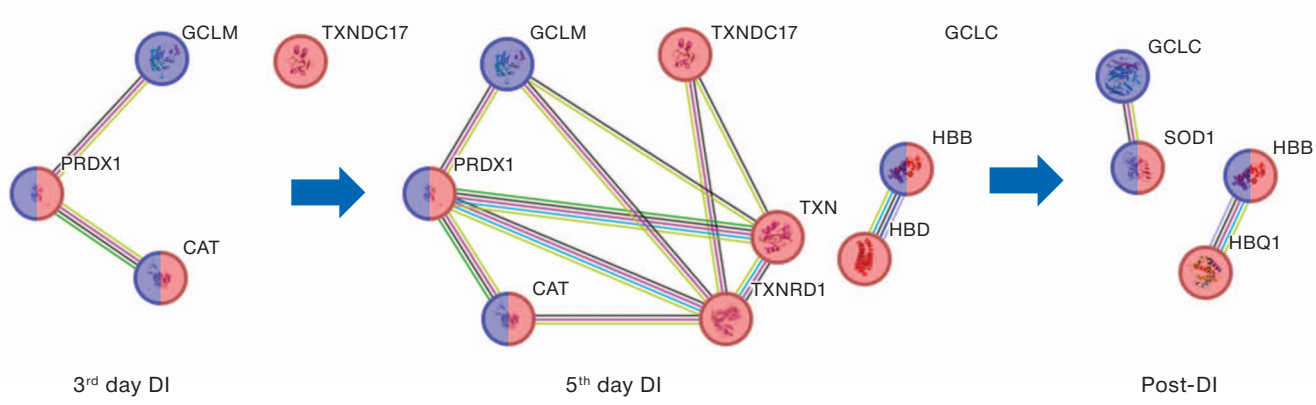

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Fig. 2. Relationship of antioxidant protection proteins
Note: red — detoxification proteins of cellular oxidants; blue — proteins of the oxidative stress response; lines of protein–protein interactions: bright green — co-mentioned in Pubmed abstracts; crimson — experimentally determined protein interaction; black — protein co-expression; light blue — the interaction is specified in the verified databases; green — close arrangement of protein genes; blue — joint occurrence of protein genes; lilac — protein homology.

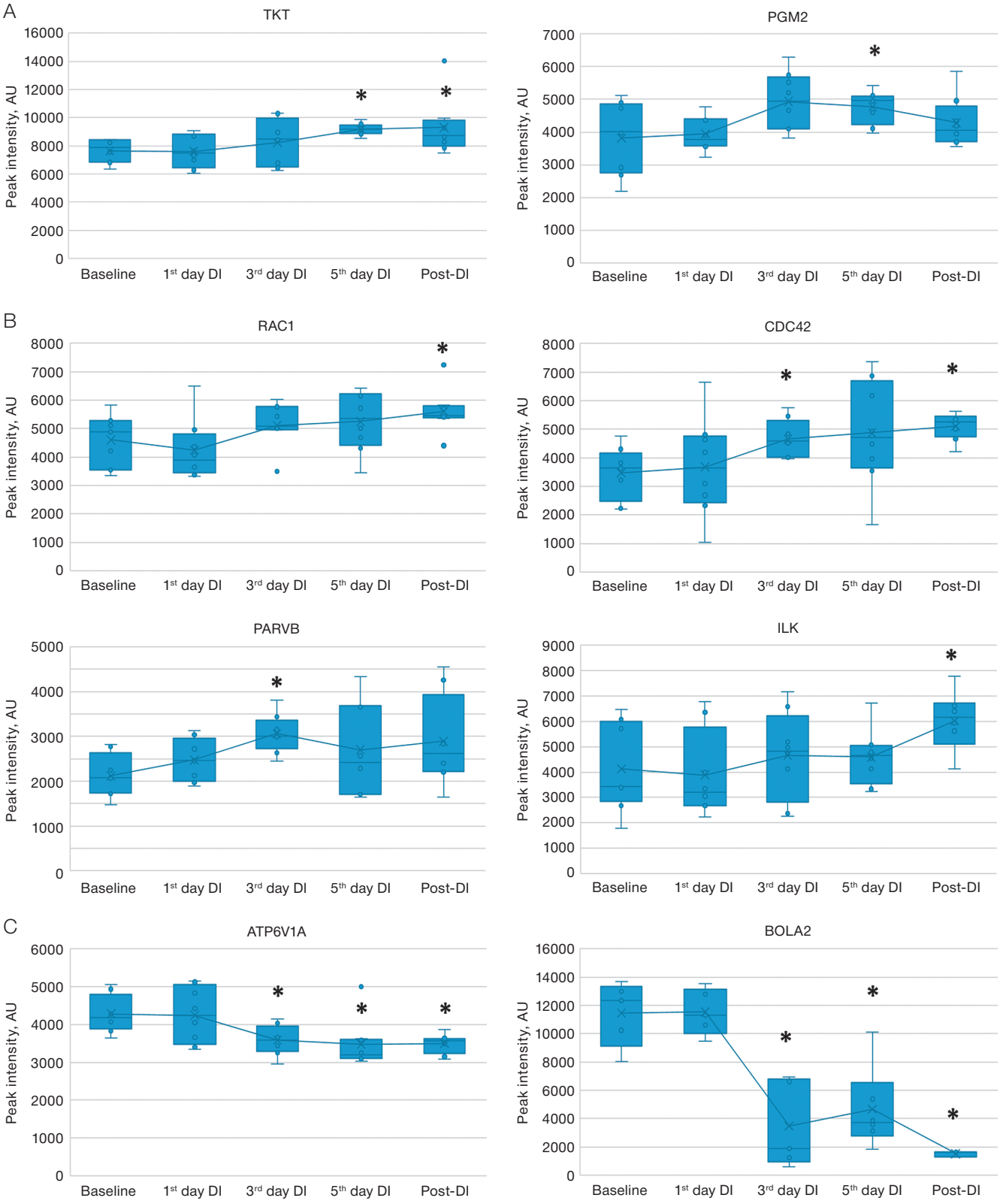

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Fig. 3. Changes in protein levels in dry blood spots of participants during a five-day DI test
Note: A — dynamics of changes in the levels of proteins of the pentose phosphate pathway; B — dynamics of changes in NADPH oxidase regulator levels; C — dynamics of changes in iron metabolism protein levels; *statistically significant changes p-value < 0.05; AU — arbitrary unit.

important for the cell to have sufficient NADPH to restore glutathione and protect against oxidative stress.

We believe that the main cause of oxidative stress observed both during space flight and in model experiments is the effects of physical inactivity and hypokinesia in muscle tissue. There are two main sources of ROS in muscle fibers, i.e., mitochondria, which form ROS during incomplete conjugation of oxidation and phosphorylation [22], and NADPH oxidase-2 (NOX-2), localized in the sarcolemma and inner membranes [23]. It was shown that muscle unloading leads

to a decrease in mitochondrial respiration and changes in the operation of NADPH oxidase-2.

NADPH oxidases as the main sources of reactive oxygen species (ROS) in cells continue to attract research interest due to their exceptional function of generating ROS under normal physiological conditions.

Increased levels of NADPH oxidase regulators RAC1_HUMAN, CDC42, as well as proteins signaling these GTPases, PARVB, and ILK during and after immersion confirm the role of NADPH oxidase in ROS generation under hypokinesia conditions and the development of oxidative stress. It is believed that oxidative stress is one of the ways to activate signaling pathways responsible for reducing muscle protein synthesis and activating proteolysis, which subsequently leads to muscle atrophy [24, 25].

The modern lifestyle of populations residing in urban agglomerations raises the importance of creating means to prevent the development of oxidative stress and its consequences caused by insufficient physical activity. Indeed, the lack of physical activity leads to increased generation of ROS by vascular cellular components and deteriorates endothelium-dependent vasorelaxation, which contributes to endothelial dysfunction and the development of atherosclerosis. Statins were reported to have a beneficial effect on the cardiovascular system, preventing cardiovascular diseases by blocking RAC1 and NADPH oxidase, thereby reducing ROS generation [26]. It should be noted that countering oxidative stress can also reduce the loss of muscle mass during physical inactivity. Thus, in animal models of hypokinesia, the addition of antioxidants prevented atrophy [27].

The second reason for increased levels of protection proteins from oxidative stress, as well as decreased levels of iron ion homeostasis proteins (ATP6V1A, BOLA2), observed in our dry immersion experiment (Fig. 3B), may be related to increased hemolysis. Activation of hemolysis was detected both in model experiments and during space flight [12, 28], although the causes of this phenomenon remain unknown. A thorough assessment of the iron status, as well as hematological reactions, was carried out in a five-day DI experiment involving 20 healthy males [12]. Immersion was found to increase the concentration of iron in the spleen, while iron reserves in the liver were not affected. Sequestration of iron in the spleen was accompanied by an increase in the level of hepcidin in the blood, which suppresses the absorption of iron in the intestine. An increase in the blood level of unconjugated bilirubin, which is normally formed as a result of the breakdown of proteins containing heme (hemoglobin, myoglobin), as well as an increase in myoglobin levels confirm that dry immersion promoted hemolysis and myolysis. These phenomena may explain the simultaneous increase in serum iron levels and transferrin saturation observed in the study [12].

Iron metabolism is strictly controlled at both the systemic and cellular levels. On the one hand, iron is necessary to support many processes in the body; on the other, iron overload can lead to the formation of highly reactive particles during the interaction of labile iron with ROS, which are naturally produced during aerobic respiration in the respiratory chain of mitochondria. These highly reactive particles induce oxidative stress. It is assumed that iron overload contributes to the development of osteoporosis and muscular atrophy by the mechanism described above [29, 30].

CONCLUSION

The molecular response to five-day immersion conditions involves changes in the protein composition of extracts of dry blood spots, which leads to increased levels of antioxidant protection proteins, catabolism, protein binding, phosphorylation of nucleoside diphosphates, metabolism of glycolysis intermediates and the pentose phosphate pathway, as well as decreased levels of negative regulators of endopeptidases, iron homeostasis proteins. An interesting finding in our study was the activation of the cellular antioxidant system. Elevated levels of proteins of the pentose phosphate pathway and antioxidant protection may indicate the development of oxidative stress during immersion. Altered iron metabolism may also contribute to ROS generation. The revealed elevated levels of NADPH oxidase activators indicate activation of NADPH oxidase under the conditions of physical inactivity.

The modification of iron metabolism and hemolysis processes, which was observed when simulating the effects of microgravity and hypokinesia, can also contribute to the development of oxidative stress. These observations underscore the importance of iron metabolism assessment in patients with limited mobility. The identified molecular participants in the body's response to immersion conditions can provide information about signaling pathways and mechanisms involved in the response to hypokinesia or simulated microgravity. The results can be used when developing pharmacological measures to support the health of both astronauts and people with lower levels of physical activity.

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POSSIBILITY OF SWITCHING OF A BIOLOGICAL DRUG WHEN TREATING CHILDHOOD ASTHMA (A CLINICAL CASE)

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Introduction. Genetically engineered biological drugs (GEBD) are widely used in the treatment of children with uncontrolled bronchial asthma (BA). In Russia, several GEBD have been registered for the treatment of children and adolescents with asthma, including anti-immunoglobulin E/anti-IgE (omalizumab), anti-interleukin 5/anti-IL-5Ra (mepolizumab), and anti-IL-4Ra (dupilumab). The choice of GEBD depends on the BA phenotype and genotype. However, in pediatric practice, the difficulty of determining a BA endotype complicates the search for an effective drug. For this reason, there is a possibility of insufficient effectiveness of the recommended expensive therapy and the need to revise the treatment of GEBD in accordance with the phenotypic features of the disease.
Clinical case description. The paper presents a dynamic follow-up of a 7-year-old child with severe asthma and concomitant atopic dermatitis (AD) receiving GEBD therapy. The initial biological drug was omalizumab. Subsequently, due to insufficient control of the symptoms of the disease and exacerbation of severe atopic dermatitis, a switch to dupilumab was performed. The change in GEBD contributed to achieving control over BA symptoms and a relief of the skin condition.
Conclusions. Our observation shows the effectiveness and safety of switching between omalizumab to dupilumab in children with severe asthma and concomitant AD. Further research is needed to clarify the clinical profile of patients in order to determine predictors of an effective choice of biotherapy and resolve the issue of switching to various monoclonal antibodies.

Keywords: bronchial asthma; atopic dermatitis; Type 2 inflammation; genetically engineered biological drugs; omalizumab; dupilumab; children

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ВОЗМОЖНОСТИ ПЕРЕКЛЮЧЕНИЯ БИОЛОГИЧЕСКОГО ПРЕПАРАТА ПРИ ЛЕЧЕНИИ БРОНХИАЛЬНОЙ АСТМЫ У ДЕТЕЙ. КЛИНИЧЕСКОЕ НАБЛЮДЕНИЕ

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Введение. В терапии детей с неконтролируемой бронхиальной астмой (БА) активно используются генно-инженерные биологические препараты (ГИБП). В России для лечения детей и подростков с БА зарегистрировано несколько ГИБП: антииммуноглобулин Е (IgE) (омализумаб), антиинтерлейкин 5 (IL-5/анти-IL-5Rα) (Меполизумаб®) и анти-IL-4Rα (Дупилумаб®). Выбор ГИБП зависит от фенотипа и эндотипа БА. Однако в педиатрической практике определение эндотипа БА затруднительно, в связи с чем поиск эффективного препарата остается непростой задачей. По этой причине существует вероятность недостаточной эффективности рекомендованной дорогостоящей терапии и необходимости пересмотра лечения ГИБП в соответствии с фенотипическими особенностями заболевания.
Описание клинического случая. В работе представлено динамическое наблюдение за ребенком 7 лет с тяжелым течением БА и сопутствующим атопическим дерматитом (АтД), получающим в терапии ГИБП. Исходным биологическим препаратом был омализумаб. В последующем в связи с недостаточным контролем симптомов заболевания и обострением тяжелого атопического дерматита проведено переключение на Дупилумаб®. Смена ГИБП способствовала достижению контроля симптомов БА и купированию кожного синдрома.
Выводы. Наше наблюдение показывает эффективность и безопасность переключения с биологического препарата омализумаб на препарат Дупилумаб® у детей с тяжелым течением БА и сопутствующим АтД. Необходимы дальнейшие исследования для уточнения клинического профиля пациентов с целью определения предикторов эффективного выбора биологической терапии и решения вопроса о переходе на различные моноклональные антитела.

Ключевые слова: бронхиальная астма; атопический дерматит; Т2-воспаление; генно-инженерные биологические препараты; омализумаб; Дупилумаб®; дети

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Соответствие принципам этики: от законных представителей пациента получено письменное информированное добровольное согласие на публикацию описания клинического случая и обезличенных медицинских данных.

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INTRODUCTION

Bronchial asthma (BA) is a chronic heterogeneous respiratory disease with the incidence of about 400 million people worldwide[1]. In Russia, according to the results of epidemiological studies, 6.9% of adults and about 10% of children and adolescents suffer from BA[2].

Currently, BA treatment is aimed at achieving control over the symptoms of the disease and preventing exacerbations. Anti-inflammatory drugs form the basis of controlled drug therapy; however, BA therapy is selected individually according to the phenotype and age of the patient. The treatment volume increases or decreases depending on the controllability of the symptoms of the disease. According to the recommendations of the Global Initiative for Asthma (GINA), inhalant glucocorticosteroids (ICS) are a basic therapy in children under five years of age, while combined drugs — fixed combinations of ICS with long-acting β2-agonists (LABA) — can be used starting from the age of six-year-olds. In addition, from two years of age, antileukotrienes (leukotrienes receptor antagonists, LTRAs) are recommended as a baseline therapy. Provided high adherence and proper inhalation technique, the majority of patients (80%) respond positively to such a therapy with the achievement of symptom control. However, 5–10% of patients are resistant to standard therapy, having a high rate of BA exacerbations and emergency treatment [1]. Currently, the drugs of choice for this patient group are genetically engineered biological drugs (GEBD), which are selected based on the phenotype and endotype of the disease [1].

The BA phenotype is a combination of features that describes clinical differences between patient groups and largely determines the BA clinical outcomes. There are five main BA phenotypes in adults, i.e., allergic, non-allergic, with late onset, with fixed airway obstruction, and BA in obese patients [1, 2]. The allergic (atopic) BA phenotype is most common in pediatric practice. This phenotype is associated with a family history of atopic diseases, early onset in childhood, the presence of concomitant allergic diseases in the patient (allergic rhinitis, pollinosis, atopic dermatitis (AD)), being characterized by severe sensitization to allergens [1].

The BA endotype is a disease subtype characterized by a unique pathogenetic or molecular mechanism. One BA endotype may underlie several phenotypes [1–4]. There are two most common BA endotypes, i.e., with the dominance of T2 inflammation (T2-BA) and without it — non-T2-BA, and a mixed endotype. As a rule, non-T2-BA is characterized by neutrophilic or paucigranulocytic inflammation, whereas T2-BA is characterized by the presence of eosinophilic inflammation of the respiratory tract [4]. Eosinophilic inflammation in T2-BA is formed due to the involvement of Th2 lymphocytes and type 2 innate lymphoid cells (ILC 2), which produce excess T2-profile cytokines IL-4, -5, -13. The secretion of these cytokines triggers IgE-related hypersensitivity reactions in the lower respiratory tract, activating and maintaining the inflammatory process. The markers of the BA T2-endotype include an increase in immunoglobulin E (IgE) in blood serum, the eosinophil blood level >150 cells/μL and/or the number of sputum eosinophils >2%, and/or the level of nitric oxide in exhaled air (FeNO) >20 particles per billion (ppb) [3].

The above inflammatory endotypes served as a theoretical basis for the development of personalized approaches to BA therapy. In this regard, the creation of GEBD is a promising direction. The GEBD mode of action consists in binding to a certain determinant, e.g., a cytokine or a receptor, and blocking the further inflammatory process. Due to this selectivity, biologics are ideally suited for personalized or targeted medicine.

In Russia, three GEBD are currently used for BA treatment in pediatric practice. Among them are anti-immunoglobulin E/anti-IgE (omalizumab), anti-interleukin-5/anti-IL-5Ra (mepolizumab), and anti-IL-4Ra (dupilumab) [3]. These drugs have demonstrated their efficacy in BA treatment in clinical trials. In the setting of GEBD therapy, most patients showed a decreased relapse rate, improved BA control and lung function [5–7]. However, the effectiveness of therapy is largely determined by the correct choice of GEBD, which is based on the assessment of the patient's BA phenotype and BA endotype. In pediatric practice, the determination of the endotype and related biomarkers may be problematic. Thus, children with eosinophilic BA may not exhibit an increase in all markers of T2 inflammation, similar to adult patients. BA patients may be phenotypically similar, but have different responses to GEBD. This results in significant difficulties in selecting GEBD and their insufficient effectiveness in actual clinical practice [3, 5]. Thus, according to [8], about 10% of patients treated with GEBD experienced insufficient clinical response during therapy. In such cases, experts recommend considering changing the drug and switching to another monoclonal antibody [1]. Unfortunately, there are currently no clear clinical criteria for selecting the most effective biological drug, as well as evidence-based recommendations regarding the timing of the transition from one biological drug to another [9]. Switching to another biological drug is possible in case of insufficient control of BA during therapy, the presence of potential adverse events (hypereosinophilia, for example), and emergence of concomitant pathology (nasal polyps, AD) [1].

Omalizumab was the first monoclonal antibody (mAb) approved as an adjunctive therapy for patients with severe persistent allergic BA. It is a recombinant humanized IgG1 mAb that inhibits the binding of freely circulating IgE to the high-affinity IgE receptor (FcεRI) on the surface of both mast cells and basophils, thereby limiting the release degree of allergic reaction mediators. The omalizumab effectiveness in patients who responded to treatment is due to its inhibitory effect on the type 2 cytokines (IL-4, IL-5, and IL-13) release and eosinophil transport.

Numerous studies performed in different countries, including the Russian Federation, have shown the effectiveness of omalizumab in the treatment of adults and children >6 years of age with severe and moderate atopic BA, uncontrolled by high doses of ICS in combination with LABA [10, 11]. The atopic nature of BA must be proven

[1] GINA. Global Strategy for Asthma Management and Prevention. Global Initiative for Asthma, 2024.
[2] Bronchial asthma. Clinical practice guideline. Ministry of Health of Russia; 2024.

by significant sensitization (positive skin tests and/or the presence of specific IgE antibodies) to allergens. Total serum IgE should range within 30–1500 IU/mL in adults and children >12 years of age and 30–1300 IU/mL in children over six years of age. Omalizumab is administered subcutaneously at a dose of 150–375 mg every two or four weeks. The dosage and frequency are calculated based on the body weight and the total serum IgE level. The first evaluation of the effectiveness of omalizumab treatment is recommended after 16 weeks of treatment[3]. After this follow-up period, treatment may be discontinued due to a lack of efficacy.

Since the approval of omalizumab, a number of randomized clinical trials (RCTs) have demonstrated the therapeutic efficacy of subcutaneous administration of omalizumab [10, 12]. According to the literature [13], the highest therapy was observed in patients with sensitization to allergens. In a combined analysis of data from five RCTs, including 2236 patients with moderate and severe persistent allergic BA who received ICS in moderate and high doses, improved clinical outcomes during omalizumab treatment were associated with a decreased peripheral blood eosinophil count, while the worst clinical outcomes were associated with an increased peripheral blood eosinophil count [14].

The researchers in [15] obtained the data indicating the possibility of switching from omalizumab to other biological drugs (mepolizumab, dupilumab) with insufficient control of BA symptoms (for example, in patients with a high level of eosinophilic inflammation). However, there are no clear clinical criteria and biological markers to identify patients in whom such a drug switch will be effective.

Dupilumab is a human monoclonal antibody capable of inhibiting IL-4 and IL-13 signaling by specifically binding to their common IL-4R receptor component. IL-4 and IL-13 are the key factors in T2 inflammation, which plays an important role in the pathogenesis of many atopic diseases. Dupilumab may be recommended for children (with BA) ≥12 years of age. According to research data, the drug effectiveness has been proven in patients with increased eosinophil count and FeNO (i.e. ≥150 cells/μL and ≥25 ppb, respectively). It is important to note that dupilumab is the only effective GEBD in children with AD.

A sufficient number of clinical studies have confirmed the feasibility of applying dupilumab in BA treatment. In case of insufficient efficiency, it is recommended to switch to another GEBD.

Mepolizumab is a humanized monoclonal antibody (IgG1k) directed against human IL-5 and preventing its interaction with a specific receptor on the surface of eosinophils, initiating recovery of the IL-5-dependent eosinophil count to the physiological norm. Mepolizumab is indicated for children ≥6 years of age as an additional supportive therapy for severe BA with an eosinophilic profile of respiratory tract inflammation[4].

The GINA report also lists additional indications for the choice of GEBD. In addition to BA, mepolizumab has indications as a nasal polyposis and eosinophilic granulomatosis with polyangiitis (from the age of 18); omalizumab has indications as a chronic idiopathic urticaria (from the age of 12) and nasal polyposis (from the age of 18); mepolizumab has chronic rhinosinusitis with nasal polyposis (from the age of 18), eosinophilic esophagitis (from 12 years old) and moderate to severe AD with insufficient response to therapy with topical medications (from six months), which is especially important given the comorbidity in BA.

Thus, the choice of GEBD is a difficult task in clinical practice. The effectiveness of biotherapy depends on the correct assessment of the patient's clinical, anamnestic, laboratory, and instrumental data. An effective biological drug can only be selected based on a comprehensive assessment of the initial data based on determination of the BA phenotype and the expected BA endotype. At the same time, the insufficient effect of GEBD therapy and the appearance of concomitant pathologies is a reason for reassessing the patient's data followed by a possible decision to switch to another GEBD.

In this article, we present a clinical case of a patient with severe BA, who underwent a change of GIBT due to a worsening of the disease course and an AD exacerbation.

CLINICAL CASE DESCRIPTION

The 7-year-old patient was observed during the 2020–2024 period at the Federal Scientific and Clinical Center for Children and Adolescents. The child had been admitted with complaints of recurrent bronchial obstruction syndrome, persistent cough, and nasal congestion for a one year.

It became known from the patient's life history that the girl was born from the second physiological pregnancy, the second spontaneous vaginal delivery at term. The birth body length was 52 cm; birth weight was 3150 g. The Apgar score was 8/9. She was breastfed on the first day and was breastfed for up to 5.5 months. She grew and developed according to her age. She was vaccinated according to the National Calendar of Preventive Vaccinations. The hereditary history of allergic diseases was burdened: the maternal brother and maternal uncle suffered from hay fever, allergic rhinoconjunctivitis; the paternal grandmother suffered from asthma.

At the age of six months, skin rashes appeared after the introduction of complementary foods and the transfer to a milk formula. The skin process was widespread, located on the face, trunk, and limbs. The child was diagnosed with atopic dermatitis. Against the background of therapy with topical corticosteroids (TCS) and moisturizers, relief of the skin syndrome with positive age dynamics was noted. By the age of 1.5 years, the skin process was limited.

From the age of two, complaints of nasal congestion, rhinorrhea, and itchy eyes in the spring appeared. Constant nasal congestion was noted throughout the year. The examination revealed significant sensitization to pollen (birch, weeds), household (dust mites), and epidermal allergens (cat epithelium). Second-generation

[3] Xolair® (omalizumab) patient information leaflet. https://grls.rosminzdrav.ru/Grls_View_v2.aspx?routingGuid=2ace7eaa-ac77-48e5-9571-529dc017235a
[4] Nucala® (mepolizumab) patient information leaflet. https://grls.rosminzdrav.ru/Grls_View_v2.aspx?routingGuid=b7a6f6cf-2e9c-4718-91c9-442c9c294777

systemic antihistamines and intranasal corticosteroids were recommended for drug therapy, which significantly improved the patient's quality of life.

The first episode of bronchial obstruction was noted at the age of 2.5 years after contact with a cat. Subsequently, episodes of bronchial obstruction recurred 1–2 times a month against the background of acute respiratory viral infections, contact with animals. In the spring and summer periods, the episodes were observed almost daily. At the age of three, the child was diagnosed with BA followed by prescription of low doses of ICS (budesonide 250 μg/day via a nebulizer) as a control therapy. Subsequently, there were complaints of bouts of bronchial obstruction during physical exertion. LTRA (montelukast 4 mg/day) were added in the therapy. During therapy, partial control of BA symptoms was observed for 1.5 years: episodes of bronchial obstruction, cough occurred during the period of frequent acute respiratory viral infections, and in the spring. However, since the age of five, episodes of bronchial obstruction had become more frequent, occurring during daytime and night hours under the action of specific (animals, dusty premises, pollen allergens) and non-specific triggers (physical exertion, acute respiratory viral infections). An increase in basic therapy was recommended: ICS doses ranged from medium to high (budesonide 500–1000 μg/day). Due to severe BA exacerbations, the child was repeatedly hospitalized. Taking into account the lack of disease control from the age of six, fixed combinations of LABA with ICS (formoterol + budesonide 4.5/80 μg) at a dose ranging 2–4 inhalations per day were included in therapy, and the course of LTRA (montelukast 5 mg/day) was continued. During the following two years, combined therapy was used to control BA symptoms without severe exacerbations.

At the age of eight, the child's condition worsened. Due to a severe exacerbation of BA, the child was admitted to a hospital, where infusion therapy was performed along with the use of systemic corticosteroids and bronchodilators. During the last hospitalization, the child complained of almost daily episodes of bronchial obstruction, nocturnal symptoms (cough, shortness of breath, distant wheezing). The assessment of BA symptom control based on the results of the ACQ-5 (Asthma Control Questionnaire) was 4 points (uncontrolled BA). The girl continued to receive formoterol + budesonide 4.5/80 μg in therapy, two inhalations twice a day with periods of increasing the dose of ICS (up to 600 μg/day) due to the additional administration of budesonide, montelukast 5 mg/day.

Objective examination during the BA exacerbation

The general appearance was severe. Nasal breathing was very difficult. The respiratory rate was 28–32 per minute. Oxygen saturation was 89%. The chest was of the usual shape. The skin was dry with foci of hypopigmentation in the elbow folds area and the presence of desquamation around the lips. The chest was swollen. The auxiliary muscles were involved in the act of breathing. Percussion sound was vesicular resonance with a boxy tinge. Auscultation: harsh breathing was heard in all departments, wheezing. Blood pressure — 105/80 mmHg, heart rate — 98/min. Height — 142 cm, body weight — 39 kg.

During a comprehensive examination, congenital pathology and hereditary respiratory diseases, immunodeficiency conditions that might occur with the phenomena of bronchial obstruction were excluded. According to the results of an allergological examination (skin test and determination of specific IgE by ImmunoCAP), high sensitization to household allergens (house dust, dust mites), pollen allergens (tree pollen: birch; weeds: wormwood), epidermal (cat) allergens was confirmed. According to a pulmonary function test performed on a JAEGER APS pro device (Germany), restrictive changes were not detected (vital capacity/VC — 92%), but bronchial obstructions were detected (forced expiratory volume in 1 sec/FEV1 — 76%; peak expiratory flow rate at the level of the medium bronchi (PEFR 50) — 48%; peak expiratory flow rate at the level of the small bronchi (PEFR 75) — 34%), the bronchodilator test (salbutamol) was positive (FEV1+20%).

Thus, based on the clinical picture, the dynamics of the disease, clinical laboratory and instrumental diagnostic tests, the child was diagnosed with severe uncontrolled allergic bronchial asthma, I-II stage of respiratory failure, seasonal allergic rhinitis, seasonal allergic conjunctivitis, pollinosis, limited mild atopic dermatitis.

Disease dynamics on the therapy

In order to relieve bronchial obstruction, the child underwent infusion therapy (saline solution, euphyllin up to 12 mg/kg/day, prednisone 2 mg/kg/day) for three days, inhalation therapy through a nebulizer (budesonide 1000 μg/day, salbutamol 8 mg/day). On the therapy, the phenomena of BA exacerbation were stopped. Further, the child was prescribed a combination therapy with LABA/ICS (formoterol + budesonide 4.5/80 μg) two doses twice a day with additional administration of ICS (budesonide 200 μg), montelukast 5 mg/day.

In January 2020, due to the BA severity and insufficient control over the symptoms of the disease, it was decided to initiate GEBD therapy. Taking into account the atopic phenotype of BA (family and personal history of atopic diseases; bronchial obstruction to causally significant allergens from an early age) and the identified BA biomarkers (total IgE 345 IU/mL, polysensitization), GEBD omalizumab became the drug of choice. The dose of the drug was calculated based on the patient's weight and the total IgE level — 300 mg (150 mg in both hands) subcutaneously once every four weeks. On the combined therapy, following 16 weeks, control over BA symptoms was achieved: nocturnal symptoms were relieved, physical activity was increased, the ICS dose was reduced (budesonide 200 μg was discontinued), the ICS dose in combination LABA/ICS was reduced to medium doses, LTRA discontinued.

Due to the sufficient control of BA symptoms, omalizumab was discontinued in June 2022. Further, the child continued to receive basic therapy LABA/ICS (formoterol + budesonide 4.5/80 μg) — one dose twice a day in combination with LABA with sufficient control over BA symptoms. During the flowering period of the trees, the

child noted a decrease in the symptoms of allergic rhinoconjunctivitis. According to the ACB-5, the control of BA symptoms has significantly improved (ACQ-5 = 2 points).

However, six months after discontinuation of omalizumab therapy, despite the continuation of LABA/ICS control therapy, the child showed a catadrome after an acute respiratory illness. Bronchial obstruction attacks appeared 1–2 times a week at night, and physical activity decreased significantly. Despite the therapy correction with an increase in the dose of LABA/ICS to four inhalations per day, episodes of coughing and distant wheezing persisted. The ACQ-5 score was 4.5 points. During the BA exacerbation, high eosinophilia in the blood (780 cells/μl) and high eosinophilia in the rhinocytogram (41%) were noted.

At the same time, the child had an AD exacerbation: common skin rashes appeared (hyperemia, excoriation on the face, body, limbs, severe pruritus, affecting the child's sleep and quality of life). TCS was treated with antihistamines without a lasting positive effect.

Due to the severe course and insufficient control over BA symptoms and the concomitant severe course of AD, it was decided to re-initiate biotherapy. Taking into account the clinical picture of the disease with concomitant AD and uncontrolled BA, laboratory test data (high eosinophilia), it was decided to initiate a targeted therapy with switching of GEBD to dupilumab. The choice of this drug was justified by the severe AD and evidence-based medical data on the positive effect of dupilumab in patients with AD. The drug was administered in an age-appropriate dosage: an initial dose of 400 mg subcutaneously, followed by 200 mg once every two weeks. After 16 weeks of therapy, control over BA symptoms was achieved, with an ACQ-5 of 1.5 points. The girl started attending sports aerobics classes, showing a high exercise tolerance. In February 2024, given the good control over the BA symptoms, the basic therapy was reduced: the child was switched to low doses of LABA/ICS (formoterol + budesonide 4.5/80 μg).

On the dupilumab therapy, a marked relieve of AD was noted after four weeks: there was no exacerbation of the skin condition and pruritus. After 16 weeks, complete relief of the skin manifestations of AD was achieved.

Currently, in the setting of dupilumab therapy, the patient has a positive trend in the absence of AD symptoms and maintains control over the BA symptoms. The disease prognosis is favorable. Taking into account the high sensitization to birch allergens, allergen-specific immunotherapy (AIT) is planned.

CLINICAL CASE DISCUSSION

To date, the issue of selecting an optimal GEBD has not lost its relevance. The emergence of new biopharmaceuticals and an increase in their availability in practical healthcare raises the importance of criteria for the prognostic effectiveness of GEBD in patients. In this regard, the determination of the phenotypic features of the disease and the inflammatory phenotype of BA would be an optimal method for selecting patients with the most complete potential response to a particular type [1, 2].

Currently, it is recommended to determine biomarkers before prescribing biotherapy to predict the clinical response, such as the level of FeNO, the number of eosinophils in the blood and, if possible, in sputum, as well as allergen-specific IgE[5] [14, 16, 17]. It is also necessary to analyze the clinical and anamnestic data, such as the frequency of exacerbations and concomitant diseases, the volume and effectiveness of inhalation therapy, adherence to the use of basic BA therapy, and triggers of asthma exacerbation. However, due to the heterogeneity of the pathogenetic mechanisms of T2 inflammation, the choice of an effective drug may be difficult, especially in pediatric practice. Thus, according to [18], up to one third of patients with severe BA have overlapping criteria for prescribing four GEBD (mepolizumab, benralizumab, dupilumab, and omalizumab), and 75% of patients meet the requirements for prescribing two or more biologics. The study [19] showed that among patients (n = 101) suitable for treatment with mepolizumab, 27–37% also meet the criteria for prescribing omalizumab. It is important to note that not all patients have the same phenotype throughout their lives. Thus, the research study [20] on the temporal stability of AD phenotypes in adults (n = 3320) after 10 years of follow-up found that the initial phenotype was preserved in 54–88% of the study participants. The phenotype can be influenced by environmental factors, allergens, environmental factors, respiratory infection, and ICS therapy [20]. The choice of GEBD also depends on the presence of concomitant pathologies, such as AD, especially severe, since dupilumab is the drug of choice in pediatrics in this group of patients.

In turn, the wrong choice of a target for therapy, and, therefore, the starting monoclonal antibody, frequently leads to a replacement of the targeted drug and an exacerbation of the allergic disease. A retrospective cohort study using data from patients (n = 3531) with severe BA from 11 countries established that 10.8% of patients needed to switch to another GP, and 10.2% stopped treatment due to inefficiency or the development of adverse events [21].

Our patient had a clinically significant sensitization with a confirmed association between allergen exposure and the development of BA exacerbations, which led to a positive response to omalizumab therapy. However, shortly after discontinuation of biotherapy, insufficient control over BA symptoms and severe exacerbation of AD were noted, which was an indication for initiation of therapy with dupilumab. The change of the GEBD contributed to the positive dynamics during both the BA and the AD.

Thus, the described GEBD switching in a patient with severe BA was necessitated by the specific features of the disease course and exacerbation of severe AD.

CONCLUSION

GEBD are the therapy of choice in patients with uncontrolled BA; at the same time, their effect on the pathogenetic mechanisms of the disease differs. The above

[5] Bronchial asthma. Clinical practice guideline. Ministry of Health of Russia; 2024.

clinical case demonstrates the need for a personalized approach when prescribing this type of therapy, taking into account the clinical course of the disease and concomitant pathology in each individual patient.

At present, when deciding on the treatment strategy, as well as when switching between different GEBD, biomarker analysis is used as a basis, including the eosinophil count in peripheral blood and sputum, FeNO measurement, and serum IgE assessment. These biomarkers provide insight into the mechanisms of pathogenesis, allowing the therapy effectiveness to be monitored and the response to treatment to be predicted. However, it should be borne in mind that in patients with BA, the appearance/exacerbation of concomitant pathology (AD, nasal polyposis) may be an important reason for switching to a more effective GEBD.

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COMPUTATIONAL PHANTOM FOR RED BONE MARROW DOSIMETRY IN ADULT MALES AND FEMALES

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Introduction. The dose assessment of internal irradiation of red bone marrow (RBM) by osteotropic radionuclides is based on dosimetric modeling using computational phantoms. Creating such phantoms for ^{89,90}Sr requires careful description of the shape and size of bones, as well as their microarchitecture. Descriptions of phantoms representing newborn, one-year-old, 5-year-old, and 10-year-old children have been published. Our study continues work on creating a set of computational skeletal phantoms for people of different ages.

Objective. Development of computational skeletal phantoms of male and female adults for estimating radiation doses of beta-emitting radionuclides incorporated in RBM.

Materials and methods. The stochastic parametric skeletal dosimetry (SPSD) method of creating phantoms was used. The skeletal sections with active hematopoiesis were divided into segments. On the basis of literature data, the parameters of segment models were evaluated: linear dimensions, cortical layer thickness, bone microarchitecture characteristics, density, chemical composition, and RBM proportion.

Results. The developed phantoms of male and female adults are composite, including 46 segments each; the parameters of 21 segments were independent of sex. The sizes of segment phantoms range within 4–66 mm; the cortical layer thickness ranges within 0.3–2.2 mm. The parameters of bone segment microarchitecture are presented.

Conclusions. The resulting phantoms simulate the micro- and macro-architecture of bone tissue, and, together with sets of additional phantoms, represent the population variability of individual skeletal bones and take sex differences into account. The developed phantoms can be used for internal dosimetry of osteotropic beta-emitters, including as part of radiopharmaceuticals.

Keywords: trabecular bone; cortical bone; bone marrow dosimetry; computational phantoms; strontium; computational phantom of an adult

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ВЫЧИСЛИТЕЛЬНЫЙ ФАНТОМ ДЛЯ ДОЗИМЕТРИИ КРАСНОГО КОСТНОГО МОЗГА ДЛЯ ВЗРОСЛЫХ МУЖЧИНЫ И ЖЕНЩИНЫ

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Введение. Оценка доз внутреннего облучения красного костного мозга (ККМ) от остеотропных радионуклидов основана на дозиметрическом моделировании с использованием вычислительных фантомов. Создание таких фантомов для ^{89,90}Sr требует аккуратного описания формы и размеров костей, а также их микроархитектуры. В настоящее время опубликованы описания фантомов новорожденного, годовалого, 5-летнего и 10-летнего детей. Данное исследование — очередной этап работы по созданию набора вычислительных фантомов скелета для людей разного возраста.

Цель. Разработка вычислительных фантомов скелета взрослых мужчины и женщины для оценки доз в ККМ от инкорпорированных бета-излучающих радионуклидов.

Материалы и методы. В работе был использован SPSD-метод (stochastic parametric skeletal dosimetry) создания фантомов. Участки скелета с активным гемопоэзом разделяли на сегменты. По литературным данным были оценены параметры моделей сегментов: линейные размеры, толщина кортикального слоя, характеристики костной микроархитектуры, плотность, химический состав и доля содержания ККМ.

Результаты. Разработанные фантомы взрослых мужчины и женщины являются составными и включают по 46 сегментов; параметры 21 сегмента не зависели от пола. Размеры фантомов-сегментов были в пределах 4–66 мм, толщина кортикального слоя — в пределах 0,3–2,2 мм. Параметры микроархитектуры костных сегментов представлены в статье.

Выводы. Полученные фантомы имитируют микро- и макроархитектуру костной ткани и вместе с наборами дополнительных фантомов отражают популяционную вариабельность отдельных костей скелета, а также учитывают половые различия. Сгенерированные фантомы могут быть использованы для внутренней дозиметрии остеотропных бета-излучателей, в том числе в составе радиофармпрепаратов.

Ключевые слова: трабекулярная кость; кортикальная кость; дозиметрия костного мозга; вычислительные фантомы; стронций; вычислительный фантом взрослого

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INTRODUCTION

Strontium isotopes accumulate in mineralized bone tissue and locally irradiate red bone marrow, which can lead to an increased risk of leukemia. ^{89,90}Sr isotopes were the main source of RBM radiation for residents of the coastal areas of the Techa River, contaminated with radionuclides in the 1950s as a result of radioactive effluents from the Mayak Production Association [1–4]. These radionuclides were also released into the environment as part of global fallout from nuclear weapons tests. In this context, the strontium isotope internal dosimetry of RBM represents a highly significant research task.

The assessment of RBM radiation doses caused by ^{89,90}Sr in the population of the coastal areas of the Techa River includes the use of biokinetic and dosimetric models. The biokinetic model for strontium isotopes developed at the Urals Research center for Radiation Medicine (URCRM) simulates the processes of exchange and transport of these radionuclides in the human body, thus allowing estimation of their specific activity in bone tissue (Bq/g). A dosimetric model is being developed to estimate the coefficients of transition from the specific activity of a radionuclide in bone tissue to the absorbed dose rate in RBM (Gy/s) [4]. Dosimetric modeling involves the use of computational phantom models that simulate the geometry and chemical composition of media in which radiation transfer is assumed, i.e., bone and RBM [5]. Creating such phantoms for beta emitters is a challenging task, due to strict requirements on the description of both the linear dimensions of bones and their microstructure.

The latest computational phantoms are based on the analysis of computed tomography (CT) images of skeletons of deceased people [5–10][1]. Such phantoms are difficult to perform and, due to the limited amount of autopsy material, are not sufficiently informative with regard to population variations in the size and microarchitecture of the skeleton [11]. As an alternative, URCRM specialists proposed an original parametric method for stochastic modeling of bone structures — stochastic parametric skeletal dosimetry (SPSD) modeling [11], suitable for internal dosimetry of ^{89,90}Sr. The parameters of SPSD phantoms are based on a large number of published bone measurement results. This makes it possible to assess the variability of skeletal characteristics within population groups and the associated DF variability, which is extremely important for dosimetric support of studies among the irradiated population of the coastal areas of the Techa River. The adequacy of the model is confirmed by a good convergence of the calculated energy dependencies for SPSD phantoms and those presented in the literature [11–13].

Previous studies reported the parameters of skeletal phantoms representing newborn, one-year-old, 5-year-old, and 10-year-old children [14–17]. The skeleton of a 15-year-old child, as well as various characteristics of the simulated areas, are similar to those of an adult, whose skeleton has been described in greater detail in previous research. Therefore, we decided first to model the skeleton of an adult and then to model the skeleton of a 15-year-old child on its basis. It should be noted that sex differences in skeletal characteristics should be taken into account for the purposes of dosimetric modeling.

Our study continues work on the creation of computational phantoms for different age groups. In the present research, we aim to develop computational skeletal phantoms representing an adult male and an adult female to estimate doses of beta-emitting radionuclides incorporated in RBM.

MATERIALS AND METHODS

The stages of creating computational phantoms for adults did not differ from those for younger age groups [11] and included:

1. Identification of simulated skeletal sites with active hematopoiesis (hematopoietic sites), assessment of the mass fraction of RBM therein;
2. Assessment of the linear dimensions and microarchitecture parameters of the simulated bones based on published data;
3. Segmentation of hematopoietic sites;
4. Generation of voxel phantoms for each segment.

PET data were used to assess the distribution of RBM within the adult skeleton and to identify the main hematopoietic sites on this basis [18]. This method is more accurate than MRI scanning, the results of which were used to analyze the RBM distribution inside the skeleton for younger age groups [19]. Hematopoietic sites are whole bones or sets of bones, they may include areas without RBM. In order to establish the presence of RBM in a particular site of a particular hematopoietic site, published MRI data were used [20–25].

The parameters of the phantoms included the average values of bone microarchitecture characteristics, including the thickness of the trabeculae (*Tb. Th.*), the size of the intertrabecular space (*Tb. Sp.*), the bone tissue proportion in the bone volume (*BV/TV*) [26], the thickness of the cortical layer (*Ct. Th.*), and the linear dimensions of the bones.

The set of published data used to estimate the phantom parameters, as well as the methodology for their collection and analysis, were described in detail earlier [26]. To assess the parameters of adult phantoms, bone measurements published in peer-reviewed publications, atlases, monographs, and dissertations were used, as well as electronic resources containing collections of X-ray images. The measurement results of people/samples identified by the authors as healthy and free of diseases leading to bone deformity were used for analysis. In cases where the published data were unavailable, bone measurements from the anatomical collections of the South Ural State Humanitarian Pedagogical University and the South Ural State Medical University were used to obtain characteristics of a specific area of the skeleton. These bones were undamaged and were not divided into groups according to sex. The measurements were carried out by the staff of the URCRM biophysical laboratory using micrometers (REXANT MK 12-9110-2, China).

[1] Pafundi D. Image-based skeletal tissues and electron dosimetry models for the ICRP reference pediatric age series. Dissertation for the degree of doctor of the philosophy. University of Florida; 2009.

The analysis used bone measurements from people belonging to ethnic groups typical of the Ural region: Caucasoid and Mongoloid aged 20 to 50 years for females and from 20 to 60 years for males.

The characteristics of the bone microarchitecture required for SPSD modeling included: the thickness of the trabeculae (*Tb. Th.*), the size of the intertrabecular space (*Tb. Sp.*), and the bone tissue proportion in the bone volume (*BV/TV*) [26]. These characteristics were evaluated based on the results of studies obtained using histomorphometry and micro-CT (computed microtomography). To assess the thickness of the cortical bone layer, published measurement results using CT, micro-CT, and micrometers were used. The linear dimensions of bones were estimated based on published measurement results using anatomical boxes, ultrasound and X-ray examinations, as well as computed tomography (CT).

In the absence of sex differences in the studied bone characteristics, the data sets for both sexes were combined. In this case, the bone section was modeled regardless of sex. In other cases, the phantoms for males and for females were modeled separately.

The skeletal areas with active hematopoiesis were divided into smaller segments. Each segment not only has a uniform microarchitecture and thickness of the cortical bone layer, but is also described by a simple geometric shape. A basic phantom of bone segment (BPS) was modeled for each such site [27]. This segmentation allowed us to take into account the heterogeneous microarchitecture inside the bone and the thickness of the cortical layer. The reduction of the phantom size by their separation allowed us to increase their resolution, thus improving the accuracy of modeling.

Table 1. RBM mass fraction (% of the RBM total mass in the skeleton) in the main hematopoietic sites of the adult skeleton

No.	Hematopoietic site	RBM mass fraction, %
1	Femur	5.9 ± 2.5
2	Humerus	3.6 ± 1.9
3	Sacrum	7.4 ± 1.8
4	Pelvic bones	23.2 ± 3.0
5	Skull	6.2 ± 2.3
6	Clavicle	0.8 ± 0.01
7	Scapula	4.7 ± 0.8
8	Ribs	9.8 ± 1.7
9	Sternum	1.8 ± 0.7
10	Cervical vertebrae	3.5 ± 1.0
11	Thoracic vertebrae	17.5 ± 2.4
12	Lumbar vertebrae	15.5 ± 2.5

Table prepared by the authors using their own data [18]

Note: the data are presented in the form of a mean value (M) and a standard deviation (σ); the source [18] shows the RBM total proportion in the collarbones, shoulder blades, and ribs equal to 15.3 ± 2.6%; the RBM proportion in each of these areas is calculated in proportion to the total volume of BPS (calculated automatically in the Trabecula software), the constituents of each hematopoietic site.

The linear dimensions and parameters of bone microarchitecture were determined separately for each segment as the mean values of bone characteristics [26].

The density and chemical composition of the simulated media (mineralized bone and bone marrow) were the same for all BPS, being estimated based on literature sources [28, 29].

The generation of BPS was carried out in the Trabecula software [30]. The BPS consists of voxel cubes, which, depending on their position, simulate one of the simulated environments.

It should be noted that further dosimetric modeling considered the trabecular (TB) and cortical bone (CB) as separate source tissues, although in the BPS they are modeled by voxels simulating mineralized bone tissue. The bone marrow was considered as a single target tissue [30]. TB is a 3D net (bone strands) of trabeculae, the thickness and relative position of which are determined randomly within the variability of microarchitecture parameters inside the simulated bone [30], estimated on the basis of published data [31–35]. This method of modeling trabeculae took their heterogeneity into account, thereby bringing the model closer to the structure of an actual bone. The bone marrow (BM) fills the space between the trabeculae, and the CB covers the BPS from the outside with a continuous layer with a thickness equal to *Ct. Th*. The voxel resolution for each BPS was selected separately, the voxel size did not exceed 70% *Tb. Th.* ranging within 50–140 μm [30]. The volumes of TB, CB, and BM were automatically calculated in the Trabecula software.

Figure 1 shows the main hematopoietic sites of an adult, the bone division into segments, as well as sections of simulated BPS using the femoral bone of an adult male as an example.

One important advantage of the SPSD methodology consists in the possibility to estimate the uncertainty of DF. To that end, for each BPS generated with parameter mean values, 12 supplementary phantoms of bone segment (SPS) were created with randomly selected parameters of the micro- and macrostructure of bone within their individual variability (within the limits of the minimum and maximum measured values). A method for estimating uncertainties when applying the SPSD approach was described in detail earlier [36].

RESULTS

The hematopoietic sites of the adult human skeleton, as well as the mass fraction of RBM therein, were determined according to PET results [18] and presented in Table 1.

Table 1 shows that the adult skeleton includes 12 hematopoietic sites. The RBM mass fraction in these sites varies from 1.8% to 23.2%. The RBM distribution within each hematopoietic site was taken from published MRI data [20–25]. No sex differences were found in the RBM distribution.

The data of the International Commission on Radiation Protection [28] for adults were used to determine the chemical composition of the simulated media; the corresponding data are presented in Table 2.

The density of mineralized bone tissue was estimated based on the published measurements of adult cortical bone density (regardless of sex), being equal to 1.9 g/cm^3

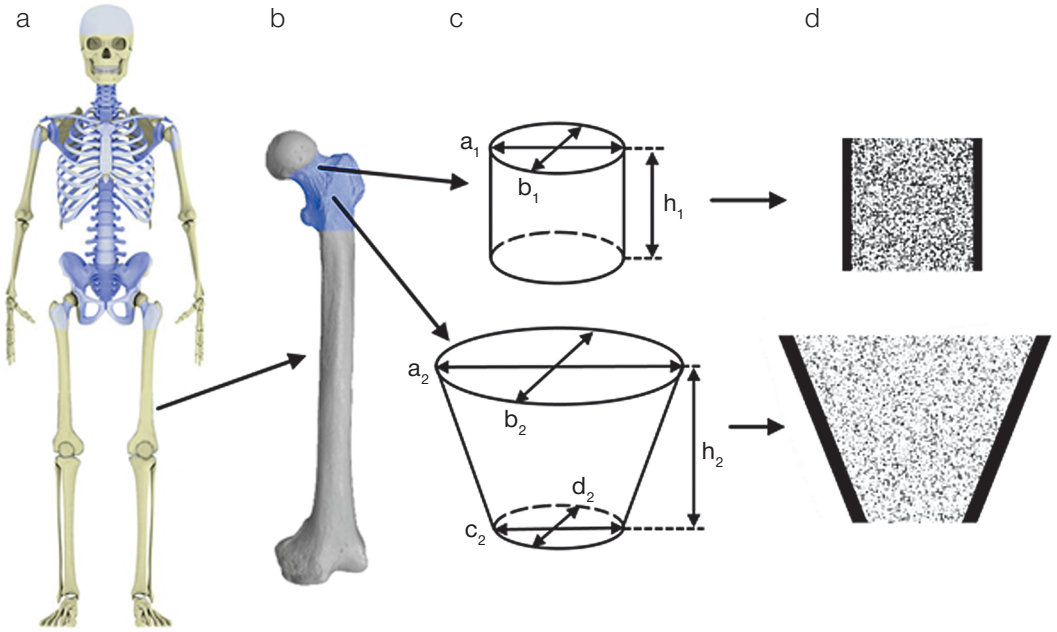

Figure prepared by the authors

Fig. 1. Hematopoietic sites of the adult human skeleton and their segmentation using the femoral bone of an adult male as an example
Note: a) skeleton of an adult male (simulated areas of the skeleton with active hematopoiesis are highlighted in blue); b) femur (simulated areas of the skeleton with active hematopoiesis are highlighted in blue); c) bone separation scheme into BPS and their linear parameters; d) femoral BPS in the voxel representation in the section (voxels are shown in black, imitating mineralized bone, white — BM).

[29]. The density of red bone marrow was assumed to be equal to the density of adipose tissue (0.98 g/cm^3) [28].

According to a review of published data [26], there are no significant sex differences in the characteristics of the bone microarchitecture; therefore, the data of males and females were combined. Table 3 shows the parameters of the BPS microarchitecture for male and female adults.

The creation of a realistic model for a 3D trabecular network requires evaluation of the variability of the microarchitecture characteristics inside the bone. Concerning this characteristic, we found published data only for the bones of the pelvis, spine, and skull. The mean value of the variability among the indicated regions was used to generate the remaining phantoms. The values of variability within the bone, accepted for different parts of the adult skeleton, are shown in Table 4.

Table 5 shows the linear dimensions and thickness of the cortical layer adopted for adult male and female BPS. The data on which these parameters are based can be found in [26].

Conversely, for a number of adult bones, the parameters of bone microstructure demonstrated significant sex differences in linear sizes. Therefore, for these areas, the sizes were estimated separately for males and females.

The SPSD skeletal phantoms of male and female adults are composite, consisting of 71 BPS. Out of the latter, 25 segments are specific to males, another 25 for females, and 21 segments were modeled identically for both sexes, as shown in Table 5.

The largest number of phantoms in a single hematopoietic site was determined for the sacrum — 10, while the skull and humerus each contain one BPS.

As far as the phantoms of younger age groups are concerned [14–17], most of the adult BPS are cylinders and rectangular parallelepipeds, the linear dimensions of which ranged from 4 mm to 66 mm. The greatest sex differences in linear size were observed for the lower part of the upper branch of the pubic bone (the area of the pubic symphysis), amounting to 66%. The lowest value of the cortical layer thickness was determined for BPS of the cervical vertebrae (0.3 mm), being more than sevenfold different from the maximum value assumed for the proximal end of the femur (2.2 mm). The parameters of the BPS microarchitecture also varied widely. The *BV/TV* value ranges from 6% to 52%, *Tb. Th.* — from 0.1 mm to 0.29 mm, *Tb. Sp.* — from 0.5 mm to 2.37 mm (Table 3).

The population variability of BPS linear sizes comprises 12%, on average; the highest value of variability is estimated for the lower branch of the pubic bone (36%), and the lowest — for the bodies of the cervical vertebrae (3%). The variability in the cortical bone layer thickness ranged from

Table 2. Chemical composition of the simulated media, accepted for all BPS

Chemical composition, relative units		
Chemical element	**Bone**	**Bone marrow**
H	0.035	0.105
C	0.16	0.414
N	0.042	0.034
O	0.445	0.439
Na	0.003	0.001
Mg	0.002	0.002
P	0.095	0.002
S	0.003	0.002
Ca	0.215	0

Table prepared by the authors using data from [28]

Table 3. Bone microarchitecture parameters adopted for adult BPS

Hematopoietic site	BV/TV, %[1]	*Tb. Th.*, mm[2]	*Tb. Sp.*, mm[2]
Femur (neck)	17 (14–22)	0.19 (19)	0.78 (13)
Femur trochanter area	11 (8–13)	0.136 (65)	0.99 (20)
Humerus	6 (1–13)	0.1 (18)	2.37 (25)
Ribs	12 (5–25)	0.14 (12)	0.82 (11)
Ilium	19 (11–25)	0.13 (15)	0.6 (20)
Ishium bone and pubic ramus inferior	25 (23–27)	0.3 (7)	1.0 (40)
Pubic ramus superior	17 (12–23)	0.29 (10)	1.0 (12)
Skull	52 (41–65)	0.29 (32)	0.57 (35)
Clavicle body	13 (8–18)	0.19 (13)	0.8 (25)
Clavicle ends	29 (15–46)	0.14 (31)	0.8 (25)
Scapula	22 (9–47)	0.24 (42)	0.96 (23)
Sternum	15 (8–22)	0.15 (29)	1.4 (9)
Cervical vertebrae	21 (16–28)	0.15 (14)	0.5 (10)
Thoracic vertebrae	15 (11–28)	0.1 (15)	0.6 (15)
Lumbar vertebrae + Sacrum	16 (11–28)	0.15 (15)	0.6 (15)

Table prepared by the authors using data from [26]

Note: [1]the range of possible values is parenthetic; [2]the coefficient of variation (CV) in % is parenthetic.

Table 4. Variability of microarchitecture parameters inside the bone

Hematopoietic site	*Tb. Th.*	*Tb. Sp.*	Data source
Pelvic bones	10	10	[25]
Vertebrae and Sacrum	48	43	[26]
Skull	8	15	[27–29]
Other bones (mean value)	22	23	[26–30]

Table prepared by the authors using data from [25–30]

Note: data is presented in %.

Table 5. Linear dimensions and thickness of the cortical layer accepted for adult BPS

Hematopoietic site	Segment	Shape[1]	Sex	Phantom parameters, mm (parenthetic CV, %)[2]					
				h	*a*	*b*	*c*	*d*	*Ct. Th.*
Femur	Neck	cyl	m	30 (13)	36 (6)	32 (4)			1.9 (5)
			f	31 (14)	29.4 (10)	23.9 (9)			1.9 (5)
	Trochanter area	dc	m	43 (26)	66 (6)	44 (6)	30 (7)	30 (7)	2.3 (15)
			f	34.5 (5)	58 (7)	39 (7)	27 (6)	27 (6)	2.3 (15)
Humerus	Proximal	dc	m	28 (12)	56 (5)	56 (5)	25 (15)	25 (15)	1.1 (18)
			f	24.9 (10)	51.3 (6)	51.3 (6)	23.9 (11)	23.9 (11)	1.1 (18)
Ribs	Ribs[4] 1–2	p	m	17 (12)	30[6]	7 (14)			0.7 (38)
			f	14 (11)	30[6]	5.5 (15)			0.7 (38)
	Ribs[4] 11–12	p	m	11 (18)	30[6]	6 (17)			0.7 (38)
			f	9.5 (11)	30[6]	4 (25)			0.7 (38)
	Ribs[4] 3, 4, 9, 10	p	m	13 (8)	30[6]	7 (14)			0.7 (38)
			f	11.3 (11)	30[6]	6 (14)			0.7 (38)
	Ribs[4] 5, 6, 7, 8	p	m	14 (14)	30[6]	8 (13)			0.7 (38)
			f	12.5 (11)	30[6]	6.8 (13)			0.7 (38)
Sacrum	Body-1	p	m	30 (7)	40 (11)	24.5 (10)			1.5 (8)
			f	30 (9)	37.8 (11)	22.2 (12)			1.5 (8)
	Body-2-3	p	m	46 (8)	28.7 (11)	15 (9)			1.5 (8)
			f	45.2 (15)	28 (11)	13.8 (13)			1.5 (8)
	Body-4-5	p	m	36 (9)	28 (11)	8.5 (13)			1.5 (8)
			f	35 (14)	28 (11)	8.5 (12)			1.5 (8)

Table 5 (continued)

Hematopoietic site	Segment	Shape[1]	Sex	Phantom parameters, mm (parenthetic CV, %)[2]					
				h	*a*	*b*	*c*	*d*	*Ct. Th.*
Sacrum	Pedicle 1	cyl	m+f	13.9 (14)	23.7 (15)	15.3 (11)			1.5 (8)
	Pedicle 2	cyl	m+f	14.2 (14)	25.0 (11)	13.6 (17)			1.5 (8)
	Pedicle 3	cyl	m+f	13.9 (14)	18.3 (11)	13.2 (14)			1.5 (8)
	Pedicle 4	cyl	m+f	13.9 (14)	14.5 (11)	11.2 (18)			1.5 (8)
	Ala 1	p	m	30 (13)	20 (10)	42 (13)			1.5 (8)
			f	30 (9)	21 (15)	38.6 (8)			1.5 (8)
	Ala 2	p	m	26 (15)	23 (17)	25 (8)			1.5 (8)
			f	26 (9)	23 (17)	22.7 (13)			1.5 (8)
	Ala 3–4	pr	m+f	19 (16)	18 (9)	38.5 (15)	38.5 (15)		1.5 (8)
Pelvic bones	Iliac ala	p	m+f	9.5 (31)	30[6]	30[6]			1 (30)
	Iliac crest	p	m	11 (15)	30[6]	13 (9)			1 (30)
			f	11 (15)	30[6]	13 (9)			1 (30)
	Iliac dorsal segment[7]	p	m+f	19 (16)	30[6]	30[6]			1 (30)
	Ischium ramus	cyl	m+f	30[6]	34 (9)	25 (8)			0.5 (30)
	Pubis ramus inferior	dc	m+f	47 (17)	16 (25)	22 (23)	26 (23)	14 (36)	0.5 (30)
	Pubis ramus superior (lower part)	p	m	32 (19)	15 (20)	29 (20)			0.7 (30)[4] 1.5 (12)[4]
			f	19 (13)	11 (7)	33 (18)			0.7 (30)[4] 1.5 (12)[4]
	Pubis ramus superior (upper)	p	m	51.2 (8)	14.5 (20)	16 (20)			0.7 (30)[4] 1.5 (12)[4]
			f	55.8 (77)	11 (18)	13 (20)			0.7 (30)[4] 1.5 (12)[4]
	Acetabulum	hc	m+f	29 (10)	26 (10)	21 (20)			0.5 (30)[5] 3.6 (30)[5]
Skull	Flat bones[4]	p	m+f	5.2 (12)	30[6]	30[6]			1.3 (33)[3] 1.5 (22)[3]
Clavicle	Shaft (acromial part)	cyl	m	56 (7)	26 (15)	24 (12)	12 (5)	12 (8)	0.8 (2)
			f	51.5 (6)	24 (16)	21 (14)	10 (10)	10 (9)	0.8 (2)
	Ends	dc	m	20	26 (15)	24 (12)			0.6 (19)
			f	20	24 (16)	21 (14)			0.6 (19
	Shaft (sternal part)	dc	m	56 (7)	22 (14)	12 (5)	12 (9)	12 (8)	0.8 (2)
			f	51.5 (6)	21 (14)	12 (10)	10 (10)	10 (9)	0.8 (2)
Scapula	Glenoid	cyl	m+f	8.8 (18)	48 (11)	26 (11)			0.8 (13)
	Acromion	p	m+f	20 (9)	36 (9)	26 (10)			0.9 (28)
	Lateral margin	p	m+f	30[6]	5 (10)	10 (13)			0.8 (13)
Sternum	Body	p	m	1 (10)	30[6]	30[6]			1.1 (42)
			f	9 (10)	30[6]	30[6]			1.1 (42)
	Manubrium	p	m	1.3 (16)	30[6]	30[6]			1.45 (22)
			f	1.1 (16)	30[6]	30[6]			1.45 (22)
Cervical vertebrae	Vertebral body 3–7	cyl	m	13 (16)	19 (14)	16 (12)			0.3 (7)
			f	12 (6)	16 (6)	15 (8)			0.3 (7)
	Vertebral body 2	p	m+f	19.2 (13)	14.3 (10)	17.5 (3)			0.3 (7)
	Lateral mass 1	p	m+f	15 (13)	11.4 (9)	10.5 (9)			0.3 (7)
Thoracic vertebrae	Vertebral body	cyl	m	27 (7)	33 (9)	28 (11)			1.3 (16)
			f	22 (6)	29 (6)	26 (8)			1.3 (16)
	Lamina+inferior articular. proc.	p	m+f	32 (12)	10.2 (14)	4.2 (13)			1.3 (16)
	Spinous process	p	m+f	10.3 (15)	50 (4)	5.1 (20)			1.3 (16)
	Superior articular process	p	m+f	11.4 (12)	11.3 (14)	4.4 (11)			1.3 (16)
	Transverse process	p	m+f	12 (9)	18 (11)	10.6 (13)			1.3 (16)

Table 5 (continued)

Hematopoietic site	Segment	Shape[1]	Sex	Phantom parameters, mm (parenthetic CV, %)[2]					
				h	*a*	*b*	*c*	*d*	*Ct. Th.*
Lumbar vertebrae	Vertebral body	cyl	m	27 (12)	35 (8)	47 (9)			1.3 (34)
			f	27 (7)	32 (8)	43 (8)			1.3 (34)
	Lamina+inferior articular. proc.	p	m+f	20.4 (10)	12.7 (13)	4.1 (13)			1.0 (34)
	Spinous process	p	m	24 (13)	31 (6)	6 (17)			0.4 (50)
			f	20 (15)	31 (6)	6 (17)			0.4 (50)
	Superior articular process	p	m+f	14 (14)	15 (13)	12 (17)			1.0 (34)
	Transverse process	p	m+f	12 (8)	23 (9)	8 (13)			0.4 (50)

Table prepared by the authors using data from [13, 28]

Note: m — male; f — female; m+f — BPS was modeled independent of sex
[1] — phantom shape was designated as follows: cyl — cylinder, dc — deformed cylinder, p — rectangular parallelepiped, pr — prism with triangle base; t — hollow cylinder;
[2] — BPS dimensions were designated as follows: *h* — height; *a* — major axis (c), major axis for a large base (dc) or side a (p) or outer diameter (t); *b* — minor axis (c), minor axis for a large base (dc) or side b (p) or inner diameter (t); *c* — major axis for a small base (dc); *d* — minor axis for a small base (dc); for prism (pr): *a, b, c* — sides of the triangle base;
[3] — cortical layer thickness was assumed to be different for the inner (medial) and outer (gluteal) surfaces of a given segment of the skull;
[4] — symphyseal surface covered by thicker cortical layer than other surfaces of BPS;
[5] — a higher *Ct. Th*. value assumed for the medial side of the acetabulum
[6] — BPS imitated only a part of the simulated bone segment, when the bone segment dimensions exceeded significantly 30 mm, since in terms of dosimetry in such cases it makes no sense to simulate the entire bone fragment
[7] — describing thick part of ilium, adjacent to sacrum

Table 6. Comparison of BPS volumes of 10-year-old children with adult males and females

BPS	Modeled media	Volume of modeled structure, cm³		
		10-year-old	Adults	
			Male	Female
Femur neck	BM	6.95	18.43	10.39
	TB	3.77	3.68	2.14
	CB	3.93	5.94	4.58
	Entire BPS	14.65	28.05	17.11
Lumbar vertebra body	BM	12.42	28.55	23.73
	TB	1.97	4.96	4.19
	CB	0.38	1.37	1.26
	Entire BPS	14.77	34.88	29.18
Cervical vertebra body	BM	1.43	2.29	1.64
	TB	0.38	0.6	0.46
	CB	0.07	0.21	0.17
	Entire BPS	1.88	3.1	2.27

Table prepared by the authors using data from [17]

2% (cervical vertebrae) to 50% (lumbar vertebrae), averaging 20%. The variability of the microstructure parameters ranged from 7% to 65%, averaging 18%.

The obtained values of the variability of phantom parameters were used to generate an SPS. The SPS volume ranged within 23–264% of the BPS volume.

DISCUSSION

The characteristics of adult human phantoms are comprehensively presented in the literature, including about 260 publications with descriptions of 28,000 people or bone samples [26]. The processes of ossification in the adult human skeleton are complete, as well as the conversion of bone marrow into yellow marrow. Those skeleton areas that are small in size and/or do not ossify in childhood, and being omitted in modeling for this reason, were modeled for adults. This is why the phantom of the adult human skeleton contains a greater number of BPS in its composition than the phantoms of younger age groups [14–17]. The distribution of RBM for adults differs significantly from that for children: the proportion of RBM in the skull is 6.2%, which is twice lower than for 10-year-old children [17]; the largest proportion of RBM in adulthood is typical for the bones of the pelvis and spine. *BV/TV* for adult BPS is, on average, 1.5-fold lower than for BPS of 10-year-old children. *Tb. Th.* practically does not change with age, while *Tb. Sp*. increases, on average, by 15% compared to the age of 10-year-old. Table 6 compares the volumes of media in the BPS for a ten-year-old child with those for adult males and females.

The total volume of adult phantoms is, on average, 17% higher than that of a 10-year-old, with a much more significant increase in skeletal size. Such small volume differences are associated with the cessation of hematopoiesis

in skeletal areas modeled by relatively large phantoms, as well as with a more detailed segmentation of the skeleton, resulting in a large number of small BPS. Nevertheless, for BPS, which are modeled in a similar manner for 10-year-olds and adults, a significant increase in the volume of media with age is observed, as shown in Table 6. For such BPS, the media volume showed, on average, a twofold increase. The sex differences in the media volume in the composition of phantoms modeled for adults for individual BPS reached 62% (the upper branch of the sciatic bone), averaging 8%.

For adults, the data on the mass ratio of lumbar vertebrae were reported in [37]. According to this information, the weight of a lumbar vertebra equals 65.3 ± 4% of the mass of the entire vertebra. Due to the small amount of RBM, the vertebral pedicles were not modeled in the framework of the SPSD. Nevertheless, the mass of BPS of the vertebral body comprises 70.0 ± 5% of the sum of the masses of all BPS simulating the lumbar vertebra, which agrees well the published measurements of actual bones.

CONCLUSION

In this paper, we present a description of SPSD skeletal phantoms for male and female adults. Each skeleton phantom consists of 46 basic phantom segments, including the proximal parts of the femur and humerus, sacrum, ribs, pelvic bones, skull, clavicles, scapulae, sternum, as well as the vertebrae of the cervical, thoracic, and lumbar regions. At the same time, sex differences in linear sizes were typical for 25 out of 46 segments. The microarchitecture parameters and cortical thickness did not differ between the male and female segment phantoms. On the basis of the estimated variability in the parameters of phantom segments, 12 SPS were generated for each basic bone phantom segment. The DF calculated for SPS will be used to estimate the population variability of DF.

The presented adult phantoms will be used to calculate the ^{89,90}Sr transition coefficients in order to improve dose estimates for residents of the Ural region. This work is the fifth in a series of papers addressing the parameters of computational skeletal phantoms of people of different ages and sexes. In future works, we aim to create SPSD phantoms for male and female 15-year-old children. The generated phantoms can be used for internal dosimetry of osteotropic beta-emitters in the population, as well as for dosimetry of other beta-emitting radionuclides, including those used in radionuclide therapy, such as ^{89}Sr, ^{32}P, ^{186}Re, ^{188}Re, ^{117m}Sn.

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ALGINATE GELS MODIFIED WITH NATURAL AMINO ACIDS FOR REGENERATIVE MEDICINE

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Introduction. Alginate is a natural polysaccharide that shows promise as a non-toxic material for the development of hydrogel wound dressings. Natural amino acids such as arginine and lysine are important components in the synthesis of the extracellular matrix and other vital bodily processes.

Objectives. Development of alginate gels modified with arginine and lysine and compatible with dermal fibroblasts. Study the release rate of these amino acids from the gel.

Materials and methods. The study was performed using three types of aqueous solutions of 3% sodium alginate containing arginine or lysine in amounts of 5, 10, and 20% of the alginate dry weight. To assess the stability of lysine gels over time, they were incubated for 1, 3, and 7 days in 600 μL of water at room temperature. The intensity of amino acid release from the alginate gel was evaluated by the spectrophotometric method after the reaction of ninhydrin. The DF2 cell line (human dermal fibroblasts, the shared research facility "Vertebrate cell culture collection" RAS) was used as model objects. Human dermal fibroblasts were cultured on the gels obtained. The cells cultured on the gels were evaluated using optical microscopy. Statistical analysis was performed using Microsoft Excel software; Student's *t*-test was used to evaluate the differences between the samples. The differences were considered statistically significant at $p < 0.05$.

Results. On the first day, the gel containing the minimal lysine concentration of 5% showed the amino acid release of almost 16% of its initial amount. A more prolonged incubation of the gel led to a decrease in the desorbed lysine proportion relative to the initial amount. Statistically significant differences were obtained between the samples of alginate gels with the lysine content of 5 and 20% ($p < 0.05$). An increase in the lysine proportion in the gel was associated with a decrease in its release, i.e., the higher concentration of lysine the gel contained, the less amount of lysin was detected after seven days of incubation. It was shown that when a gel sample with a lysine content of 5% was incubated in a large volume of water (the gel-to-water ratio of 11%), more than 40% of lysine was desorbed into water during the first hour of incubation. Therefore, lysine was 4.3% more intensively desorbed from the alginate gel compared to the arginine gel. The following pattern was established: a twofold decrease in the amount of lysine release under an increase in the gel-to-water ratio from 11% to 20%.

Conclusions. During the study, alginate gels modified with amino acids were obtained. It was found that the higher the concentration of an amino acid in the gel, the less intensively it leaves the gel. The rate of amino acid release from the gel is directly proportional to the volume of liquid in which the gel was incubated. Human dermal fibroblasts adhered better to alginate gels modified with amino acids compared to cells on alginate gels without modification. As a result of the study, gels with controlled desorption of amino acids were obtained, which promote the adhesion of human dermal fibroblasts.

Keywords: alginate gels; lysine; arginine; human dermal fibroblasts

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АЛЬГИНАТНЫЕ ГЕЛИ, МОДИФИЦИРОВАННЫЕ ПРИРОДНЫМИ АМИНОКИСЛОТАМИ, ДЛЯ РЕГЕНЕРАТИВНОЙ МЕДИЦИНЫ

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Введение. Альгинат — природный полисахарид, который зарекомендовал себя как перспективный нетоксичный материал для формирования раневых покрытий в виде гелей. Такие природные аминокислоты, как аргинин и лизин, являются важными составляющими при синтезе внеклеточного матрикса, а также участвуют во многих жизненно важных процессах в организме.

Цель. Формирование альгинатных гелей, совместимых с фибробластами кожи и модифицированных природными аминокислотами аргинином и лизином, а также исследование скорости выхода данных аминокислот из геля.

Материалы и методы. Работа выполнена на 3 видах водных растворов 3% альгината натрия с содержанием аминокислоты аргинина или лизина в количестве 5, 10 и 20% от сухой массы альгината. Для оценки стабильности гелей с лизином во времени их инкубировали в течение 1, 3 и 7 сут в 600 мкл воды при комнатной температуре. Интенсивность выхода аминокислот из альгинатного геля оценивали спектрофотометрическим методом после проведения нингидриновой реакции. В качестве модельных объектов в работе была использована клеточная линия DF2 (фибробласты кожи человека, «Коллекция культур клеток позвоночных» ИНЦ РАН). На полученных гелях культивировали дермальные фибробласты человека. Оценку клеток на гелях проводили с помощью оптической микроскопии. Статистический анализ выполнен с использованием программного обеспечения Microsoft Excel, для оценки различий между образцами использовали *t*-тест Стьюдента. Различия считали статистически значимыми при $p < 0,05$.

Результаты. Установлено, что для геля с минимальной концентрацией лизина (5%) выход аминокислоты в первые сутки составил почти 16% от исходного ее количества, а при длительном инкубировании геля доля десорбированного лизина относительно исходного количества уменьшалась. Получены статистически достоверные отличия между образцами альгинатных гелей с содержанием лизина 5 и 20% ($p \leq 0,05$). При увеличении доли лизина в геле его выход снижался: чем большая концентрация лизина была в геле, тем меньшее количество его обнаруживали по истечении 7 сут инкубирования. Показано, что в образце геля с содержанием лизина 5% при инкубировании в большом объеме воды (соотношение геля

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по отношению к воде — 11%) уже за первый час инкубирования более 40% лизина десорбировалось в воду, т.е. лизин на 4,3% более интенсивно десорбируется из альгинатного геля по сравнению с аргинином. Установлена закономерность в виде снижения процента выхода лизина практически в два раза при увеличении соотношения геля к воде с 11 до 20%.

Выводы. В ходе выполнения исследования были сформированы альгинатные гели, модифицированные аминокислотами. Установлено, что чем больше концентрация аминокислоты в составе геля, тем менее интенсивно она выходит из него. Скорость выхода аминокислоты из геля прямо пропорциональна объему жидкости, в котором инкубировали гель. Фибробласты кожи человека лучше адгезировали на альгинатных гелях, модифицированных аминокислотами, по сравнению с клетками на альгинатных гелях без модификации. В результате исследования получены гели с контролируемой десорбцией аминокислот, способствующие адгезии фибробластов кожи человека.

Ключевые слова: альгинатные гели; лизин; аргинин; фибробласты кожи человека

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Финансирование: исследование не имело спонсорской поддержки.

Соответствие принципам этики: исследование не требовало заключения локального биоэтического комитета. В работе использована клеточная линия DF2 (фибробласты кожи человека из коллекции клеточных культур ИНЦ РАН).

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INTRODUCTION

Wound healing is a major challenge for healthcare systems worldwide [1]. The use of wound dressings can provide a physical barrier to further infection during wound healing [2]. Ideal wound dressings should be biocompatible and biodegradable, being capable of preventing the loss of biological fluids, removing exudate, protecting the wound from pathogens, demonstrating good breathability and moisture permeability, promoting cell proliferation, and accelerating wound healing [3].

Alginate is an anionic linear block polysaccharide consisting of repeating monomeric units of (1-4)-β-D-mannuronic acid (M) and (1-4)-α-L-guluronic acid (G), capable of forming fairly user-friendly gels [4]. Alginate wound dressings are available in the form of hydrogels, foams, films, nanofibers, and sponges. Alginate dressings absorb wound fluid, resulting in the formation of gels that maintain a physiologically moist environment and thereby minimize the attachment/development of bacterial infections in the wound [5]. They can be modified to meet the requirement of chemical stability or degradation upon contact with biological fluids over a period of time. Hydrogels are used for wound healing due to their biocompatibility, as well as their ability to load and release bioactive substances of moderate porosity, high water content, and flexibility [6].

Successful wound healing is accompanied by the synthesis of extracellular matrix components. Arginine and lysine are essential amino acids that are involved in the synthesis of the extracellular matrix. Arginine is an α-amino acid with the L-form, being one of the twenty most common naturally occurring amino acids. L-arginine exhibits a number of biological activities [7]. Arginine acts as one of the key metabolites involved in the processes of nitrogen metabolism, in particular, in the ornithine cycle, characteristic of mammals. Arginine participates in the synthesis of nitric oxide (NO), which has multiple effects, ranging from anti-inflammatory to vascular effects (vasodilation) and stimulation of angiogenesis. Due to its biocompatibility and biodegradability, L-arginine is used in the development of components of numerous biomedical matrices [8]. It was shown that an overdose of L-arginine is not accompanied with the development of serious side effects, since its excess is excreted in the urine within a few hours [8]. At the same time, L-arginine is actively involved in wound regeneration, making controlled administration of this amino acid an urgent task of regenerative medicine [9].

It should be noted that lysine can also improve wound healing in the body. Lysine participates in the formation of collagen, a protein that acts as a scaffold by supporting and imparting structure to skin and bones. Lysine can act as a binder, increasing the number of new cells in the wound [10].

The process of repairing damaged tissues is quite lengthy; in this regard, it is possible to increase the efficiency of regeneration, including the synthesis of a new extracellular matrix, by dosing amino acids into the wound bed. Creating a wound dressing that exhibits not only protective and bactericidal, but also regenerating properties is an urgent task of modern regenerative medicine.

This study was aimed at developing alginate gels modified with arginine and lysine, natural amino acids, that are compatible with dermal fibroblasts and to study the release rate of these amino acids from the gel.

MATERIALS AND METHODS

Formation of gels

Three types of aqueous solutions of 3% sodium alginate (Sigma-Aldrich, USA) containing arginine or lysine amino acids (Sigma-Aldrich, USA) in amounts of 5, 10, and 20% of the alginate dry weight were prepared for experiments.

Amino acids were added in dry form followed by mixing the resulting solution thoroughly on a magnetic stirrer (Tagler MM 135H, Russia) at room temperature for 10 min. Next, an alginate solution with an amino acid was added to a 24-well plate with 300 μL of gel per well. To form a gel,

the alginate in the wells was sprayed with a 10% solution of calcium chloride ($CaCl_2$) from a spray bottle and left for 20 min at room temperature.

After gelling, the gels were washed three times with deionized water to remove amino acids that were not absorbed by the gel. The gels were then filled with a fixed volume of water (600–2500 µL) for subsequent incubation and measurement of the content of amino acids released from the gels.

To assess the stability of lysine gels over time, they were incubated for 1, 3, and 7 days in 600 µL of water at room temperature.

To assess the effect of the volume of liquid in which the gel was located on the rate of arginine release from the gel, each sample with a volume of 300 µL with an arginine content of 5, 10, and 20% by weight of alginate was kept in 600, 1200, and 2500 µL of water at room temperature for 1 h; alginate gel in relation to water occupied 33, 20, and 11%. To compare the release of arginine and lysine, the gels were incubated for 1 h in 600 µL of water at room temperature.

Determination of amino acid release from gels

The content of amino acids released from the gels was estimated by spectrophotometry (PE-5400UF spectrophotometer, Ekros, Russia). To that end, a ninhydrin reaction was carried out, during which the amino groups interacted with ninhydrin. Next, the optical density of the reaction product was measured: a violet-colored complex at a wavelength of 400 nm. This method highly very sensitive, allowing determination of amino acids in low concentrations [11].

To construct calibration graphs of the dependence of optical density on the concentration of amino acids, the following solutions were prepared: 0.2% ninhydrin solution (Sigma-Aldrich, USA) in deionized water, 0.2% lysine and arginine solutions in deionized water. Solutions of lysine and arginine at a concentration of 0.2% were used in five samples with a volume of 50, 100, 200, and 300 µL, which contained 0.1, 0.2, 0.4, and 0.6 mg of the amino acid, respectively. 250 µL of ninhydrin solution was added to each tube. The tubes were then placed in a thermostat and incubated at 100°C for 3 min. The volume of solutions in each tube was adjusted to 6 mL of deionized water and the optical density of the solutions was measured at a wavelength of 400 nm. The values obtained were used to construct calibration curves to depict the dependence of the optical density of the solution on the amount of amino acids in the solution.

The content of amino acids released from the gels in the experiments was calculated using calibration curves. To that end, 500 µL of liquid in which the gels were incubated was taken in each experiment. 250 µL of ninhydrin solution was added to tubes with liquid followed by their placement in a thermostat at 100°C for 3 min. The volume of solutions in each tube was adjusted to 6 mL of deionized water; the optical density of the solutions was measured using a spectrophotometer at a wavelength of 400 nm.

In vitro studies

The DF2 cell line (human dermal fibroblasts, INC RAS cell culture collections) was used as model objects. For experiments with cells, alginate was previously sterilized by ozone. After preparation of sterile gels with 5% amino acid, a 300 µL cell suspension was applied to gels with a diameter of 1 cm, the cell content of which was 30,000 cells per well, and incubated in a DMEM/F-12 medium (Biolot, Russia) containing 10% fetal bovine serum FBS (Gibco, USA) for 3 days. The results were recorded using an inverted microscope (Nikon eclipse, TS 100, Japan).

Statistical analysis was performed using Microsoft Excel software. Student's *t*-test was used to evaluate statistically significant differences between the samples. The differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Effect of incubation duration on the percentage of lysine release from the alginate gel

Initially, the kinetics of lysine desorption from alginate gels was investigated. Gels with a different lysine content (5, 10, and 20%) were incubated in water for 7 days. When comparing the effect of incubation duration on the amount of lysine bound to the gel, the gel with the minimum lysine concentration (5%) showed the amino acid release on the first day of almost 16% of its initial amount; the corresponding data are shown in Fig. 1. Under a longer incubation of the gel, the proportion of desorbed lysine relative to the initial amount decreased. However, statistically significant differences were found between the samples with a lysine content of 5 and 20%. This result is likely to be related to the concentration equilibrium reached in the system during prolonged incubation of gels in water.

Then, for 7 days, the amount of lysine released from the gel remain at the same level. An increase in the proportion of lysine in the gel led to a decrease in its release throughout the week: the more lysine the gel contained, the less lysine was detected after 7 days of incubation. This can be explained by the establishment of a concentration equilibrium between the gel and the solution.

The study of the effect of the volume of liquid in which the gel was incubated on the amount of lysine release found that larger volumes of water, in which the gel was incubated, were associated with a more intense desorption of amino acids from the gel. Figure 2 shows that lysine release from the gel increased with an increase in the volume of the liquid in which it was incubated.

Thus, in a gel sample with a lysine content of 5% incubated in a large volume of water (the gel-to-water ratio of 11%), more than 40% of lysine was desorbed into water during the first hour of incubation. However, a threefold increase in the proportion of gel relative to water to 33% led a decrease in the intensity of lysine release to 15%. This is approximately three times less than in the sample with the gel-to-water ratio of 11%. This case also demonstrates the pattern of reducing the amount of lysine release by half with an increase in the gel-to-water ratio from 11% to 20%.

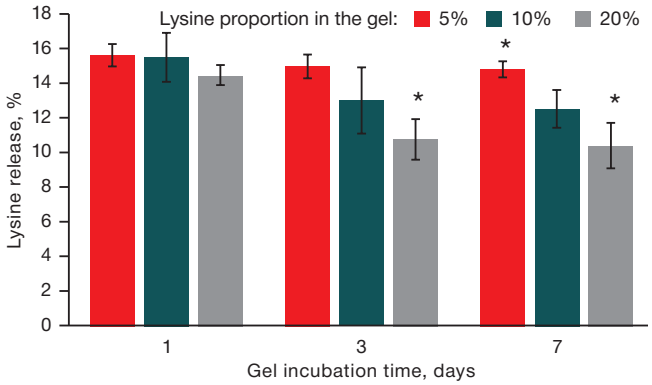

Figure prepared by the authors using their own data

Fig. 1. Kinetics of lysine release from gels
Note: * — statistical significance level $p \leq 0.05$.

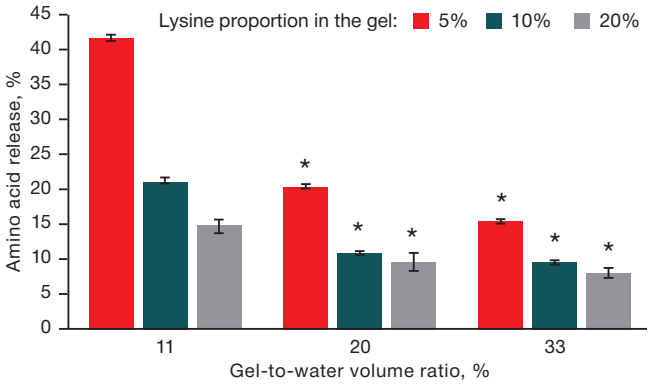

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Fig. 2. Effect of the gel-water ratio on the amount of lysine desorbed from the gel
Note: * — statistical significance level $p \leq 0.05$.

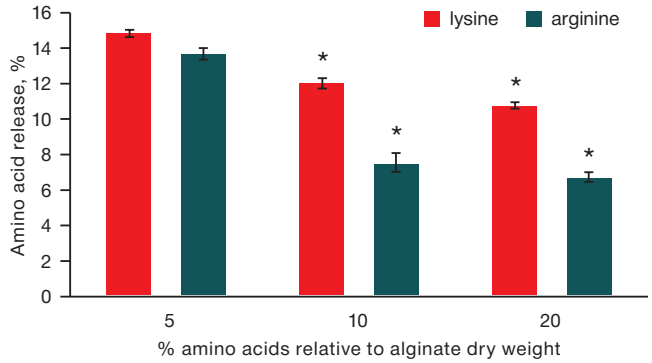

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Fig. 3. Dynamics of amino acid release from gels with a different ratio of amino acid and alginate after 1 h of incubation in 600 µL of liquid
Note: * — statistical significance level $p \leq 0.05$.

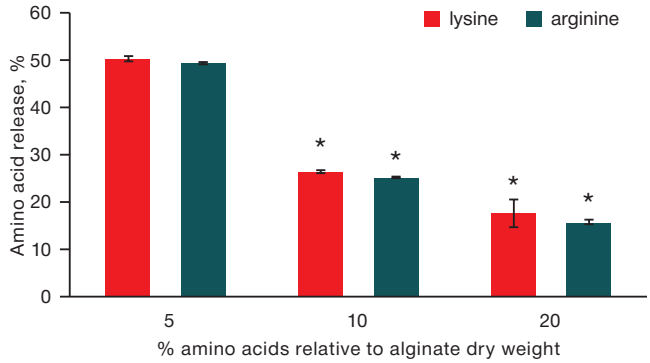

Figure prepared by the authors using their own data

Fig. 4. Dynamics of amino acid release from gels with a different ratio of amino acid and alginate after 1 h of incubation in 2500 µL of liquid
Note: * — statistical significance level $p \leq 0.05$.

These changes can be explained by saturation of the surrounding aqueous solution with lysine during its release from the gel, which process decreased the rate of lysine desorption.

Comparison of the amount of lysine and arginine release from the gel after 1 h of incubation

There are two amino acids in the human body that are involved in metabolism and construction of new proteins. Therefore, the presence of both amino acids can promote faster tissue regeneration. Since lysine and arginine have different structural features, it should be expected that their ability to desorb from gels will also be different.

When incubating gels with both lysine and arginine in 600 µL of water, lysine was found to release out of gel more intensively than arginine (Fig. 3). Moreover, the lysine release varied from 15 to 11%, depending on its initial content, and the arginine release from 14 to 7% for gel samples with an amino acid content from 5 to 20%.

When the volume of water was increased from 600 µL to 250 µm during gel incubation, no difference in the intensity of desorption between arginine and lysine was noted.

Figures 3 and 4 show that when incubating gels in a small volume of water (600 µL), lysine left the gel faster than arginine, by an average of 4.3%. When the volume of water was increased to 2500 µL, no statistically significant differences between the desorption of arginine and lysine was observed. It should also be noted that for both amino acids, the intensity of their desorption from the alginate gel increases several times with an increase in the volume of water from 600 to 2500 µL.

In order to explain the results obtained, it is necessary to consider the structure of the molecules of these amino acids. Due to its structure, the arginine molecule is capable of imparting greater stability to proteins than lysine [10]. The guanidine group of arginine interacts with other molecules in three different directions, while the additional amino group of lysine interacts in only one direction. This feature allows arginine to form many electrostatic and hydrogen bonds and provide a stronger interaction than lysine [12].

The ionic interaction should also be taken into account. In this respect, arginine should be more stable, especially at higher pH values, due to the higher acid dissociation constant (pKa) compared with lysine [13].

It should be noted that a further increase in the amino acid content in the alginate solution prevented the formation of a gel. During the experiment, it was necessary to take into account that the viscosity of alginate depends on the acidity of the medium. Thus, according to Lee KY& Mooney, it increases with a decrease in acidity, reaching a maximum at a pH value of 3–3.5 [14].

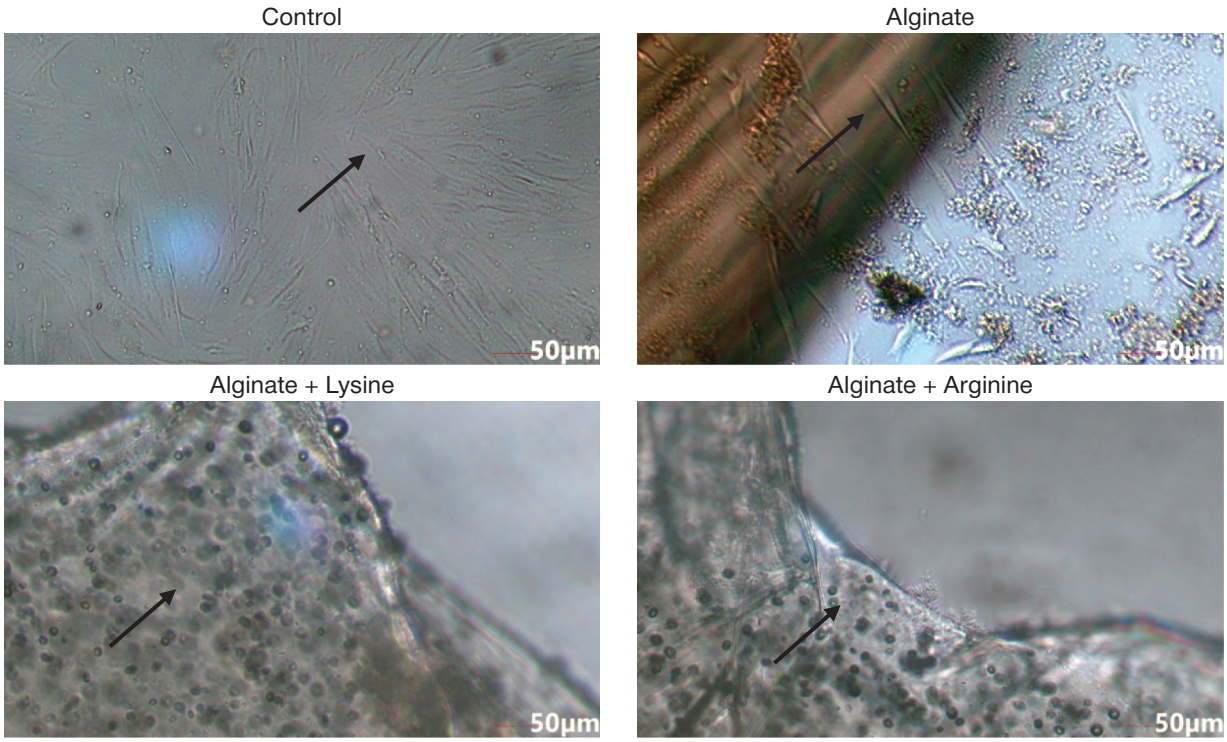

Figure prepared by the authors using their own data

Fig. 5. Optical microscopy of DF2 cells on alginate gels
Note: the arrows indicate the cells on the surface of the cup and inside the gel.

Under physiological conditions (pH=7), the L-arginine and L-lysine amino acids have a positive charge. However, when measuring the pH of alginate solutions with these amino acids, this value turned out to be 11. Despite the high importance of pKa, especially for arginine, this could have an effect on both the amino acid charge and the stability of the alginate gel [15, 16].

Human dermal fibroblasts were applied onto the surface of alginate gels followed by analysis of the gels using optical microscopy (Fig. 5). The surface of the culture plastic was used as a control. Figure 5 showed that after 3 days of culture, the cells on the plastic had a fusiform shape, characteristic of fibroblast-like cells. When applying a cell suspension to alginate gels without modification, no cells on the gels were observed: all cells migrated to the surface of the culture plastic and proliferated only on the surface of the culture vessel. When applying a cell suspension to alginate gels modified with amino acids, the cells adhered to the gels; however, their morphology was spherical. Indeed, this form is typical for cells cultured on alginate gels, since the negative charge of alginate prevents the cells from spreading, but the presence of positively charged amino acids such as lysine and arginine in the gels contributed to a sufficiently high adhesion of cells to modified alginate gels. Therefore, the introduction of amino acids into alginate gels modified with amino acids promotes cell adhesion.

CONCLUSION

In this study, gels modified with natural amino acids — arginine and lysine — were obtained. It was found that the rate of amino acid desorption from the gel can be managed by varying the concentration of amino acids in the modified alginate gel: the higher the amino acid concentration, the less amino acid is released from the gel. In addition, the intensity of amino acid desorption can be increased by increasing the volume of water. The lysine desorption rate is higher than that of arginine from the alginate gel. The sufficiently high adhesive ability of human dermal fibroblasts to alginate gels modified with amino acids suggests that such wound dressings can be used not only as carriers for transplanted cells, but also as facilitators of migration of the patient's own cells from the surrounding tissues bordering the wound bed.

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Authors' contributions. All authors confirm that their authorship meets the ICMJE criteria. The greatest contribution was distributed as follows: Valentina A. Konson — conducting experiments on gel formation and assessment of amino acid desorption; Ilya A. Barsuk — laboratory studies, fibroblast culturing; Yuliya A. Nashchekina — research concept, scientific guidance, manuscript writing.

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APPLICATION OF ALGINATE POLYMER POLYSACCHARIDE HEMOSTATIC HYDROGEL FOR ONGOING ARTERIAL BLEEDING FROM PEPTIC ULCER OF GASTROENTEROANASTOMOSIS (A CLINICAL CASE)

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Introduction. Treatment of ulcer gastroduodenal bleeding is a challenging problem of emergency surgery. The combined technique of endoscopic hemostasis in ongoing arterial ulcer bleeding reduces the rate of recurrences in only 21.3% of cases, which indicates the need for improved endoscopic hemostasis technologies. In this regard, the use of triple therapy based on conventional methods (injections, coagulation, clipping) and local hemostatic systems seems promising.

Clinical case description. We describe a clinical case of ongoing arterial ulcer bleeding from a peptic ulcer of gastroenteroanastomosis treated by endoscopic hemostasis using a 5% solution of aminocaproic acid with adrenaline in combination with argon plasma coagulation and pneumatic insufflation of an alginate polymer polysaccharide hemostatic hydrogel on the bleeding site.

Conclusions. The use of a personalized approach to endoscopic hemostasis of arterial bleeding from peptic ulcer of gastroenteroanastomosis using an alginate polymer polysaccharide hemostatic hydrogel as part of combined therapy improves the treatment outcome by ensuring final hemostasis, avoiding emergency surgery for massive bleeding, and facilitating the healing process of the ulcerous defect.

Keywords: gastrointestinal bleeding; peptic ulcers of gastroenteroanastomosis; endoscopic hemostasis; alginate polymer polysaccharide hemostatic hydrogel

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Compliance with the ethical principles: a written informed voluntary consent was received from the patient to publish a description of the clinical case, anonymized medical data and photographs.

Potential conflict of interest: the authors declare no conflict of interest.

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ПРИМЕНЕНИЕ АЛЬГИНАТНОГО ПОЛИМЕРНОГО ПОЛИСАХАРИДНОГО ГЕМОСТАТИЧЕСКОГО ГИДРОГЕЛЯ ПРИ ПРОДОЛЖАЮЩЕМСЯ АРТЕРИАЛЬНОМ КРОВОТЕЧЕНИИ ИЗ ПЕПТИЧЕСКОЙ ЯЗВЫ ГАСТРОЭНТЕРОАНАСТОМОЗА (КЛИНИЧЕСКИЙ СЛУЧАЙ)

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Введение. Проблема лечения гастродуоденальных кровотечений язвенной этиологии — одна из наиболее сложных в экстренной хирургии. Даже при использовании комбинированной технологии эндогемостаза продолжающегося артериального язвенного кровотечения частота рецидивных кровотечений может достигать 21,3%, что указывает на необходимость совершенствования технологий эндогемостаза кровоточащей гастродуоденальной язвы. В совершенствовании комбинированных технологий эндогемостаза перспективным представляется использование тройной терапии, включающей применение традиционных способов (обкалывание, коагуляция, клипирование) с дополнительным включением местных гемостатических систем.

Описание клинического случая. Описан клинический случай применения комбинированной технологии эндоскопического гемостаза продолжающегося артериального кровотечения из пептической язвы гастроэнтероанастомоза с применением обкалывания 5%-ным раствором аминокапроновой кислоты с адреналином в сочетании с аргоноплазменной коагуляцией и пневмоинсуффляцией альгинатного полимерного полисахаридного гемостатического гидрогеля на источник кровотечения.

Выводы. Использование персонализированного подхода к эндоскопическому гемостазу артериального кровотечения из пептической язвы гастроэнтероанастомоза с применением альгинатного полимерного полисахаридного гемостатического гидрогеля в комплексной терапии позволило улучшить результаты лечения пациента: обеспечить окончательный гемостаз, избежать неотложной операции на «высоте» кровотечения и улучшить качество заживления язвенного дефекта.

Ключевые слова: желудочно-кишечные кровотечения; пептические язвы гастроэнтероанастомоза; эндоскопический гемостаз; альгинатный полимерный полисахаридный гемостатический гидрогель

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Соответствие принципам этики: от пациента получено письменное информированное добровольное согласие на публикацию описания клинического случая, обезличенных медицинских данных и фотографий.

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INTRODUCTION

Peptic ulcer disease (PUD) of the stomach and duodenum is a widespread problem affecting mainly young and able-bodied patients. From 2006 to 2017, the occurrence and prevalence of PUD in the Russian Federation decreased from 128.7 to 79.5 per 100,000 population[1]. However, its complication rate shows no downward trend. The most common PUD complication is gastrointestinal bleeding. According to Amiran S. Revishvili, the Chief Surgeon of the Russian Federation, 47,224 cases of ulcer upper gastrointestinal bleeding (UGIB) were registered in 2023 in Russia, with the total mortality rate of 1.49–17.81%. The postoperative mortality rate reached the level of 6.17–62.5%[2].

Patients with bleeding peptic ulcers of gastroenteroanastomosis (GEA) form a separate group. According to statistical data, the current incidence of post-gastrectomy peptic ulcers ranges within 0.5–15%. At the same time, about 90–98% of GEA peptic ulcers are known to develop after partial gastrectomy for duodenal ulcers (DU). The main reasons for the formation of GEA peptic ulcers include preservation gastrectomy (less than 2/3 of the stomach); reservation of a part of the antrum gastric mucosa in the duodenal stump after Billroth II operation; vagus hypertonus and incomplete vagotomy if performed in combination with preservation gastrectomy; adverse effects of the biliary and pancreatic secretions on the gastric stump mucosa in anastomoses other than isolated Roux loop. Complications of GEA peptic ulcers are observed in 75–80% of patients, presenting a significant healthcare problem. Gastrointestinal bleeding is one of the most common complications of GEA peptic ulcers [1, 2].

UGIB patients with ongoing arterial bleeding are classified as Forrest Ia cases according to J. Forrest's Classification for Bleeding Peptic Ulcer [3]. In the structure of UGIB, this type of bleeding accounts for about 7.7% of cases; however, their treatment is associated with significant difficulties. In the management of such patients, it is extremely important to reliably stop the ongoing arterial UGIB and create conditions for the prevention of its recurrence [4, 5].

Despite the recent progress in the development of endovascular hemostasis technologies, therapeutic endoscopy remains to be the leading approach to the treatment of such patients. The modern methods of endoscopic hemostasis (EH) for ongoing arterial ulcer UGIB include injectable, physical, mechanical, and combined EH. Endoscopic injections around the bleeding site with various solutions reduce or eliminate arterial ulcer UGIB due to vasoconstriction and local tamponade. The effectiveness of injectable EH in ongoing bleeding reaches 85–92%. However, injectable monotherapy is not recommended due to its temporary effect and a high rate of recurrences that reach about 17.6–24.0%. Argon plasma coagulation (APC) occupies a special place among the EH physical methods. This non-contact method does not involve welding of the diathermy probe (electrode) to the coagulation scab. The effectiveness of APC in primary EH reaches 95–100%. At the same time, the APC method possesses a number of drawbacks: thus, similarly to other coagulation techniques, the violation of the protocol may lead to perforation of the organ wall and bleeding relapses, which are observed in 12.5–25.0% of cases [6, 7].

Clipping, i.e., constriction of the bleeding point by a metal clip, is an effective EH approach in the case of arterial ulcer UGIB. The EH using clips is similar to the surgical retroclusion of a bleeding vessel. The clip is passed through the instrumental channel of the endoscope and fixed by a clip applier across the vessel, thereby reliably stopping the bleeding. Clipping is effective in bleeding from destructed large vessels (more than 2 mm in diameter), as well as in patients with severe hypocoagulation. However, the disadvantage of clipping includes the complexity of its implementation. In addition, endoclips are difficult to use in hard-to-reach anatomical areas, such as the lesser curvature of the stomach, the cardia, and the duodenum posterior wall. The use of endoclips is associated with ulcer UGIB relapses, observed in 8–37% of cases [8, 9].

Combined EH is currently considered to be the most advanced EH of arterial ulcer UGIB. Injections around the bleeding site with subsequent clipping of the destructed vessel or injections in combination with APC are often used. Combination (dual) therapy, i.e., infiltration in combination with coagulation of the bleeding point or mechanical compression using hemoclips, remains the endoscopic

[1] Clinical guidelines for Peptic Ulcer disease. Ministry of Health of the Russian Federation; 2024.
[2] Revishvili AS, Olovyannyi VE, Gogia BS, Gurmikov BN, Markov PV, Ruchkin DV, et al. Surgical care in the Russian Federation. Moscow. 2024.

therapy of choice recommended in the main international clinical guidelines for the treatment of patients with ulcer UGIB. However, according to N.V. Lebedeva, the use of combined EH technologies for ongoing arterial ulcer UGIB is also associated with a high recurrent bleeding rate of about 21.3%, which indicates the need to improve EH technologies [9–11].

The use of hemostatic powders and gels is a promising EH of ulcer UGIB. Hemostatic powders absorb blood, exhibit a mechanical tamponing effect, and activate the blood coagulation cascade [12]. Hemospray (TC-325) and EndoClot endoscopic powder systems have become widespread in clinical practice. However, the action of Hemospray and EndoClot is limited to less than 24 h; moreover, they do not promote tissue regeneration [13]. It should be noted that the Voronezh City Specialized Center for the Treatment of Patients with Gastroduodenal Bleeding (Russia) has accumulated extensive experience in the use of powdered granular sorbents (Sephadex®, Gelevinum, Aseptisorb®, etc.) in the GIB treatment. The application of these sorbents to the bleeding point forms a soft elastic gel, which remains on the surface of the defect for up to four days and improves the healing of gastroduodenal ulcers. However, the use of powdered hemostatic systems in the EH of ongoing arterial ulcer UGIB was shown to have low efficiency [14].

The possibility of applying the Diovine granular sorbent with the Gelplastan and NovoSeven® lyophilizate hemostatic agents in the combined endoscopic treatment of ulcer UGIB was described by M.N. Romantsov et al. This technology produced positive results by reducing the ulcer UGIB recurrence risk to 5.01% and mortality to 1.7% [15].

Japanese scientists T. Uroka, N. Ueda, et al. reported the successful application of innovative EH technology using a gel-like hemostatic matrix for GIB after endoscopic submucous dissection. The gel-like hemostatic matrix is a viscous transparent peptide that forms a sealing layer after contact with tissue or blood. The use of this matrix reduces the need for coagulation to stop gastroduodenal bleeding [16].

The use of a triple therapy, which combines conventional EH methods (injections, coagulation, and clipping) with local hemostatic systems, seems to be a promising direction. One such domestic technology of combined EH of ulcer UGIB consists in the use of an alginate polymer polysaccharide hemostatic hydrogel (APPHH) [17]. Previously, we reported an experimental study into the possibility of using APPHHs in the treatment of bleeding stomach defects [18] and the first experience of APPHH clinical use in the combined endoscopic treatment of unstably stopped ulcer bleeding [19]. The clinical research into the use of APPHHs in a combined EH of ulcer UGIB is currently underway.

In this article, we describe the use of an APPHH in the combined treatment of ongoing arterial ulcer UGIB in a patient with GEA peptic ulcer.

CLINICAL CASE DESCRIPTION

On 13.10.2024 at 7:56 a.m., a 73-year-old patient was urgently admitted the Voronezh City Clinical Emergency Hospital No. 1. On admission, the patient complained of vomiting blood with clots, black tarry stools, discernible weakness, and dizziness.

The patient had been ill since 1995, after a Billroth II partial gastrectomy due to duodenal ulcer profuse bleeding. During the 1995–2023 period, the patient had no gastrointestinal complaints. In 2023, GEA peptic ulcer bleeding was detected, which was surgically treated in Kazan. His state worsened on 13.10.2024 with the following symptoms: discernible weakness, dizziness, fainting, decrease in blood pressure to 90/60 mm Hg, black liquid stools, and vomiting blood with clots. He had a history of acute myocardial infarction, type 2 diabetes mellitus, gout. At the moment of admission, the patient was taking Aspirin® on a regular basis.

Objective data. Examination in the emergency department. On admission, the patient's condition was severe. Consciousness was clear, 15 points on the Glasgow Coma Scale (GCG), blood pressure (BP) — 90/60 mm Hg, heart rate (HR) — 110 per minute, respiratory rate (RR) — 18 per minute, SPO_2 — 98%. The overall health status was severe; the skin was pale. During auscultation of the lungs, vesicular respiration occurred in all pulmonary fields, no wheezing. The heart tones were muted and rhythmic. The tongue was dry, covered with a white coating. The stomach was not swollen. An old postoperative scar on the anterior abdominal wall after an upper-midline incision was present. On palpation, the abdomen was soft and painless in all parts. Infiltrates in the abdominal cavity; liver, spleen, kidneys were not palpated. Peritoneal symptoms were negative in all parts of the abdomen. During percussion, hepatic dullness was normal, displaced dullness of percussion sound in the lateral abdominal regions was not detected. Normal peristaltic sounds were listened by auscultation. No costovertebral angle tenderness. *Per rectum*: traces of liquid black feces on the glove. A nasogastral tube was inserted, and altered blood was obtained through the tube. Simultaneously with the emergency care, diagnostic tests were performed in the emergency department: general and biochemical blood tests, hemostasis system examination, blood type and Rh factor determination, electrocardiographic examination, abdominal ultrasonography and examination by a therapist.

Predominant diagnosis: peptic ulcer of gastroenteroanastomosis with bleeding (ICD 10 — K28.4, chronic or unspecified gastrojejunal ulcer with hemorrhage). Complication of the main diagnosis: severe haemorrhage (A.I. Gorbashko, 1982), 3 Grade blood loss anemia.

Secondary diagnosis: coronary heart disease; postinfarction and atherosclerotic cardiosclerosis; coronary artery aortic atherosclerosis; stage 3 hypertension; Class I chronic heart failure, functional class I; diabetes mellitus type 2; individual target HbA1c level less than 7.5%; myocardial dystrophy; diabetic polyneuropathy; diabetic encephalopathy; gout is not acute.

Medical interventions. Emergency esophagogastroduodenoscopy (EGDS) was performed urgently (30 minutes after admission) (Fig. 1).

Examination of gastroenteroanastomosis (Billroth II operation): the gastric remnant contained a moderate amount of altered blood, which made a detailed

examination difficult. The gastric folds were low, longitudinal-and-convoluted, and expanded by air. Peristalsis was satisfactory, with smooth waves in all parts. The mucosa closer to the gastroenteroanastomosis was covered with fresh blood. Gastroenteroanastomosis was wide and freely passable; incomplete visualization of the mucous membrane due to blood residues was noted. In the anastomotic spur area, a spurting bleeding from a destructed vessel 1.0 mm in diameter was noted, the base of the vessel was not brought into the field of view (Fig. 1A). A 5% solution of aminocaproic acid with 0.1% epinephrine solution was injected paravasally into the area of the anastomosis using a disposable endoscopic injector with a needle diameter of 0.7 mm and a needle length of 4 mm until an infiltrate was created (Fig. 1B). The massive bleeding was stopped. After washing, a flat-surface ulcerous defect 7 mm in diameter and 1.5 mm deep was revealed on the anastomosis spur, with a fixed blood clot 5 mm in diameter at the bottom. An attempt to remove the clot with an irrigator and visualize the destructed vessel had no effect. Clipping was technically impossible.

The APC of the bleeding point through the clot was performed using an electrosurgical coagulator with ERBE APC 2 (Elektromedizin) accessories: operating mode FORCED APC, power 30 W (max), flow rate 1.0 L/min; probe for argon plasma coagulation — Flexible Argonsonde REF 932-149 (BOWA), diameter 2.3 mm. Diffuse blood leakage was noted after APC (Fig. 1B). In order to achieve final hemostasis to the bleeding point using an endoscopic pump for pumping an air mixture (EndoClot Air Compressor, EPAC-2, EndoClot Plus. Inc., USA), in an air supply mode of 1.3 L/min through an endoscopic catheter 2.5 mm of diameter, insufflation of powdered APPHH was performed in the amount of 0.5 g. After insufflation to the bleeding point, upon contact with blood and mucus, the powdered hemostatic was actively soaked in blood, turning into a hydrogel. The final hemostasis had been achieved; blood leakage had been stopped (Fig. 1 D).

Further treatment was carried out in the intensive care unit (ICU). The patient refused to perform endoscopic monitoring after the EH. A nasogastric tube was used to control the recurrence of GIB.

On the day 3 of inpatient treatment (15.10.2024), a control EGDS was performed (Fig. 2). The gastric remnant contained a moderate amount of foamed bile. No blood and hematin was present. The ulcerous defect on the anastomosis spur was 0.7 cm in diameter, superficial, and the ulcer base was covered with an alginate hydrogel soaked in bile (Fig. 2A). The hydrogel was removed, and fibrin was present at the ulcer base. In order to prevent recurrence of bleeding and cytoprotective treatment for the ulcerous defect using an endoscopic pump to inject an air mixture in an air supply mode of 1.3 L/min through an endoscopic catheter 2.5 mm of diameter, APPHH insufflation in the amount of 0.5 g was performed (Fig. 2B). A rapid test for the presence of *Helicobacter pylori* was positive. After the dynamic EGDS, the patient was transferred to a general ward.

During the following three days, the patient's status remained unchanged. On day 6 of inpatient treatment (18.10.2024), a control EGDS was performed (Fig. 3).

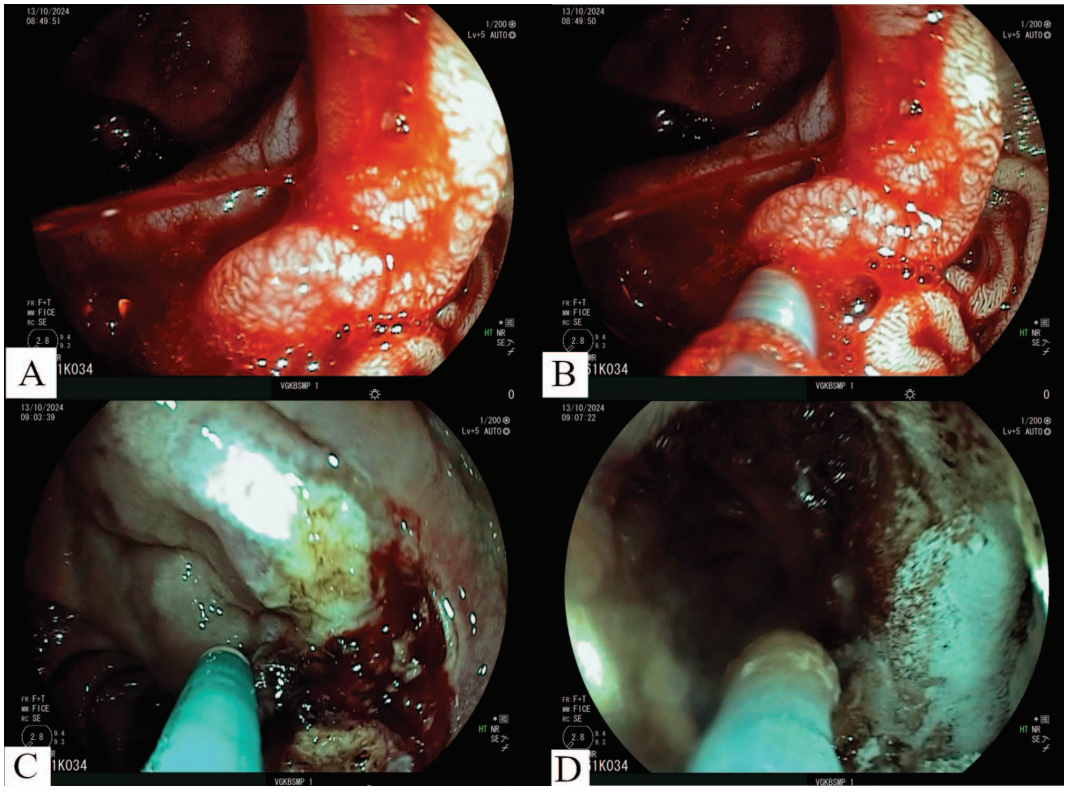

Photo taken by the authors

Fig. 1. Endoscopic images of gastroenteroanastomosis, white light examination: A — ulcerous defect of gastroenteroanastomosis with massive spurting bleeding from the destructed vessel; B — injection compression of the destructed vessel; C — argon plasma coagulation of the bleeding point; D — APPHH insufflation for the ulcerous defect

No traces of blood or hematin in the examined sections of the gastric remnant (foamed bile) were noted. The ulcer of the gastroenteroanastomosis spur decreased in size to 5×4 mm, superficial, covered with a transparent hydrogel soaked in bile (Fig. 3A), after which the hydrogel was removed (Fig. 3B). The ulcer base was covered with fibrin with islands of granulation tissue; marginal epithelialization was observed.

In order to prevent bleeding and cytoprotective treatment using an endoscopic pump for pumping an air mixture in an air supply mode of 1.3 L/min through an endoscopic catheter 2.5 mm of diameter, APPHH insufflation was performed (Fig. 3C, D). The patient was discharged from the hospital and forwarded for outpatient treatment to a polyclinic at his place of residence. On 29.10.2024, a control EGDS was performed at the outpatient stage of treatment (Fig. 4).

On examination: the stomach was resected according to the Billroth II type, its stump was small, the gastroenteroanastomosis did not gape widely. A delicate whitish linear scar on the gastroenteroanastomosis spur was seen. The patient had a moderate risk of recurrent gastroduodenal bleeding according to the digital program "Prevention of gastroduodenal bleeding: an individualized risk assessment scheme, the formation of recommendations on patient management tactics" [20]. The patient was given personalized recommendations for the prevention of recurrent gastroduodenal bleeding.

CLINICAL CASE DISCUSSION

The number of patients with PUD in the Russian Federation is demonstrating a pronounced downward trend, largely due to the current achievements of the state programs. These include a medical examination program with a wide population coverage, the availability of endoscopic examination, as well as the use of highly effective treatment regimens. However, the rate of complicated forms of peptic ulcer disease, and above all, ulcer UGIB, remains at a high level in Russia, showing no reduction in recent years[3].

Modern surgical achievements in the treatment of ulcer UGIB (EH, improvement of drug-induced hemostasis, endovascular hemostasis, etc.) have significantly improved the treatment outcomes of patients with ulcer UGIB. However, in situations where ulcer UGIB cannot be stopped using conservative methods, surgeons are forced to perform emergency surgery under the conditions of massive bleeding. The main types of surgical interventions in such patients are retroclusion of a bleeding vessel in an ulcer and, less often, partial gastrectomy [21, 22].

At present, there is no doubt that therapeutic endoscopy plays a leading role in the treatment of patients with ulcer UGIB. The international community is actively engaged in the development and application of local hemostatic systems in the form of powders, gels, etc., for the treatment of gastroduodenal ulcer bleeding [23]. Most modern powdered endoscopic systems are non-Russian products. The development of domestic endoscopic hemostasis technologies is an important direction in the context of raising the technological sovereignty and leadership of the country. This highlights the importance of exploring the possibility of using alginate polymer polysaccharide hemostatic hydrogels (APPHHs) in clinical practice.

Alginate polymer polysaccharide hemostatic hydrogel is a powdered, finely dispersed hemostatic agent, the derivatives of which are sodium alginate, potassium sorbate, sodium benzoate, iodoform, and tricalcium phosphate. Upon contact with the bleeding surface and mucus, this hemostatic turns into a hydrogel followed by its tight adhesion to the area of the bleeding point, which provides for prolonged local hemostasis. Preliminary experimental studies into the use of APPHH in the treatment of stomach bleeding have shown its efficacy in stopping experimental bleeding and facilitating the healing process of upper digestive tract defects, which was confirmed by morphological studies [18].

It is important to note that PUD is a chronic disease, which makes the healing quality of the ulcerous defect

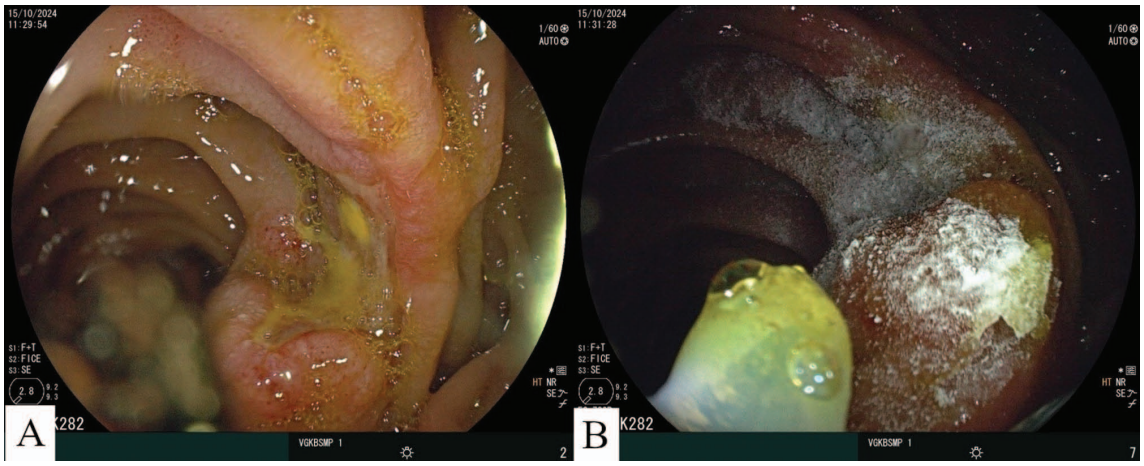

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Fig. 2. Endoscopic images of gastroenteroanastomosis on day 3 of inpatient treatment, white light examination: A — fixed APPHH soaked in bile in the area of the ulcerous defect; B — additional APPHH insufflation for ulcerous defec.

[3] Kotova EG, Kobyakova OS, Alexandrova GA, Golubev NA, Oskov Yul, Polikarpov AV, Shelepova EA. Morbidity of the entire Russian population in 2021: statistical materials. Moscow: Central Research Institute of the Ministry of Health of the Russian Federation. 2022.

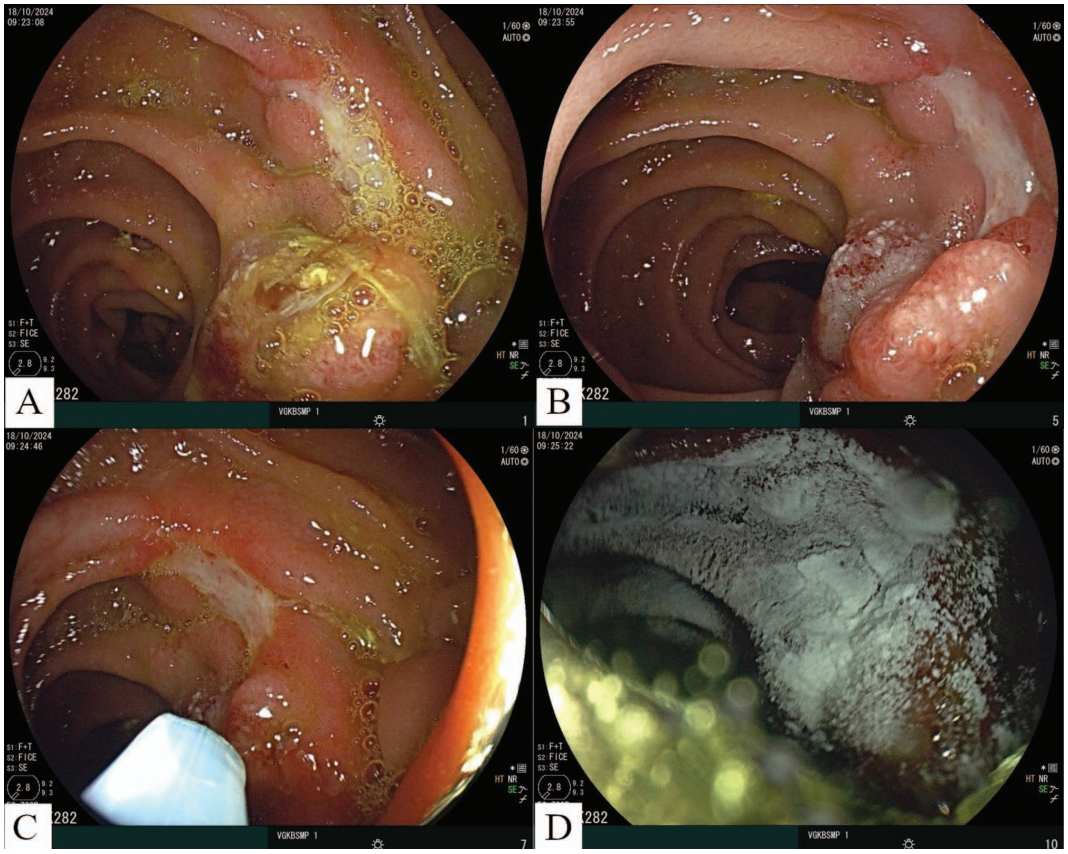

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Fig. 3. Endoscopic images of gastroenteroanastomosis on day 6 of inpatient treatment, white light examination: A — fixed APPHH soaked in bile in the area of the ulcerous defect; B — ulcerous defect on the gastroenteroanastomosis spur after washing the APPHH; C — the onset of APPHH insufflation for ulcerous defect; D — fixed APPHH in the area of the ulcerous defect

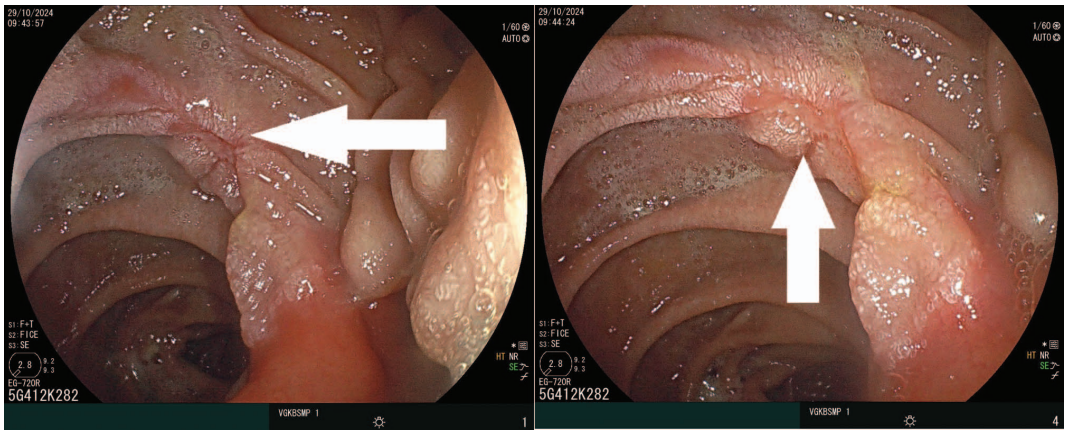

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Fig. 4. Endoscopic images of gastroenteroanastomosis on day 17 of treatment, white light examination. The arrow indicates a delicate whitish linear scar on the gastroenteroanastomosis spur

particularly important in the treatment of gastroduodenal ulcers. According to L.I. Aruin, it is the rough scar that acts as the pathomorphological substrate for PUD exacerbation [24].

In the present article, we describe the experience of using APPHH in the combined treatment of ongoing arterial ulcer UGIB. The discussed clinical case was complicated by massive arterial ulcer UGI, which was treated using a combined EH by injections to the bleeding point followed by APC of the destructed vessel. Following APC, diffuse blood leakage persisted, and the endoscopist had the opportunity to continue APC of the bleeding point. It should be borne in mind that intensive prolonged coagulation can lead to perforation of the hollow organ wall and the development of peritonitis. This complication is extremely serious and can lead to death against the background of acute blood loss. The APPHH application to the bleeding defect allowed us to solve the problem of blood leakage and achieve final hemostasis. Repeated EGDS revealed the absence of bleeding relapses and

pronounced cytoprotective properties of APPHH. Due to its pronounced adhesive properties, the APPHH composition used remained on the ulcerous defect for up to three days. EGDS on day 6 revealed signs of ulcerous defect healing in the form of marginal epithelialization, and on day 17, complete healing of the GEA peptic ulcer in the form of a delicate scar.

Summarizing the presented clinical case, the use of a personalized approach to endoscopic hemostasis of arterial bleeding from GEA peptic ulcer in a patient with severe somatic pathology and a high-degree surgical risk, the use of APPHH as part of combined therapy improved the treatment outcome by ensuring final hemostasis, avoiding urgent surgery for massive bleeding, and improving the healing quality of the ulcerous defect. These aspects are extremely important in the treatment of UGIB patients with various concomitant somatic pathologies. In such patients, the impossibility to stop bleeding using conservative methods often requires surgical interventions, which may lead to fatal outcomes.

CONCLUSION

The use of an alginate polymer polysaccharide hemostatic hydrogel (APPHH) as part of the combined endoscopic treatment of ongoing ulcer UGIB ensured reliable hemostasis and allowed recurrent bleeding and emergency surgery to be avoided. It should be noted that this publication describes the first experience of using APPHH technology in a patient with ongoing arterial bleeding from a GEA peptic ulcer. The accumulation of data from larger patient cohorts and their detailed analysis will elucidate the feasibility and effectiveness of this EH technology for clinical surgical practice.

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EDN: PBZWJR

Authors' contributions. All the authors confirm that they meet the ICMJE criteria for authorship. The most significant contributions were as follows. Sergey V. Barannikov — literature analysis, medical intervention, patient supervision, preparation of a draft manuscript; Evgeniy F. Cherednikov — research concept; Galina V. Polubkova — manuscript editing; Igor N. Banin — data collection; Alexey E. Bolkhovitinov — endoscopic examinations; Sergey I. Berezhnoy — medical intervention; Eva E. Slyusareva — literature analysis.

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POTENTIALLY SIGNIFICANT MARKERS OF IMMUNE SYSTEM AND BLOOD CLOTTING RESIDUAL DISORDERS IN ATHLETES WITH A HISTORY OF COVID-19

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Introduction. The increase in the number of patients with post-acute sequelae of COVID-19, including athletes, requires improved diagnostic methods for the long-term effects of this disease. The study of changes in the blood-clotting and immune regulation in a limited but homogeneous group of athletes (intense physical activity and careful health monitoring) with post-acute sequelae of COVID-19 can contribute to identification of reliable diagnostic and prognostic biomarkers of this condition.

Objective. A comparative analysis of the cytokine profile and hemostasis system features as promising prognostic markers for the diagnosis of post-acute sequelae of COVID-19 in athletes.

Materials and methods. 60 athletes (24 men and 36 women, average age 20.8 ± 1.86 years) were examined. All participants were divided into two groups: group 1 — 40 athletes who had suffered from coronavirus infection; group 2 (control) — 20 athletes who had not had COVID-19. The athletes specialized in various sports: figure skating, rhythmic gymnastics, athletics, rugby, and wrestling. To assess the residual effects of COVID-19, biochemical parameters were studied in all participants: alanine aminotransferase activity, aspartate aminotransferase, C-reactive protein level, troponin-I level; hemostasis parameters: prothrombin time (PT), prothrombin index (PTI), activated partial thromboplastin time (aPTT), international normalized ratio (INR), D-dimer; immune status indicators: interleukins-6, -8, -10 (IL-6, -8, -10, respectively), tumor necrosis factor-α (TNF-α). Statistical data processing was carried out using the Statistica 10 software.

Results. An increase in clotting time was revealed in terms of aPTT, prothrombin time, international normalized ratio, and a decrease in the prothrombin index ($p < 0.05$). Statistically significant differences in the functional state of the immune system were also found: an increase of IL-8 from 2.5 to 7.42 times [3.08; 9.96] pg/mL and IL-10 from 2 to 5.08 times [2.93; 6.66] pg/mL compared to similar indicators in athletes who had not suffered from COVID-19 — 3.05 [1.86; 8.15] pg/mL and 2.53 [1.0; 5.68] pg/mL ($p < 0.05$) in the group of athletes who had undergone COVID-19. A direct correlation was established between an increase in IL-8 levels and an increase in PT ($r_s = 0.355$; $p < 0.05$) and INR ($r_s = 0.420$; $p < 0.05$) in athletes who had undergone a coronavirus infection. At the same time, a negative association was found between an increase in IL-8 levels and a decrease in PTI ($r_s = -0.323$; $p < 0.05$).

Conclusions. Higher levels of activating cytokines and lower values of parameters of the anti-inflammatory immune system indicate residual dysregulatory phenomena in the immune system in post-acute sequelae of COVID-19. The revealed relationships between the coagulation profile and the components of the immune response allow these relationships to be considered as possible diagnostic markers of residual phenomena after coronavirus infection. The data obtained confirm the validity of IL-8 and IL-10 indicators as potential markers of residual disorders after COVID-19 in athletes. However, these findings should be verified on larger samples, where the observed ratios may show a different dynamics.

Keywords: athletes; diagnosis; post-acute sequelae of COVID-19; cytokines; coagulation profile

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ПОТЕНЦИАЛЬНО ЗНАЧИМЫЕ МАРКЕРЫ РЕЗИДУАЛЬНЫХ НАРУШЕНИЙ СИСТЕМЫ ИММУНИТЕТА И СВЕРТЫВАНИЯ КРОВИ У СПОРТСМЕНОВ, ПЕРЕБОЛЕВШИХ COVID-19

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Введение. Увеличение числа пациентов с постковидным синдромом, в том числе среди спортсменов, требует усовершенствования методов диагностики долгосрочных последствий данного заболевания. Исследование изменений в системе свертывания крови и иммунной регуляции у ограниченной по численности, но однородной по ключевым характеристикам группы спортсменов (интенсивные физические нагрузки и тщательный мониторинг здоровья) с постковидным синдромом может помочь выявить надежные диагностические и прогностические биомаркеры этого состояния.

Цель. Сравнительный анализ особенностей цитокинового профиля и системы гемостаза как перспективных прогностических маркеров диагностики постковидного синдрома у спортсменов.

Материалы и методы. Выполнено обследование 60 спортсменов (24 мужчины и 36 женщин, средний возраст 20,80 ± 1,86 года). Все участники были распределены на 2 группы: группа 1 — 40 атлетов, перенесших коронавирусную инфекцию; группа 2 (контроль) — 20 атлетов, не болевших COVID-19. Спортсмены специализировались в различных видах спорта: фигурное катание, художественная гимнастика, легкая атлетика, регби и спортивная борьба. Для оценки резидуальных явлений COVID-19 у всех участников исследовали биохимические показатели: активность аланин-

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аминотрансферазы, аспартатаминотрансферазы, уровень С-реактивного белка, уровень тропонина-I; показатели гемостаза: протромбиновое время (ПТВ), протромбиновый индекс (ПТИ), активированное частичное тромбопластиновое время (АЧТВ), международное нормализованное отношение (МНО), D-димер; показатели иммунного статуса: интерлейкины-6, -8, -10 (ИЛ-6, -8, -10 соответственно), фактор некроза опухолей-α (ФНО-α). Статистическая обработка данных проводилась с помощью программного обеспечения Statistica 10.

Результаты. Выявлено повышение времени свертывания по показателям АЧТВ, протромбинового времени, международного нормализованного отношения и снижение протромбинового индекса ($p < 0,05$). Также были обнаружены статистически значимые различия в функциональном состоянии иммунной системы повышение ИЛ-8 в 2,5 раза до 7,42 [3,08; 9,96] пг/мл и ИЛ-10 в 2 раза до уровня 5,08 [2,93; 6,66] пг/мл в группе атлетов, переболевших COVID-19, по сравнению с аналогичными показателями у не болевших COVID-19 спортсменов — 3,05 [1,86; 8,15] пг/мл и 2,53 [1,0; 5,68] пг/мл ($p \leq 0,05$). Установлена прямая корреляционная связь между повышением уровня ИЛ-8 и повышением ПТВ ($r_s = 0,355$; $p < 0,05$) и МНО ($r_s = 0,420$; $p < 0,05$) у спортсменов, переболевших коронавирусной инфекцией. В то же время отрицательная ассоциация была выявлена между повышением уровня ИЛ-8 и снижением ПТИ ($r_s = -0,323$; $p < 0,05$).

Выводы. Более высокие уровни активирующих цитокинов и низкие значения параметров в противовоспалительном звене иммунитета указывают на остаточные дизрегуляторные явления в иммунной системе при постковидном синдроме. Выявленные взаимосвязи между показателями коагулограммы и компонентами иммунного ответа позволяют рассматривать их как возможные диагностические маркеры остаточных явлений после перенесенной коронавирусной инфекции. Полученные данные подтверждают состоятельность показателей ИЛ-8 и ИЛ-10 в качестве потенциальных маркеров резидуальных нарушений после COVID-19 у спортсменов. Однако стоит учитывать, что объемы выборок были небольшими и при увеличении количества наблюдений соотношения могут измениться.

Ключевые слова: спортсмены; диагностика; постковидный синдром; цитокины; коагулограмма

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INTRODUCTION

The term “post-acute sequelae of COVID-19” (PASC) was proposed in February 2020 to describe the residual manifestations detected in individuals with a history of SARS-CoV-2 infection. Further, in October 2020, this syndrome received a separate ICD-10 code: U09.9 — post COVID-19 condition, unspecified.

During the development of PASC, symptoms that cannot be explained by an alternative diagnosis appear, on average, three months after the onset of the disease. At the moment, the literature proposes no model for the diagnosis of PASC for both the general population, whose degree of motor inertia has increased significantly over the years of the pandemic, and athletes, whose share in the global population is extremely insignificant [1]. However, although the positive effect of regular physical activity on the body’s resistance to infectious agents is generally recognized, which is essential during periods of epidemiological outbreaks, the effects of excessive physical exertion, characteristic of high-performance sports, are not that unambiguous. Thus, the so-called “open window effect” occurs during certain stages of one-year training, expressed in an increase in the body’s susceptibility to infectious diseases after significant physical exertion [2].

The PASC clinical manifestations are highly diverse: a total of 55 long-term symptoms associated with COVID-19 have been described. Most of them correspond to clinical symptoms or syndromes of the central nervous system and the mental health, respiratory, cardiovascular, immune, digestive systems, etc. A meta-analysis of studies ($n = 15$), which included characterization of the PASC signs, showed that up to 80% of patients who have suffered COVID-19 experience long-term consequences in the form of monosymptoms and their associations [3]. However, other values were obtained for the cohort of athletes. Thus, the study [4] examining the long-term effects of the disease among 11,518 athletes of various skill levels found that the incidence of PASC persistent symptoms was only 8.3%. The tendency towards a milder (often asymptomatic) course of COVID-19 in a cohort of athletes with a lower probability of complications, including viral pneumonia, than in the general population, can be considered as a probable cause of such pronounced differences.

In addition to routine markers, diagnostic search programs for the sports contingent were advised to include an assessment of laboratory parameters of cardiac function, hormonal status, immune response, and the coagulation system. In most cases, the values of the studied parameters remain within the reference ranges; however, long-term deviations are also possible, more often of minor severity [4].

Data on the immune system parameters and coagulation profile in patients with the post-acute sequelae of

COVID-19 are heterogeneous. A recent meta-analytical study [5], which covered 23 publications, showed that patients with suspected PASC are more likely to have elevated levels of leukocytes, C-reactive protein (CRP), and D-dimer; however, the prognostic effect of deviations was assessed as insignificant. At the same time, the authors [6] proposed to consider the D-dimer and CRP as some non-cytokine markers, the values of which increase in patients infected with SARS-CoV-2. At the same time, more pronounced differences in the D-dimer level are associated with concomitant pathology revealed by imaging and functional testing methods [7].

In addition, after COVID-19, there may be a slight increase in the activity of other components of the blood-clotting sequence [5], as well as changes in the effector link of the immune response, namely increased activation of T cells, as evidenced by the level of the soluble interleukin-2 receptor [8].

The study [9] reported changes in the immunoregulation system, which were multidirectional in some cases. Thus, it was noted that the levels of interleukin-6 (IL-6), often considered as the leading indicator of proinflammatory cytokine activity, were higher in patients with PASC compared to practically healthy patients and individuals without long-term consequences from COVID-19. The authors in [10] proposed this cytokine, as well as tumor necrosis factor alpha (TNF-α), to be considered as potential predictors of COVID-19 severity in acutely infected patients without concomitant diseases. It should be noted that IL-6 can be actively produced in muscle tissue along with other myokines (myostatin, insulin-like growth factor, fibroblast growth factor, etc.) associated with the level of physical activity [11]. This fact makes it difficult to validate IL-6 levels as a PASC marker in the athlete cohort due to its significant dependence on the level of training and competitive activity.

At the same time, the interleukin-8 (IL-8) level, which exhibits proinflammatory activity, is associated with the COVID-19 severity in the acute period and may be directly involved in the pathogenetic pathways of the formation of post-acute sequelae of COVID-19 [12].

One of the main anti-inflammatory cytokines, interleukin-10 (IL-10), has a pleiotropic effect on the immune response. The researchers in [13] emphasized that IL-10 is a predictor of the severity and mortality of patients with acute infection and residual COVID-19 effects. In this context, IL-10 can act as an endogenous anti-inflammatory component secreted by damaged tissues in response to a hyperinflammatory state, which also allows this indicator to be assumed as a PASC marker.

The growing number of patients with post-acute sequelae of COVID-19, including athletes with a high probability of latent post-infectious myocardial damage, substantiates the need to optimize approaches to diagnosing the long-term consequences of COVID-19. Research into the dynamics of changes in hemostasis and immune regulation in the target group of athletes with PASC, which may be insignificant in number but fairly homogeneous in terms of the characteristics taken into account (significant physical activity and targeted/constant monitoring of health status), is likely to contribute to establishing valid diagnostic and prognostic markers of this condition.

In this study, we set out to compare the features of the cytokine profile and the hemostasis system as promising prognostic markers of post-acute sequelae of COVID-19 in athletes.

MATERIALS AND METHODS

In total, 60 athletes were examined as part of the research work. The study included 24 male and 36 female athletes, with an average age of 20.8 ± 1.86 years. All participants were divided into two groups: group 1 — 40 athletes who had suffered from coronavirus infection (according to medical records); group 2 (control) — 20 athletes who had not suffered from coronavirus infection (according to medical records). All athletes were at the stages of improvement and possessed higher sports skills, specializing in various sports: figure skating (n = 16), rhythmic gymnastics (n = 15), athletics (n = 10), as well as rugby (n = 10) and wrestling (n = 9). The study was conducted during the preparatory period of the training process.

The inclusion criteria were age from 18 to 45 years, history of coronavirus infection, informed voluntary consent of the subjects. The exclusion criteria were the patient's refusal to participate in the study.

For residual COVID-19 estimation, a venous sampling was performed in all study participants. Blood draw was performed in 30 sportsmen in 2023 (19.05.2023–22.09.2023) and in 30 sportsmen in 2024 (14.06.2024–10.07.2024). Venous sampling was performed following an overnight fast from the peripheral vein with the help of a vacuum system in tubes with anticoagulant. The following parameters were evaluated: biochemical — alanine aminotransferase (ALT), aspartate aminotransferase (AST), C-reactive protein (CRP), troponin-I level (highly sensitive method); hemostasis parameters — prothrombin time (PT), prothrombin index (PTI), activated partial thromboplastin time (aPTT), international normalized ratio (INR), D-dimer; immune status indicators — interleukins-6, -8, -10 (IL-6, -8, -10, respectively), tumor necrosis factor-α (TNF-α).

The immune parameters were evaluated by enzyme-linked immunosorbent on a Real R microplate photometer (Vector-Best, Russia) and tubes with plasma and EDTA Na_2 solution (6.7%) with Tween-20. To evaluate the levels of troponin-I, C-reactive protein, ALT, AST, and coagulation profile, a Cobas 501c analyzer (Roche, Germany) was used along with a tube with plasma treated with Kz-ETDA. The assay was carried out using the following reagent sets: ALT and AST — "ALT" and "AST" in accordance with IFCC without pyridoxal phosphate activation, CRP — "CRP4" Tina-quant C-Reactive Protein IV, troponin-I — "Elecsys Troponin I" (Roche Diagnostics GmbH, Germany); aPTT — "Pathromtin SL", PTB/PTI — "Thromborel S", D-dimer — "INNOVATION D-Dimer" (Siemens Healthcare Diagnostics, Germany).

The STATISTICA 10 software was used for statistical data processing. The samples were checked for compliance with the normal distribution law by Shapiro–Wilk test statistics. The variables were represented as the median (Me) and the interquartile range [$Q25$; $Q75$]. In the presence of a normal distribution, Student's t-test was calculated for unrelated samples. When the distribution was different

from normal, the nonparametric Mann–Whitney *U*-test was used for comparative intergroup analysis. Spearman's rank correlation coefficient (r_s) was used to identify intragroup correlations. The statistical significance level was assumed to be 0.05.

RESULTS AND DISCUSSION

During the study, athletes from group 1 showed a statistically significant increase in clotting time: an increase in aPTT to 36.6 [34.3; 39.9] s, aPTT of 12.2 [11.5; 12.8] s and INR of 1.11 [1.08; 1.16], a decrease in PTI of 89.9 [85.6 93.3] % compared with the indicators of athletes from the second group — 34.9 [32.8; 35.7] s, 11.5 [11.1; 11.9] s, 1.06 [1.02; 1.11], 95.9 [88.5; 101,4] % ($p < 0.05$), respectively. The relevant data is shown in Fig. 1.

At the same time, the direction of the changes observed contradicts the literature data that people with a history of COVID-19 are more likely to have a procoagulant condition. The affinity of SARS-CoV-2 to angiotensin converting enzyme 2 (ACE2) receptors expressed on endothelial cells triggers a cascade of events provoking endothelial damage, which leads to dysregulation of thrombo-inflammatory reactions characterized by an increased release of von Willebrand factor, impaired fibrinolysis, and subsequent hypercoagulation [14]. The group of researchers [15], who examined the first patients hospitalized in Wuhan, found elevated levels of aPTT, PTV, and D-dimer. Subsequently, a tendency to hypercoagulation was also noted in a number of studies in patients who had suffered from COVID-19 [16-18]. It is likely that the level of physical activity and fitness of athletes contributes to a less pronounced effect of SARS-CoV-2 on the hemostasis system.

No statistically significant intergroup difference was found in the athletes in terms of ALT, AST, D-dimer, TNF-α parameters, probably because the study included the athletes without significant cardiorespiratory disorders. No comparative analysis was performed for the highly sensitive troponin-I index due to the low variance of values.-

When considering the functional state of the immune system, statistically significant differences were obtained for IL-8 and IL-10 levels, indicating disorders in the form of an imbalance of regulatory cytokine activity with the predominance of pro-inflammatory effects. Thus, athletes with a history of COVID-19 showed a 2.5-fold increase in IL-8 to 7.42 [3.08; 9.96] pg/mL and IL-10 2-fold to 5.08 [2.93; 6.66] pg/mL compared to similar indicators in athletes without a history of COVID-19 — 3.05 [1.86; 8.15] pg/mL and 2.53 [1.0; 5.68] pg/mL ($p < 0.05$), respectively (Fig. 2), which indicates a relative lack of activation of the anti-inflammatory immune system in athletes who have undergone COVID-19.

The data obtained on the level of cytokines in post-acute sequelae of COVID-19 are consistent with the data presented in the scientific literature. Thus, the study [19] found a significant increase in the levels of most regulatory chemokines, including IL-8 and IL-10, in patients with COVID-19. The researchers in [20] conducted an analysis of the cytokine profile in patients with PASC within 2–3 months after COVID-19 and detected high levels of cytokines IL-8 and IL-10. It was shown that their average levels in those with the decease history were twice higher than in healthy individuals. In our study, however, no significant changes in IL-6 levels were found in athletes who had undergone COVID-19 compared to athletes without such a history, the content of which is directly associated with the development of post-COVID-19 residual disorders [21]. It is likely that in the cohort of athletes, taking into account the level of physical activity, IL-8 and IL-10 are more correlated with the development of post-COVID disorders.

In the course of the study, we identified the dependence of coagulative hemostasis parameters and immune disorders in athletes who had a history of coronavirus infection (Table 1).

Thus, a weak direct correlation was established between an increase in IL-8 levels and an increase in PT ($r_s = 0.355$; $p < 0.05$) and INR ($r_s = 0.420$; $p < 0.05$). At the same time, a moderate negative association was found between an increase in IL-8 levels and a decrease in PTI ($r_s = -0.323$; $p < 0.05$). Given that such correlations have not been found in athletes who have not had COVID-19, the ratio of IL-8 levels with various coagulation profiles can probably be considered as a potential marker of residual COVID-19 effects.

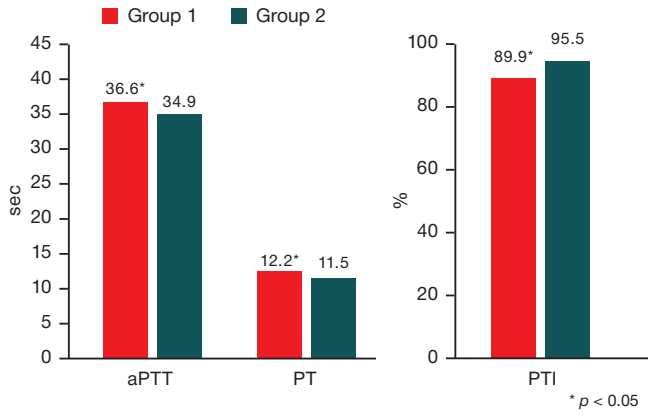

Figure prepared by the authors using their own data

Fig. 1. Coagulation profile changes among the study participants
Note: aPTT — activated partial thromboplastin time, PT — prothrombin time, PTI — prothrombin index, INR — international normalized ratio.

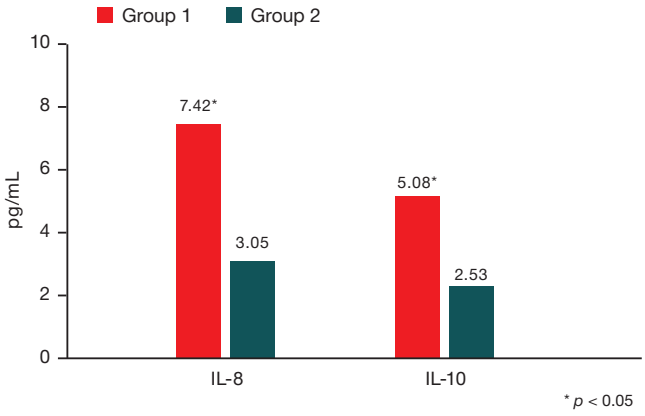

Figure prepared by the authors using their own data

Fig. 2. Changes in some indicators of immune status among the study participants
Note: IL-8 — interleukin-8; IL-10 — interleukin-10.

Table 1. Correlation analysis of the studied indicators in the group of athletes with a history of COVID-19

Variables	IL-8	IL-10	aPTT	PT	PTI	INR
IL-8		-0.087	0.112	0.355	-0.323	0.420
IL-10	-0.087		0.136	-0.184	0.214	-0.056
aPTT	0.112	0.136		0.065	0.115	0.099
PT	0.355	-0.184	0.065		-0.920	0.871
PTI	-0.323	0.214	0.115	-0.920		-0.770
INR	0.420	-0.056	0.099	0.871	-0.770	

Table prepared by the authors using their own data

CONCLUSION

A comparative analysis of the functional state of the coagulative hemostasis system and the immune profile in athletes who have had coronavirus infection revealed multi-vector changes in the parameters of the coagulation system in the form of an increase in blood clotting time according to the main parameters of the coagulation profile of aPTT, PT, INR, as well as a decrease in PTI.

Athletes with a history of coronavirus infection are characterized by a relative predominance of pro-inflammatory cytokine activity over anti-inflammatory activity, which is manifested by a more pronounced increase in the level of IL-8 than IL-10, identifying an imbalance in the regulatory link of the immune response.

In the course of the correlation analysis, a direct moderate relationship was established between an increase in IL-8 levels and an increase in PTV and INR, as well as a negative correlation between an increase in IL-8 levels and a decrease in PTI, which partially confirms the validity of IL-8 and IL-10 indicators as potential markers of residual disorders after COVID-19 in athletes. The associations between the parameters of the coagulation hemostasis system and the immune profile allow us to consider their ratios as diagnostic criteria for residual effects in athletes who have undergone COVID-19.

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APPLICABILITY OF INDIVIDUAL METABOLITES OF THE TRICARBOXYLIC ACID CYCLE IN ATHLETES (A LITERATURE REVIEW)

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Introduction. Various metabolites of the tricarboxylic acid cycle (Krebs cycle, TCA cycle, TCAC) find application in medicine. The emergence of improved chemical synthesis methods and the more affordable production of individual TCA metabolites make them promising candidates for developing effective compositions capable of increasing the adaptive potential of the human body.

Objective. Identification of the physiological effects of the main TCA metabolites. This knowledge is important for informed application of TCA metabolite-based products in the medical and biological support of athletes.

Discussion. The conducted literature review investigated the physiological effects of TCA metabolites — energy metabolism substrates — used in sports medicine. At present, succinate, citrate, malate, and oxaloacetate have found reasonable use. A number of publications have reported the anti-catabolic effect of alpha-ketoglutarate; however, the current level of evidence is insufficient. Isocitrate dehydrogenase is promising for use in sports medicine, which substantiates its further detailed study.

Conclusions. Due to their physiological effects, the majority of TCA metabolites can be used in the compositions of antihypoxic, antioxidant, neuroprotector, and metabolic correction agents. A number of TCA metabolites are promising substances for creating new products for the medical and biological support of athletes, which validates additional research of their physiological effects.

Keywords: tricarboxylic acid cycle; Krebs cycle; tricarboxylic acids; energy metabolism; energy metabolism substrates; high-performance sport; antioxidants; antihypoxic agents, isocitric acid

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ВОЗМОЖНОСТИ ПРИМЕНЕНИЯ ОТДЕЛЬНЫХ МЕТАБОЛИТОВ ЦИКЛА ТРИКАРБОНОВЫХ КИСЛОТ У СПОРТСМЕНОВ (ОБЗОР ЛИТЕРАТУРЫ)

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Введение. Многие метаболиты цикла трикарбоновых кислот (цикл Кребса, ЦТК) применяются в медицине. Развитие методов химического синтеза и удешевление производства отдельных метаболитов ЦТК делает их перспективными субстанциями для создания средств повышения адаптационного потенциала организма человека.

Цель. Выявление физиологических эффектов основных метаболитов ЦТК, позволяющих использовать продукцию на их основе для медико-биологического обеспечения спортсменов.

Обсуждение. На основе анализа литературы изучены физиологические эффекты субстратов энергетического обмена — метаболитов ЦТК, которые применяются в спортивной медицине. В настоящее время нашли обоснованное применение сукцинат, цитрат, малат, оксалоацетат. В ряде исследований отмечался антикатаболический эффект альфа-кетоглутарата, но уровень доказательности его недостаточен. Перспективным для использования в спортивной медицине, но недостаточно изученным на настоящий момент является изоцитрат.

Выводы. Физиологические эффекты большинства основных метаболитов ЦТК позволяют использовать продукцию на их основе в качестве антигипоксантов, антиоксидантов, нейропротекторов, средств коррекции метаболизма. Ряд метаболитов ЦТК являются перспективными субстанциями для создания новой продукции для медико-биологического обеспечения спортсменов, но их физиологические эффекты требуют дополнительного изучения.

Ключевые слова: цикл трикарбоновых кислот; цикл Кребса; трикарбоновые кислоты; энергообмен; субстраты энергетического обмена; спорт высших достижений; антиоксиданты; антигипоксанты; изолимонная кислота

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INTRODUCTION

In modern sports medicine, the search for new safe substances capable of optimizing metabolic processes in athletes experiencing high professional loads represents a relevant research direction. The incidence of energy metabolism disorders with profound hypoxic and ischemic changes can be reduced through the timely use of antihypoxic agents that directly affect redox processes [1–3].

The metabolites of the tricarboxylic acid cycle (TCA cycle, Krebs cycle, citric acid cycle, TCAC) are promising agents for preventing energy metabolism disorders resulting from professional sports loads.

The tricarboxylic acid cycle is the central pathway for the conversion of organic acids during the anaerobic oxidation of glucose in the cell with the release of energy in the form of ATP. This cycle plays a key role in cellular respiration, being the main source of energy in aerobic conditions[1]. Under anaerobic conditions, glucose is oxidized to pyruvic acid (Pyr), which, under the action of enzymes, is converted into acetyl-coenzyme A (acetyl-CoA), where the TCA begins (Fig. 1).

The TCA cycle in athletes has a number of specific characteristics. Thus, during physical exercise with an intensity above 50% of maximal oxygen uptake (VO_2 max), the total concentration of TCA intermediates in skeletal muscles increases. This phenomenon is referred to as anaplerosis [4–6]. Anaplerosis is commonly associated with an increase in the activity of the enzyme alanine aminotransferase (ALT) due to increased availability of pyruvate. As a result of glycolysis, its formation rate exceeds the oxidation rate: glutamate + pyruvate ↔ alanine + alpha-ketoglutarate. One currently leading hypothesis explaining the phenomenon of anaplerosis under the action of high athletic loads consists in the following. The observed increase in the pool of TCA intermediates in muscles is necessary to achieve higher rates of providing the aerobic pathway of energy formation. That is why an increase in the pool of TCA intermediates by exogenous addition of individual TCA metabolites may be a factor in improving the peak muscle oxidative capacity, which is especially important for professional athletes [5, 6].

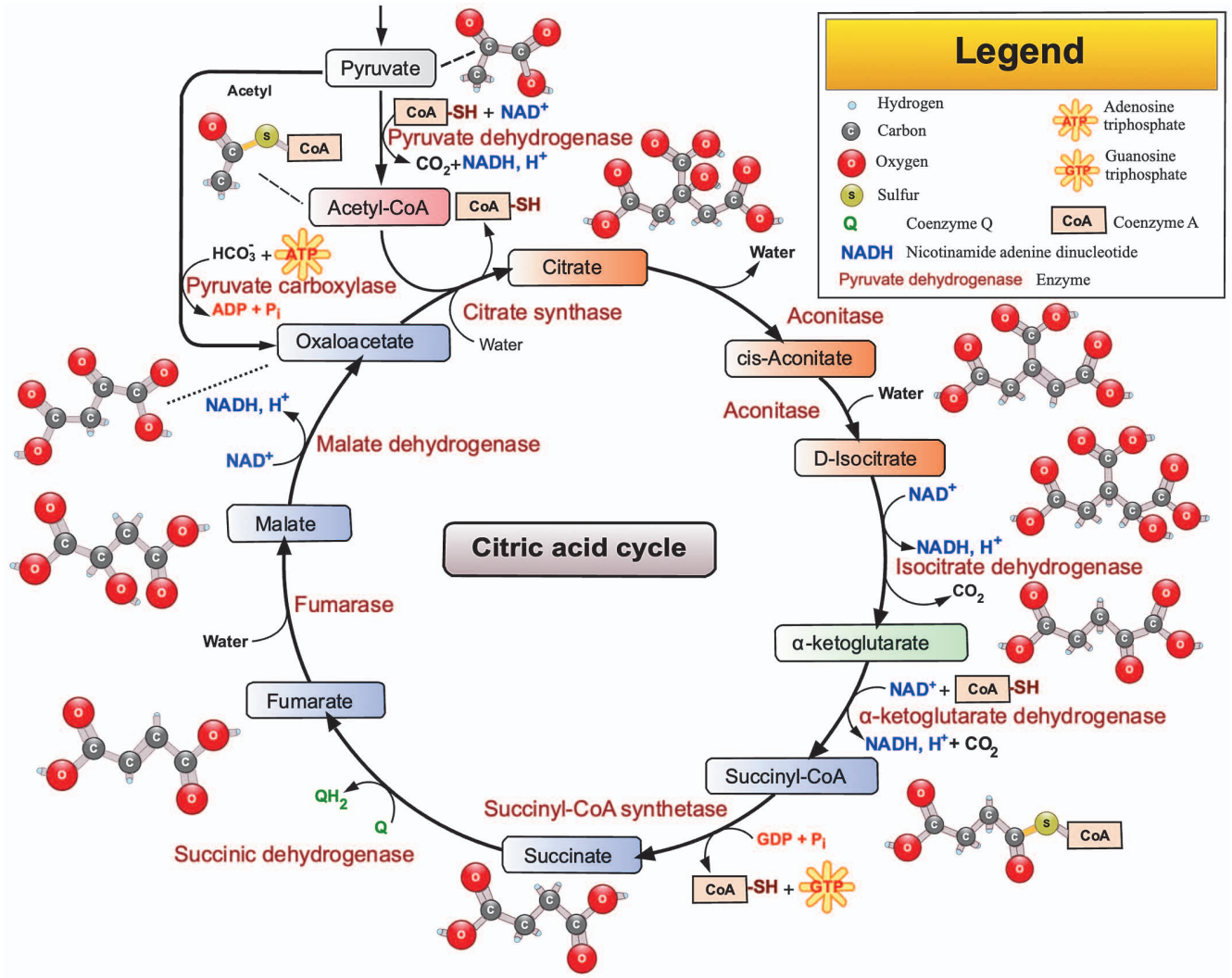

Figure prepared by the authors using data from Refs.[2]

Fig. 1. Tricarboxylic acid cycle scheme

[1] Nesen EN, Volkov NI, Osipenko AA, Korsun SN. Biochemistry of muscle activity. Kyiv: Olympic literature; 2013.
[2] https://chemicalportal.ru/compounds/tsikl-trikarbonovyh-kislot

In this study, we aim to identify the physiological effects the main TCA metabolites. This knowledge forms the basis for developing TCA metabolite-based products for the medical and biological support of athletes.

MATERIALS AND METHODS

The relevant scientific publications were retrieved from electronic bibliographic databases in the Russian (eLibrary, CyberLeninka) and English (Web of Science, Scopus, PubMed) languages. The search queries included the following keywords: Krebs cycle; tricarboxylic acids; tricarboxylic acid cycle; energy metabolism; energy metabolism substrates; high-performance sports; antioxidants; antihypoxants; isocitric acid. The search depth was 30 years. The inclusion criteria were the availability of data on the results of randomized controlled trials, including data from preclinical studies.

RESULTS AND DISCUSSION

The tricarboxylic acid cycle is the central link in the cell energy metabolism. A number of substances related to the TCA metabolism have found application in medicine in general and in sports medicine in particular. Such substances can be used by athletes to increase the adaptive potential of the body, optimize energy production in muscle cells, and protect the body from the effects of various adverse factors, including increased stress. Further, we summarize the information on TCA metabolites.

Citric acid (citrate) is the first TCA metabolite formed by condensation of two Pyr molecules[3]. An increase in the amount of glucose leads to the formation of a large amount citrate. As a result, the activity of phosphofructokinase is inhibited and glycolysis slows down, which is energetically beneficial for the cell. The high concentration of citrate indicates the presence of a large supply of precursor molecules; therefore, phosphofructokinase does not release fructose-6-phosphate molecules into glycolysis, thus saving energy substrates [7].

Citric acid and citrate preparations are widely used in medicine. For example, citrates are used to alkalize urine (as an alternative to sodium bicarbonate) under the conditions where, for health reasons, it is desirable to maintain its pH at an alkaline level for a certain period of time. This property is relevant for athletes who, when taking citric acid derivative-based medications, experience an increase in the buffer capacity of body fluids and, therefore, a delayed onset of fatigue due to a decrease in the body acidity level [8, 9].

Citrate also exhibits antioxidant properties, having a synergistic effect with vitamin E [8, 9]. The use of citrate reduces the load on the body's antioxidant system (AOS), which is manifested by a decrease in the activity of superoxide dismutase (SOD), catalase, glutathione peroxidase, a reduced glutathione level, as well as the activity of some NADPH-generating enzymes, including during strenuous physical work [8]. In addition, citrate is capable of exhibiting cytoprotective properties and act as an activator of fatty acid biosynthesis and a supplier of acetyl fragments for cell membrane repair [7]. Another important citrate property is its ability to retain magnesium inside mitochondria, thereby protecting them from damage [9, 10]. All of the above-mentioned physiological properties of citrate and its derivatives make the use of medications on its basis highly promising for mitigating the effects of high loads typical of professional sports.

Isocitrate (isocitric acid, ICA) is the next important TCA metabolite. ICA is synthesized from citric acid through the intermediate cis-aconitic acid under the action of the aconitase enzyme. It is believed that the reaction of conversion of isocitric acid to alpha-ketoglutarate is the reaction that limits the rate of the entire TCA cycle. Despite this fact, isocitric acid is currently one of the least studied TCA metabolites in terms of its effects on the human body. For a long time, isocitrate was used only as a specific biochemical reagent for analyzing the activity of aconitate hydratase, NAD-isocitrate dehydrogenase, NADP-isocitrate dehydrogenase, isocitrate lyase, and other enzymes [11, 12]. Relatively recently, ICA has been studied as a natural preventive and therapeutic agent, with its effectiveness in the treatment of iron deficiency anemia and therapeutic thrombolysis being reported [12].

A number of studies have demonstrated the effectiveness of isocitric acid in anemia of chronic disease and inflammation (ACDI), as well as in the setting of professional sports activities [13, 14]. Using isocitrate, erythropoiesis can be therapeutically manipulated without using iron preparations. This is especially true in cases where the body's iron load is undesirable or ineffective [13, 14].

In an experimental rat model, the antioxidant properties of isocitrate were demonstrated. Monopotassium isocitrate was found to be a more effective antioxidant than ascorbic acid. Monopotassium isocitrate also mitigated the neurotoxic effect of lead and molybdenum salts, reduced learning and memory inhibition in rats poisoned with heavy metals, and counteracted oxidative stress caused by heavy metals [15].

In the study [16], a 10-day intake of isocitric acid during antihypertensive therapy showed a stress-protective effect. This is likely to be related to the capacity of isocitrate to influence the processes of excitation and inhibition in the central nervous system. The researchers also noted a previously undescribed antihypertensive effect, which was manifested in a significant decrease in the average daily diastolic blood pressure when taking isocitric acid.

In addition, the isocitrate dehydrogenase (IDH) enzyme plays a central role in the TCA-cycle control during physical exercise [17]. IDH isoforms play an important role in protecting cells from oxidative damage due to the reaction of direct oxidative decarboxylation. In addition, these may serve as a source of NADPH [17].

Another area of research into the physiological effects of isocitrate concerns its antihypoxic effects. Hypoxia affects the enzymes involved in the TCA cycle in different ways. In particular, under hypoxia conditions, aconitase is suppressed, while IDH is not affected or activated. IDH was shown to be essential for alternative metabolic pathways that support cell function under hypoxic conditions. It is assumed that the addition of exogenous isocitrate

[3] Kulinenkov OS, Lapshin IA. Biochemistry in the practice of sports.: Guide. Moscow.: Sport; 2019.

removes aconitase inhibition and serves as an IDH substrate to provide an alternative energy source in hypoxia [18, 19]. This isocitrate effect can be used in sports where hypoxic conditions are present, as well as during training in the mountains.

Isocitrate is a promising substance for the creation of medications and specialized food products for use in the medical and biological support of athletes. However, for the justified use of this organic acid in sports medicine, further study of its effect on the body is required, including under the action of increased physical and psychoemotional stress.

Alpha-ketoglutaric acid (alpha-ketoglutarate, AKG, 2-oxoglutarate) is an important intermediate in the TCA cycle, passing from isocitrate to succinyl-CoA [15, 19]. Anaplerotic reactions can replenish the TCA cycle at this stage by synthesizing alpha-ketoglutarate as a result of glutamate transamination or under the action of glutamate dehydrogenase. Another function of this metabolite is to prevent nitrogen overload of cells through the capacity of alpha-ketoglutorate to combine with excess nitrogen and directs it into the urea cycle [19]. In addition, alpha-ketoglutarate reacts with glutamine to form the excitatory neurotransmitter glutamate. Then glutamate can be decarboxylated (vitamin B_6 is required) into gamma-aminobutyric acid (GABA), an inhibitory neurotransmitter [20].

In the work [21], alpha-ketoglutaric acid was shown to exhibit antioxidant properties in mice, being capable of preventing damage to mitochondrial DNA caused by free radicals in nerve cells.

Alpha-ketoglutaric acid can also function as a signaling molecule by regulating the G protein function. Signaling through this pathway mobilizes intracellular Ca^{2+}, which acts as a diffusive second messenger regulating a wide range of vital cell functions, including cellular metabolism and growth, as well as cell division and differentiation [22]. There are also studies demonstrating the effectiveness of AKG in accelerating tissue repair after surgery, injury, and burns [19, 23].

As a precursor to glutamine, alpha-ketoglutarate is a molecule with a high potential for correcting conditions with increased protein catabolism, including those caused by prolonged aerobic exercise in athletes. AKG supplements were shown to improve the body nitrogen balance and contribute to maintaining the level of anabolic hormones and hormone-like compounds (insulin, growth hormone, and insulin-like growth factor) during surgical interventions, injuries, and burns [23]. In addition, AKG may protect liver cells from damage and prevent a decrease in the activity of the cytochrome P-450 family. This hepatoprotective effect may also be relevant in sports medicine [23].

The positive effect of alpha-ketoglutaric acid on bone metabolism described in a number of studies suggests its potential use in the prevention of bone matrix formation disorders, in the treatment of diseases with progressive bone loss, such as osteoporosis, or in improving the body's bone mass, which is also relevant for professional athletes [24].

The study [25] showed that a significant accumulation of blood AKG is a metabolic signal of the effectiveness of resistance training. Interestingly, its plasma level negatively correlates with the body mass index. *In vivo* experiments in mice found that an increase in circulating AKG in the blood caused by its exogenous intake into the body promotes hypertrophic changes in muscle tissue, thermogenesis, due to brown fat and lipolysis of white fat. AKG was also found to stimulate the release of adrenaline. The results of this study demonstrate an underestimated mechanism of AKG as a molecule in adrenal stimulation for muscle hypertrophy and fat loss in resistance training [25].

Currently, in the practice of sports medicine, AKG is used in combination with the L-arginine amino acid as a component of specialized food products under the common name of AAKG. In some studies, the anabolizing and anti-catabolic effects of AKG application were identified [26]. However, currently, due to insufficient knowledge and a limited number of studies on athletes, AAKG belongs to specialized food products with a low level of evidence-based effectiveness when used in professional sports [27].

Succinic acid (SA, succinate) is formed from alpha-ketoglutarate via succinyl-CoA. All metabolic pathways that are interconnected with the TCA cycle, including the metabolism of carbohydrates, amino acids, fatty acids, cholesterol, and heme, depend on the temporary formation of succinate.

Succinate can exit the mitochondrial matrix and function both in the cytoplasm and in the extracellular space, altering gene expression patterns, modulating the epigenetic landscape, or demonstrating hormone-like signaling. For example, in adipocytes, the signaling cascade activated by succinate inhibits lipolysis. Most often, succinate signaling occurs in response to hypoxic conditions [26].

When oxidized, succinate monopolizes the respiratory chain, which leads to rapid ATP resynthesis by cells and increases the amount of reduced mitochondrial NAD^+ more markedly than other TCAC substrates, stimulating the course of reductive synthesis in the cell and supporting calcium transport. Its positive effect on organ functions is associated with an energizing effect on the functional state of structures that exert a central regulatory effect [28].

This metabolite is the re-entry point for the GABA shunt into the TCA cycle, where GABA is synthesized and processed, which explains its antistress effect [29]. In addition, succinate can enhance adaptive immunity by triggering the activity of antigens that activate T cells. It was also shown that accumulated succinate regulates the production of inflammatory cytokines [30].

The researchers in [30] studied the antihypoxic and antioxidant effects of succinate. These effects are not based only on its ability to activate IDH (the ATP resynthesis pathway), reduce the level of NAD-dependent TCA substrates and fatty acids, but are also associated with the stimulation of cytochrome oxidase, which is a key enzyme of the mitochondrial respiratory chain. Succinate is helpful in normalizing the concentration of histamine and

serotonin in the blood and epidermis, while having a beneficial effect on the microcirculatory system without affecting blood pressure (BP) and heart function.

The work [31] addressed the hepatoprotective effect of succinate, which consists in activating the enzyme succinate dehydrogenase (SDH) in the mitochondria of hepatocytes. This leads to normalization of urea synthesis disorders, mitigation of hepatic cholestasis, prevention of fatty degeneration of the liver, and the development of collagenous tissue in the liver.

The adaptogenic effect of succinate was described in experiments using models of immobilization stress and stress provoked by burns, electric shock, and hypothermia [31]. It is also known that succinate promotes accelerated recovery during heavy physical exertion.[4]

One of the factors that limit the use of succinate in sports medicine is its bioavailability, which is lower than that of fumarate, malate, or citrate. To increase the bioavailability of succinate, its compounds with other TCA metabolites in the form of salts can be used.[5]

Currently, succinate is widely applied in domestic sports medicine. Thus, Mexidol® (3-hydroxy-6-methyl-2-ethylpyridine succinate), a medication of the metabolic type of action has a powerful inhibitory effect on lipid peroxidation processes, as well as the effects of neutralizing free radicals and activating SOD. Mexidol® promotes the activation of the succinate oxidase oxidation pathway, due to which a certain level of oxidative phosphorylation is maintained in mitochondria at the initial stages of hypoxia under conditions of inhibition of NAD-dependent oxidation [32]. However, it should be noted that the use of Mexidol® by professional athletes is allowed only in tablet form, whereas intravenous infusions and/or injections of more than 100 mL over a 12h period are prohibited by the World Anti-Doping Agency (WADA) Code. The same rule applies to the Reamberin® medication, which contains meglumine sodium succinate in its composition.[6]

In addition to Mexidol®, Cytoflavin®, the active ingredients of which include succinic acid in combination with inosine, nicotinamide, and riboflavin, is currently widely used as a component of the medical and biological support for athletes in Russia. Cytoflavin® was found to enhance cellular respiration during strenuous physical activity, providing an optimal level of oxygen uptake by cells [32, 33]. The course use of Cytoflavin® was found to have a positive effect on metabolic processes in the body, such as supporting protein-synthetic function, promoting the absorption of glucose and fatty acids by cells, improving cellular energy supply, and restoring the activity of enzymes of the antioxidant system. This medication can be classified as a drug exhibiting adaptogenic and stress-protective properties [32, 34, 35]. The study [33] revealed the positive effects of Cytoflavin® on the performance of professional hockey players in the pre-competition period [33, 34].

Fumaric acid (fumarate) is formed as a result of the oxidation of succinate with the participation of the SDH enzyme, which is also involved in the mitochondrial electron transport chain (respiratory complex II). Fumarate functions as an intermediate product of urea synthesis and oxidation of phenylalanine, tyrosine, leucine, tryptophan, and lysine [36, 37]. There is evidence that fumarate derivatives act as appetite-enhancing agents and possess antifungal effects, being also used as tranquilizers and radiopaque medications, as well as agents for blood-clotting disorders (bencyclane hydrofumarate) and rhinitis [36–38].

Fumarate penetrates readily through membranes and is easily disposed of in mitochondria. This compound, similar to lactate and sodium acetate, helps eliminate acidemia by chemically neutralizing acidic metabolic products. However, fumarate has an advantage over lactate and acetate, since it is metabolized during severe hypoxia. Moreover, its utilization is accompanied by the formation of ATP [37, 38].

Fumarate-based medications that support the activity of the succinate link during hypoxia of various origins is increasingly finding practical application as antihypoxic agents. One of these medications is mafusol (1 L of an aqueous solution for injection contains 6.0 g sodium chloride, 0.3 g potassium chloride, 0.12 g magnesium chloride and 14.0 g sodium fumarate) [40]. Mafusol can also be used in sports medicine provided that it does not contradict the following WADA anti-doping rule: intravenous infusions and/or injections in a volume of more than 100 mL during a 12-hour period are prohibited.

Malic acid (malate) is formed from fumarate under aerobic conditions. Malate has the properties of a cellular protector, being also capable of increasing the activity of enzyme complexes, such as SOD and glutathione peroxidase, by enhancing the expression of messenger RNA [3].

It was noted that the activity of the malate-aspartate transporter in the heart muscle is more than 10 times higher than that of all other known electron transport systems. The study of cardiomyocytes in ischemia and at the time of post-ischemic reperfusion showed the tremendous importance of this system in the adequate supply of cells with energy. In addition, the malate-aspartate mechanism is a link in antioxidant protection and an agent in insulin synthesis [3, 39, 40].

Malate performs various functions of switching metabolic pathways: it participates in glycolysis, beta-oxidation of fatty acids, synthesis of amino acids, playing an important role in transport communication between mitochondria and the cytosol, exerting anaplerotic or cataplerotic effects on the central nervous system [3, 39, 40]. It is known that lettuce can indirectly have an antihypoxic effect by pre-dehydrating to fumarate [3, 39, 40].

In sports medicine, a compound of malic acid with the citrulline amino acid is used. This compound has a tonic effect, reducing fatigue and increasing endurance [41, 42]. An example of such a medication is Stimol®, which is widely used by sports physicians to accelerate the recovery of an athlete after heavy loads. In [43], this medication accelerated lactate excretion from muscles in sprinting athletes.

Oxaloacetic acid (OA, oxaloacetate) is another important metabolite of the TCA cycle. Its synthesis is

[4] Isakov VA, Sologub TV, Kovalenko AL, Romantsov MG. Reamberin in the treatment of critical conditions. Guide for doctors. St. Petersburg.: JV Minimax; 2001.
[5] Ibid.
[6] Ibid.

maintained mainly due to pyruvate carboxylation; therefore, a reduced intensity of glycolysis during hypoglycemia and depletion of glycogen stores leads to a deficiency of pyruvate and, as a result, to a lack of oxaloacetate. This limits not only the entry of acetyl-CoA into the TCA cycle, but also the course of other important adaptive reactions [3, 44].

Oxaloacetate participates in gluconeogenesis, the urea cycle, the glyoxylate cycle, amino acid synthesis, and fatty acid synthesis. It is important for the formation of essential and non-essential amino acids, including aspartate, asparagine, methionine, lysine, and threonine [44, 45].

Bioenergetic medications based on oxaloacetate derivatives were developed to increase the cell energy level. Such medications exhibit protective and promitochondrial effects, preventing neuroinflammation and non-degeneration [44, 45]. Oxaloacetate acts as a neuroprotector due to its ability to reduce the content of glutamate in the brain as a result of activation of the glutamate oxaloacetate transaminase enzyme, which catalyzes the reversible conversion of oxaloacetate and glutamate into aspartate and alpha-ketoglutarate. It promotes recovery after traumatic brain injury (TBI), causing neurorehabilitation effects, which also has prospects for use in sports medicine in rehabilitation after TBI sustained during training or competition [45].

Among other properties of oxaloacetate, its participation in the processes of gluconeogenesis and glycerogenesis should be noted. Oxaloacetate is capable of increasing the volume of mitochondria in striated muscles, which has a positive effect on endurance by reducing muscle fatigue [44, 45]. Another important advantage associated with the introduction of oxaloacetate is an increase in mitochondrial biogenesis [45].

CONCLUSION

The majority of TCA metabolites are actively used in medicine for antihypoxic, antioxidant, neuroprotector, and metabolic therapies. Succinate and its derivatives (Cytoflavin®, Mexidol® ergogenic, antihypoxic medications), citrate (as a buffer agent and magnesium carrier), alpha-ketoglutarate (to suppress catabolism, stimulate anabolism, hepatoprotection), malate (in the form of citrulline malate to increase endurance, the Stimol® medication) have found their use in sports medicine, oxaloacetate (as a component of medications whose action is aimed at recovery after TBI, reducing fatigue, and metabolic regulation of gluconeogenesis).

At present, there is a lack of data on a number of TCA metabolites, which limits their application in the medical and biological support of professional athletes. For example, alpha-ketogluturate as a component of specialized food products in combination with the L-arginine amino acid, despite the anti-catabolic and anabolic effects shown in some studies, currently has a low degree of evidence-based effectiveness when used in professional sports. Isocitrate, despite its safety and promise as a component of medications and specialized food products used in sports medicine, has not yet been widely used due to its rather expensive synthesis. However, the recent progress in making the production of isocitrate more affordable offers the opportunity for its wider use in the practice of sports medicine physicians.

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IN VITRO EVALUATION OF *ACINETOBACTER BAUMANNII* RESISTANCE TO TIGECYCLINE IN IRAN: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Introduction. *Acinetobacter baumannii (A. baumannii)* is a widespread and exclusively hospital-acquired microorganism whose new mutation is resistant to most available antibiotics, with the exception of tigecycline. Clinicians are concerned about recent evidence of resistance to this antibiotic in Iran.

Objective. This study evaluates the resistance of *Acinetobacter baumannii* (*A. baumannii*) to tigecycline in Iran, considering its clinical significance in treating multi-drug-resistant infections.

Methods. The MEDLINE, PubMed, Web of Science (WOS), and Scopus databases were searched for studies published over all this time to January 2024. The advanced search using Medical Subject Headings (MeSH) terms for "*Acinetobacter baumannii*" and "Tigecycline" was performed. The title, abstract, and full text of the articles were screened based on eligibility criteria. The cross-sectional studies reporting Tigecycline resistance in sequential isolates of *A. baumannii* in patients admitted to the hospitals in Iran were included.

Results. A total of 16 studies were included for meta-analysis. The overall prevalence of *A. baumannii* strains resistant to tigecycline in Iran equals 18.1%. Among the reviewed studies, distinct variances of resistance were detected. Although investigations were conducted in limited regions, the studies reported a wide range of resistance in Tehran (0%) and in Tabriz (100%) as minimum and maximum, respectively.

Conclusion. Despite the high level of resistance in some cities of Iran, tigecycline is still one of the most effective antibiotics for the treatment of *A. baumannii* infection. Improved control over the use of antibiotics may contribute to hampering the spread of resistance to these agents.

Keywords: *Acinetobacter baumannii*; tigecycline; drug resistance; *in vitro*; meta-analysis; multiple drug resistance

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ОЦЕНКА УСТОЙЧИВОСТИ *ACINETOBACTER BAUMANNII IN VITRO* К ТИГЕЦИКЛИНУ В ИРАНЕ: СИСТЕМАТИЧЕСКИЙ ОБЗОР И МЕТААНАЛИЗ

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Введение. *Acinetobacter baumannii (A. baumannii)* — это распространенный и исключительно госпитальный микроорганизм, новая мутация которого устойчива к большинству доступных антибиотиков, за исключением тигециклина. Клиницисты выражают озабоченность в связи с недавними данными о резистентности к этому антибиотику, зафиксированными в Иране.

Цель. Оценить устойчивость *Acinetobacter baumannii* (*A. baumannii*) к тигециклину в Иране, учитывая его клиническое значение в лечении инфекций с множественной лекарственной устойчивостью.

Материалы и методы. В базах данных MEDLINE, PubMed, Web of Science (WOS), Scopus проведен поиск исследований, опубликованных за все время до января 2024 г. Выполнен расширенный поиск с использованием медицинских тематических рубрик (MeSH) по таким терминам, как «*Acinetobacter baumannii*», «тигециклин». В соответствии с критериями приемлемости были отобраны название, аннотация и полный текст статей. Также были включены перекрестные исследования, в которых сообщалось об устойчивости к тигециклину у последовательных изолятов *A. baumannii* среди госпитализированных пациентов иранских клиник.

Результаты. В метаанализ было включено в общей сложности 16 исследований. Общая распространенность штаммов *A. baumannii*, устойчивых к тигециклину, составила 18,1%. Выявлены отчетливые различия в устойчивости. Несмотря на то, что исследования проводились в ограниченном количестве регионов, сообщалось о широком диапазоне резистентности: от минимальной в исследовании в Тегеране (0%) до максимальной в исследовании в Тебризе (100%).

Заключение. В результате проведенного исследования установлено, что, несмотря на высокий уровень резистентности в некоторых городах Ирана, тигециклин по-прежнему остается одним из наиболее эффективных антибиотиков для лечения инфекции *A. baumannii*. Существует вероятность, что, улучшив контроль за применением антибиотиков, можно замедлить или остановить распространение устойчивости к ним.

Ключевые слова: *Acinetobacter baumannii*; тигециклин; антибиотикорезистентность; *in vitro*; метаанализ; множественная антибиотикорезистентность

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INTRODUCTION

Acinetobacter baumannii (*A. baumannii*) is a ubiquitous, aerobic, gram-negative coccobacillus. This bacterium is widely spread in water, soil, and hospital environments, capable of surviving there for a long time [1, 2]. In addition, *A. baumannii* is a common pathogen identified in the blood, skin, urine, pleural fluid, and sputum [3]. Due to its capacity to transfer between non-living and living objects, this pathogen is increasingly responsible for hospital-acquired infections [4]. Moreover, the emergence, proliferation, and spread of a new drug-resistant *A. baumannii*, which is capable of transferring some genetic elements and is resistant to different antibiotics, has worsened the situation [5]. Various studies have reported the resistance of *A. baumannii* to different classes of antimicrobial agents, such as aminoglycosides, β-lactams, and quinolones, as well as carbapenems. Given the scarcity of alternatives available for treating drug-resistant infections caused by *A. baumannii*, tigecycline is currently attracting research attention [2, 6].

In comparison with other tetracyclines, tigecycline is a bacteriostatic agent with a higher binding affinity to the bacterial 30S ribosomal subunit. Tigecycline, an antibiotic based on minocycline, has a wide spectrum of action and is capable of overcoming the main mechanisms of bacterial resistance to tetracyclines, such as efflux and ribosome protection. This is achieved by adding a glycylamide fragment to the minocycline molecule [7]. The mechanism of resistance to tetracyclines is generally mediated by the following systems: the attainment of genetic sections transferring the genes particularly resistant to tetracyclines, mutations inside the attaching region of the ribosome, and/or mutations in chromosomes leading to intensified expression of fundamental resistance mechanisms. Various processes of bacterial resistance were described in [8].

Tigecycline is a semisynthetic agent known as the primary exclusive antibiotic of the glycylamide class. Tigecycline overcomes key tetracycline resistance mechanisms — namely, efflux pump activity and ribosomal protection — through the addition of a glycyclamide group to its minocycline-based structure. This structural modification contributes to its broad-spectrum antibacterial activity [7]. Tigecycline is an available drug for treating multidrug-resistant *A. baumannii* [9], showing activity against multiple drug resistant (MDR) pathogens such as *Enterobacteriaceae*, *Staphylococcus aureus*, and *Acinetobacter* species [10]. In 2005, the US Food and Drug Administration (US FDA) approved this drug for treating community-acquired pneumonia, skin infections (except for diabetes foot infection), and complicated intra-abdominal infections [11].

Tigecycline exhibits a noticeable activity against extensive drug resistance (XDR) and MDR gram-negative bacteria, particularly *A. baumannii*. The resistance of the latter to tigecycline has been reported relatively recently [12–14]. Some mechanisms, such as the chromosomal or supplemental encoding process of genes, are responsible for tigecycline resistance [7]. Since tigecycline is one of the few remaining drugs for the treatment of *A. baumannii* infection with MDR, it is necessary to determine its potential resistance in a timely manner. In comparison with developed countries, where effective preventive health measures are used, developing countries, such as Iran, require the development and implementation of measures to control the process of drug prescription, disseminate information about antibiotic resistance among patients, and regulate the proper use of medicines [15]. Thus, the study of *A. baumannii* resistance to tigecycline in Iranian patients is an important and urgent task.

The purpose of this study was to evaluate the resistance of *Acinetobacter baumannii (A. baumannii)* to tigecycline in Iran, taking into account its clinical significance in the treatment of multidrug-resistant infections.

MATERIALS AND METHODS

This research was designed in accordance with Cochrane's standard methodology and presented based on the PRISMA checklist (Preferred Reporting Items for Systematic Reviews and Meta-Analyses).

Search strategy

The literature search was conducted across the Google Scholar, PubMed, Web of Science, and Scopus databases, covering all available data up to January 2024. The search algorithm based on keywords, their synonyms, and related Medical Subject Headings (MeSH terms) was as follows: ((*Acinetobacter baumannii* [title/abstract]) OR (Acinetobacter [Title/Abstract])) OR (*A. baumannii* [Title/Abstract]) AND (Tygacil [Title/Abstract])) OR ((Tigecycline [title/abstract]). In addition, a backward and forward citation search was conducted to raise the comprehensiveness of the conducted literature search.

Inclusion and exclusion criteria

The records achieved from searching the databases were combined in the EndNote X9 library (Thomson Reuters, Toronto, ON, Canada); duplicates were deleted.

The resulting sample included *in vitro* cross-sectional studies investigating the resistance of *A. baumannii* to tigecycline in sequential *A. baumannii* isolates from the patients admitted to Iranian hospitals by using different methods, such as broth microdilution, disk diffusion, and *E*-test [16]. Moreover, only patients with MDR resistance were eligible for this study. Multidrug resistance is defined as not being susceptible to at least one antibiotic in three antimicrobial classes acknowledged as treatment options for the disease associated with *A. baumannii* [17]. Biological and biomedical research studies on animal models, as well as case reports, case series, case-controls, and studies evaluating variables irrelevant to resistance rate, were excluded.

Statistical analysis

The statistical analysis was conducted using the Comprehensive Meta-Analysis software package, version 3.0 (Biostat Inc., Englewood, NJ, USA). 95% confidence intervals (CIs) and point estimates for the resistance rate to tigecycline were calculated.

The degree of existing heterogeneity between different meta-studies was assessed using the I^2 value and the *p*-criterion. The I^2 value is a quantitative indicator of heterogeneity that shows a degree of inconsistency in research results. I^2 describes the percentage of total variation between studies, which is due to heterogeneity rather than randomness. The I^2 indicator is calculated based on the basic results obtained as a result of a typical meta-analysis, as

$$I^2 = 100\% \times (Q - df) / Q, \quad (1)$$

where Q is the Cochran heterogeneity statistic and df is the degree of freedom.

Negative I^2 values were equated to zero such that I^2 were ranging from 0 to 100%. A 0% value indicates the absence of heterogeneity, with higher values indicating an increase in heterogeneity. I^2 statistics and Cochran's *Q*-test were used to assess the heterogeneity between studies. Due to the high level of heterogeneity between studies ($I^2 > 50\%$ or $p < 0.1$), a random effect model was used. To assess the reliability of the publication, we used the Egger criterion. Accordingly, the value of $p < 0.05$ was considered a statistically significant indicator for the reliability of the publication.

RESULTS

Study selection

The conducted systematic search across scientific databases produced 365 relevant articles that evaluate the prevalence of tigecycline-resistant *A. baumannii* in Iran. The first screening identified 190 articles by title and abstract, while the second screening based on full text found 53 articles. Following application of the inclusion and exclusion criteria, 16 articles were deemed satisfactory, thus being included in the current systematic review and meta-analysis (Figure 1).

Characteristics of the included studies

All selected articles reported cross-sectional studies conducted from 2014 to 2022 and spanning different geographical domains—capital (n = 10), north (n = 2), south (n = 3), and southwest (n = 1) — with various types of samples, including burn wound, urine, sputum, blood, and other body fluids (Table 1).

The quantity of MDR isolates ranged from 26 to 200. Nine, four, three, and one studies used disk diffusion, broth microdilution, E-test methods, and a combination of disk diffusion and broth microdilution methods for antimicrobial susceptibility testing on *A. baumannii*, respectively (Table 2).

Prevalence and Genetic Mechanisms of Tigecycline Resistance in *A. baumannii*

The studies under analysis reported differing data on the resistance prevalence of *A. baumannii*. Thus, although Saadati et al. [13] found all of the isolates to be MDR (100 out of 100) and reported the highest resistance rate (100%) against tigecycline in the northwest of Iran, Salehi et al. [33] identified 1.6% of MDR clinical isolates (2 out of 125) resistant to tigecycline in the North of Iran. Bahador et al. [31] conducted a five-year-long study in the north of Iran and found a notable rise in resistance to tigecycline, with all isolates demonstrating susceptibility in 2006. However, by 2011, 8% of isolates had exhibited resistance. In contrast to southern regions, a study by Kooti et al. conducted in 2015 [30] detected resistance to tigecycline in 2% (4 out of 200) of MDR isolates. However, in 2016, Alaei et al. [27] reported an estimated resistance rate of 8.8% (4 out of 45) within the same region. In investigations conducted within the capital of Iran from 2016 to 2018, a wide scattering of results spanning from 1.6 to 84% was observed. Jasemi et al. [29] carried out multi-center research to evaluate the prevalence and trajectory of drug-resistant *A. baumannii* phenotypes from 2011 to 2013 in Tehran. Eventually, the researchers observed a remarkable decrease in the resistance of this pathogen to tigecycline.

The resistance mechanism to this antibiotic refers to the genotypic profiles of multidrug-resistant *Acinetobacter baumannii* (MDR-AB) isolates and involves genes in the resistance process.

In the study by Kooti et al. [30], 0.5, 7, and 40% of isolates possessed $bla_{\text{OXA-58-like}}$, $bla_{\text{OXA-24-like}}$, and $bla_{\text{OXA-23-like}}$ (class D beta-lactamase family) genes, respectively, using multiplex-polymerase chain reaction (PCR). In another study, by Bahador et al. [31], IS*Aba1* and IS*Aba4* (transposase family) were identified upstream of $bla_{\text{OXA-23-like}}$ genes in 45.1% and 12.9% of isolates, respectively. Moreover, Sarhaddi et al. [25] estimated the occurrence rates of bla_{TEM} (class A beta-lactamase family), $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, bla_{VIM}, and bla_{IMP} (subclass

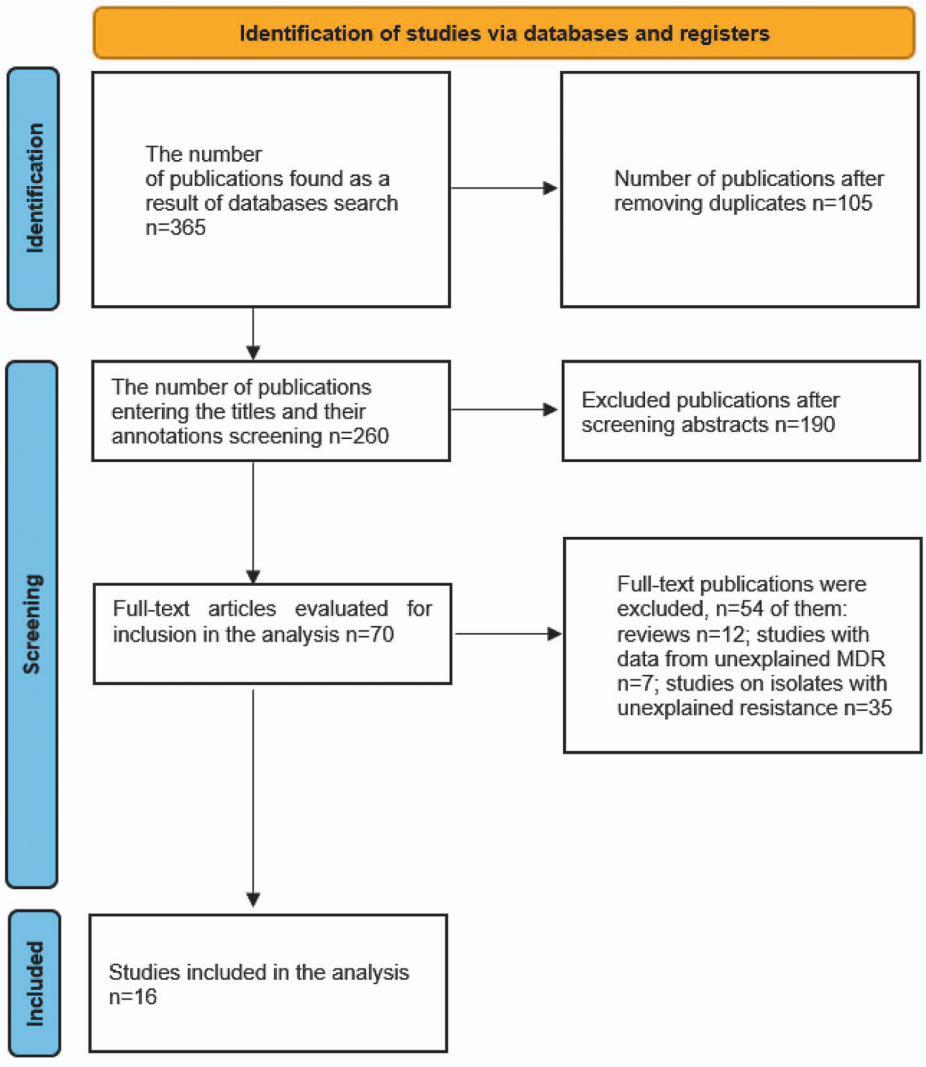

Figure prepared by the authors

Fig. 1. Flowchart diagram of the literature search procedure

B1 metallo-beta-lactamase family) at the level of 64.8, 66.7, 68.5, 70.4, and 70.4%, respectively. The presence of these genes might demonstrate a correlation with the acquisition of antimicrobial resistance.

The forest plot presented in Fig. 2 based on the meta-analysis results, demonstrates an 18.1% antimicrobial resistance to tigecycline among MDR *A. baumannii* species. Concerning the heterogeneity test, the obtained results showed *Q*-value = 305.712, *df* (*Q*) = 16, *p*-value = 0.000, and I^2 = 94.766 for the selected studies. The included studies demonstrated a publication bias, based on significant results of Egger's test (*p*-value < 0.05) and the asymmetrical funnel plot (Fig. 3).

Subgroup analysis

The overall resistance rate in ICU and non-ICU was 0.072 and 0.222, respectively (Fig. 4). The ICU subgroup had a lower heterogeneity (I^2 = 14.18%), while non-ICU patients showed a high heterogenicity (I^2 = 95.28%).

The overall resistance rate in Shiraz and Tehran was 0.079 and 0.161, respectively (Fig. 5). I^2 for these analyses was 91.31% for Shiraz and 95.14% for Tehran.

DISCUSSION

A. baumannii is a key agent of hospital-acquired infections due to its resistance to various classes of antibiotics [34, 35]. For this reason, the use of effective antibiotics, and the ongoing monitoring of antimicrobial resistance may contribute to *A. baumannii* eradication. Tigecycline, a potent semi-synthetic derivative of tetracycline, is recognized as the primary option among novel pharmaceuticals for infections caused by MDR strains of *Acinetobacter spp.* and carbapenem-resistant *A. baumannii* [7, 36]. The varied resistance rates could be explained by prescribing patterns and differences in regional epidemiology. Since 2005, the resistance rate to tigecycline has risen significantly as a result of long-term administration of this drug as a monotherapy and FDA approval.

In 2006, tigecycline was approved by the European Medicines Agency (EMA); in 2011, it was introduced in China. Since 2007, global reports have been published on tigecycline resistance. The *Acinetobacter* resistant strains had been reported before 2011 [37]. Overprescription of antibiotics has been associated with a higher rate of resistance [38]. There is probably an indirect correlation

Table 1. Characteristics of the included studies

	Study	Published year	Year of study	Type of study	City of study	Study population
1	Sepahvand et al. [18]	2022	no data	Cross-Sectional	Shiraz	hospital patients
2	Saadati et al. [13]	2021	August 2017 to February 2018	Cross-Sectional	Tabriz	hospital patients
3	Alavi-Moghaddam et al. [19]	2020	January 2016 to November 2018	Cross-Sectional	Tehran	hospital patients
4	Salehi et al. 2019 [20]	2019	August 2016 and February 2017	Cross-Sectional	Tehran	hospital patients
5	Tafreshi et al. [21]	2019	between 2016 and 2018	Cross-Sectional	Tehran	hospital patients
6	Yazdansetad et al. [22]	2019	during 2013	Cross-Sectional	Tehran	hospital burned patients
7	Salehi et al. 2018 [23]	2018	no data	Cross-Sectional	Tehran	patients, staff, and environment of an educational hospital
8	Zafari et al. [24]	2017	September 2015 to June 2016	Cross-Sectional	Tehran	hospital patients
9	Sarhaddi et al. [25]	2017	January and December 2014	Cross-Sectional	Mashhad	hospital patients
10	Ansari et al. [26]	2017	September 2015 to April 2016	Cross-Sectional	Shahrekord	hospital patients
11	Alaei et al. [27]	2016	February 2010 and March 2011	Cross-Sectional	Shiraz	ICU patients
12	Pourhajibagher et al. [28]	2016	no data	Cross-Sectional	Tehran	hospital patients
13	Jasemi et al. [29]	2016	August 2011 to December 2013	Cross-Sectional	Tehran	hospital patients
14	Kooti et al. [30]	2015	December 2012 to May 2013	Cross-Sectional	Shiraz	hospital patients
15	Bahador et al. 2015 [31]	2015	2012	Cross-Sectional	Tehran	Burned patients
16	Bahador et al. 2014 [32]	2014	2011	Cross-Sectional	Tehran	ICU patients
17	Bahador et al. 2014 [32]	2014	2006	Cross-Sectional	Tehran	ICU patients

Table prepared by the authors using data from the included studies [13, 18–32]

Table 2. List of selected samples and research methods

	Study	Sample source	MDR isolates	TGC /MDR	Diagnostic test
1	Sepahvand et al [18]	blood, wound, respiratory and urine samples	100	22	disc diffusion
2	Saadati et al [13]	tracheal secretion, blood, wound, catheter, bronchial washing, CSF, urine, sputum, and ascites fluid	100	100	disk diffusion
3	Alavi-Moghaddam et al [19]	blood, trachea, urine, cerebrospinal fluid, catheter and pleural fluid	109	35	disc diffusion
4	Salehi et al 2019 [20]	various specimens mostly sputum	180	152	disk diffusion
5	Tafreshi et al [21]	burn wound infection	84	28	broth microdilution
6	Yazdansetad et al [22]	burn wound	63	22	broth microdilution
7	Salehi et al 2018 [23]	wet swab from clothes and hands of staff, medical equipment, and patients' environment	125	2	disk diffusion
8	Zafari et al [24]	blood, wound, urine, sputum, and respiratory tract	100	2	disc diffusion
9	Sarhaddi et al [25]	burnt wound	54	2	*E*-test
10	Ansari et al [26]	clinical samples	30	18	disk diffusion
11	Alaei et al [27]	urine, sputum, blood, postoperative wound, cerebrospinal fluid, nasal secretion, eye secretion	45	4	broth microdilution
12	Pourhajibagher et al [28]	burn wound	33	2	disk diffusion and broth microdilution
13	Jasemi et al [29]	clinical specimens	26	8	disk diffusion
14	Kooti et al [30]	urine, wound, blood, sputum, ETT, body fluid, nose, throat and eye	200	4	disk diffusion
15	Bahador et al 2015 [31]	clinical samples	62	11	broth microdilution
16	Bahador et al 2014 [32]	wound, respiratory tract, urine, blood, and CSF	50	4	*E*-Test
17	Bahador et al 2014 [32]	wound, respiratory tract, urine, blood, and CSF	50	0	*E*-Test

Table prepared by the authors using data from the included studies [13, 18–32]

Note: TGC — Tigecycline; CSF — cerebrospinal fluid, ETT — endotracheal tube.

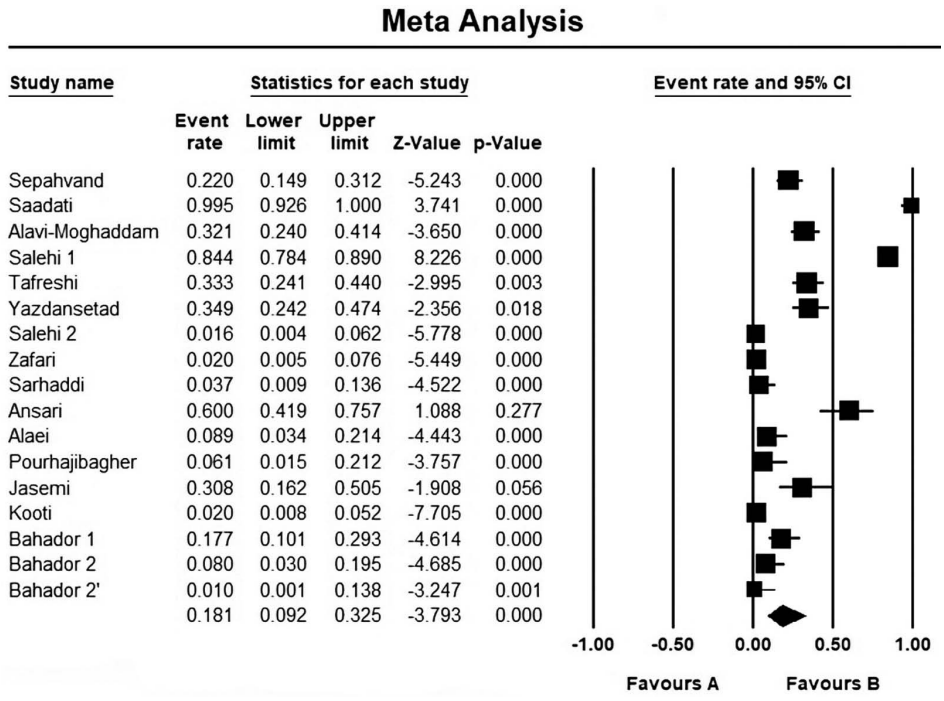

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Fig. 2. The results of the meta-analysis evaluating the resistance of different isolates of *A. baumannii* to tigecycline in Iran

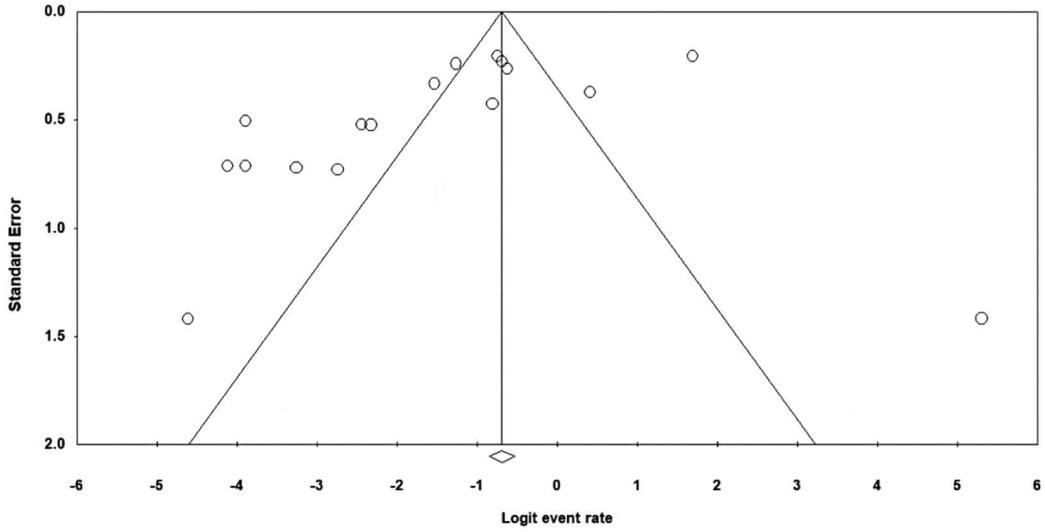

Figure prepared by the authors

Fig. 3. Funnel plot

between the previous use of other antibiotics and tigecycline resistance because of transportation by the similar efflux pump [39]. In addition, treatment by a wide-spectrum antibiotic instead of a narrow-spectrum drug, e.g., due to the inaccurate diagnosis of infection, inappropriate differentiation between virus or bacterium and the resulting improper prescription, as well as self-medication, leads to a growth in drug resistance [40].

The resistance mechanism to tigecycline is mediated by efflux pumps such as *AdeABC*. The overexpression in *AdeABC* caused by amino acid and nucleotide changes in the *AdeRS* two-component system and modified expression of *AdeA* and *AdeB* by the BaeSR system is another possible mechanism. In addition, mutations in genes encoding 1-acyl-sn-glycerol-3-phosphate acyltransferase and S-adenosyl-L-methionine (SAM)-dependent methyltransferase result in lower susceptibility [7].

Despite our findings, other review studies indicated conflicting results. In the review of Ni et al. [17], administration of tigecycline was discouraged based on assessment of cohorts and RCT studies. This review demonstrated a higher in-hospital mortality rate, a lower rate of bacterial eradication, and insufficiency of combination therapy in treatment groups compared to the control. Sodeifian et al. applied an approach similar to that used in our work to analyze observational studies. As a result, tigecycline was not recommended for treatment regimens. The researchers found that the overall efficacy of tigecycline in patients was comparable with other antimicrobial agents. Furthermore, a higher death rate and a lower bacterial

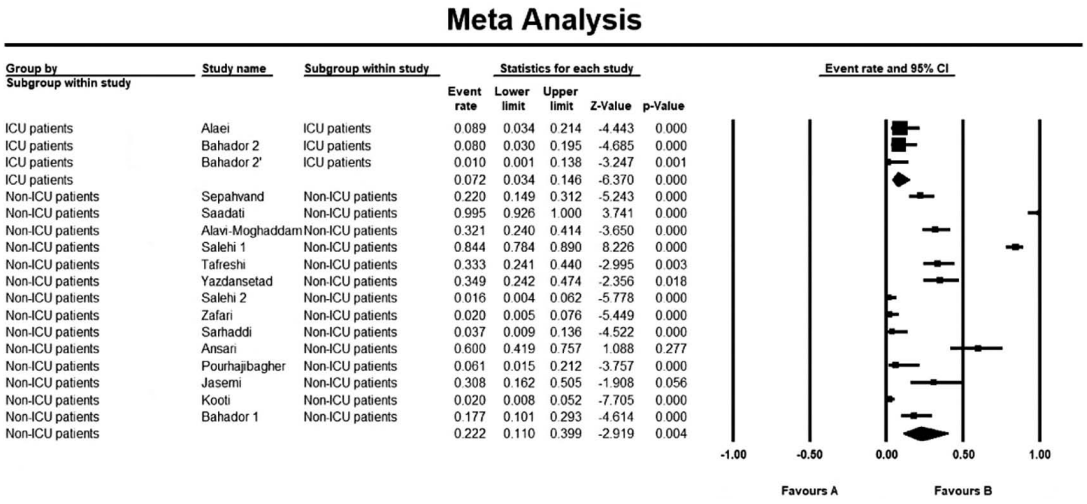

Figure prepared by the authors

Fig. 4. Subgroup analysis of patients admitted to ICU and non-ICU wards

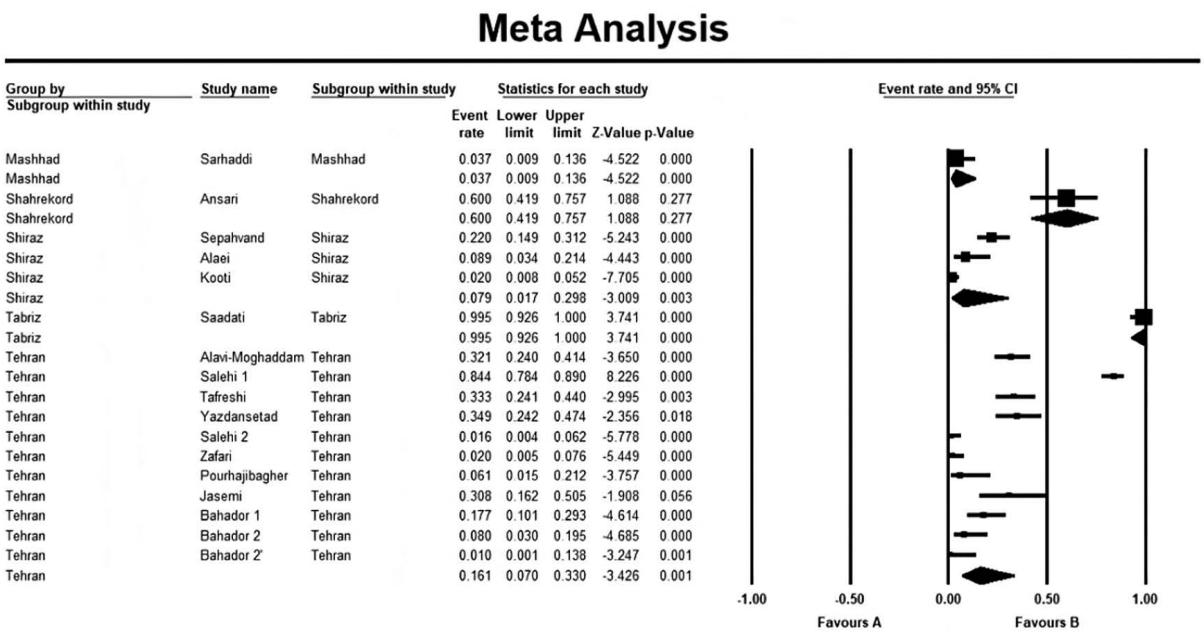

Figure prepared by the authors

Fig. 5. Subgroup analysis based on location

eradication rate compared to medication regimens based on colistin were established [41].

Care should be taken when interpreting the results due to the limited data. There are few studies on the prevalence of *A. baumannii* with MDR in Iran, with their majority covering large cities, Tehran in particular. Therefore, more data from other Iranian locations should be obtained to verify the results. In addition, the small size of samples for a subgroup analysis of statistical tests should be considered. There is a high potential of bias when evaluating research studies based on the JBI checklist. None of the studies mentioned confounding factors and the respective corrective approaches; in some studies, statistical methods were not described appropriately. The study setting and validity, as well as the reliability of outcome measurement, remain unclear, thus rendering the interpretation unreliable. Moreover, the clinical samples were obtained from intensive care unit (ICU) patients and individuals hospitalized in various wards, including the burn unit. Variations in isolate extraction methods and the challenging conditions experienced by ICU patients may have potentially impacted the outcomes of antimicrobial susceptibility testing. Additionally, the choice of antimicrobial susceptibility testing methodologies, such as disc diffusion, *E*-test, or broth microdilution, could also have contributed to discrepancies in results. This variability poses challenges in drawing unequivocal conclusions. This study contributes to the information about the susceptibility of different *A. baumannii* isolates to tigecycline, thus facilitating a grounded choice of antibiotics for MDR strains. However, further research is needed to obtain more reliable data on the level of such resistance.

CONCLUSION

In the present review and meta-analysis, we evaluated the resistance rate among patients infected by *A. baumannii* and admitted to hospitals in some cities of Iran. Our findings indicate a high resistance rate of *A. baumannii* strains against tigecycline; however, tigecycline is still considered an effective drug against MDR bacteria. The meta-analysis results show that the reviewed publications do not provide clear evidence of the overall effect of tigecycline on the resistance rate. In other words, the increase in *A. baumannii* resistance to tigecycline is not statistically significant, which is confirmed by the results of other studies conducted earlier in Iran.

Increased resistance of *A. baumannii* to most antibiotics, established in the present study, may be due to improper use or unjustifiably high consumption of broad-spectrum antimicrobial agents, the lack of access to clean water, irregularity of algorithms for sanitary and hygienic and disinfection measures, and administration of antimicrobial combinations in fixed doses, even without knowledge of proven advantages over individual medicinal compounds. There are also social factors, such as self-medication, over-the-counter antimicrobial use, inadequate prevention of infections and diseases, and limited access to high-quality, affordable medicines, vaccines, and diagnostic tools. Given the current situation with the spread of resistant isolates, it is necessary to introduce comprehensive infection control programs aimed at localizing and limiting the spread of *A. baumannii* strains in medical institutions.

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