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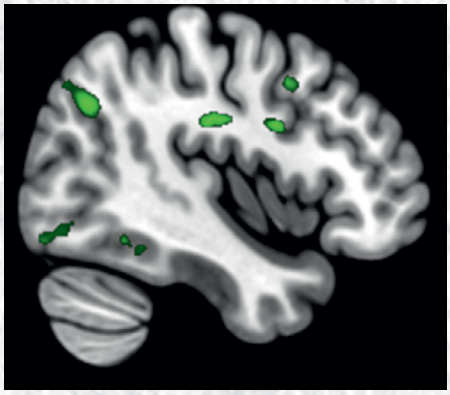

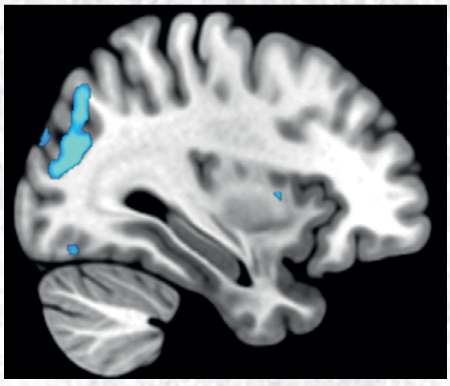

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On the front cover: part of the Figure 1 from the article of A.A. Raskurazhev et al. (p. 68).

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Vol. 19 No. 1 2025

Table of Contents:

Original articles

Clinical neurology

Experimental neurology

Review articles

Reviews

Technologies

Case reports

Анналы клинической и экспериментальной неврологии
Том 19 № 1 2025

В номере:

Оригинальные статьи

Клиническая неврология

Экспериментальная неврология

Обзоры

Научный обзор

Технологии

Клинические разборы

Intracranial Atherosclerosis: Structure, Clinical Aspects and Risk Factors

Marine M. Tanashyan, Andrey S. Mazur, Anton A. Raskurazhev, Anna N. Berliand, Marina V. Dreval, Polina I. Kuznetsova, Olga V. Lagoda

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Abstract

Introduction. *Atherosclerosis is a complex pathophysiological process with a wide range of clinical manifestations. Active research is underway to determine the prevalence of intracranial atherosclerosis across different ethnic groups, the role of modifiable and non-modifiable risk factors in its pathogenesis, and the advances in diagnostic algorithms for patients with extra-/intracranial atherosclerosis.*

The ***aim*** *was to evaluate manifestations of intracranial atherosclerosis (patterns of intracranial artery lesions, including pathomorphological findings) and identify potential associations between known risk factors and intracranial atherosclerosis in patients with cerebrovascular disease.*

Materials and methods. *During the first phase, a retrospective analysis of autopsy protocols was conducted for 166 patients (66% men) hospitalized at the Research Center of Neurology between 1976 and 2007. The second phase involved clinical, laboratory, and imaging data from 120 patients (59% men) with atherosclerotic disease of the brachiocephalic arteries. These patients were divided into two subgroups: a main subgroup with intracranial artery involvement combined with extracranial atherosclerosis (n = 60) and a control subgroup with isolated extracranial artery involvement (n = 60).*

Results. *Pathomorphological assessment revealed a high rate of atherosclerotic lesions in the carotid artery system at both extra- and intracranial levels. One-third of patients had ⩾50% atherosclerotic stenosis in intracranial arteries without significant extracranial stenosis. Multivariate logistic regression analysis identified obesity (odds ratio [OR] 3.22), male sex (OR 6.17), and low-density lipoprotein levels (OR 2.5) as the most significant independent clinical laboratory factors associated with intracranial atherosclerosis.*

Conclusion. *The role of intracranial atherosclerosis in cerebrovascular events is underestimated. Overlapping neurological and generalized manifestations of isolated intra- and extracranial atherosclerosis may give a misleading impression of the true prevalence of intracranial atherosclerosis.*

Keywords: *intracranial atherosclerosis; risk factors; ischemic stroke*

Ethics approval. The study was conducted with the informed consent of the patients. The research protocol was approved by the Ethics Committee of the Research Center of Neurology (protocol No. 9-5/22, October 19, 2022).

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Интракраниальный атеросклероз: структура, клинические аспекты и факторы риска

М.М. Танашян, А.С. Мазур, А.А. Раскуражев, А.Н. Берлянд, М.В. Древаль, П.И. Кузнецова, О.В. Лагода

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Аннотация

Введение. *Атеросклероз – сложный патофизиологический процесс с огромным диапазоном клинических проявлений. Активно изучается распространённость интракраниального атеросклероза среди населения различных этнических групп, роль модифицируемых и немодифицируемых факторов риска в развитии данной патологии, совершенствуются алгоритмы обследования пациентов с экстра-/интракраниальным атеросклерозом.*

Цель *исследования – оценка проявлений интракраниального атеросклероза (характер поражения интракраниальных артерий, в том числе с учётом патоморфологических данных), а также выявление у пациентов с цереброваскулярными заболеваниями возможных ассоциаций известных факторов риска.*

Материалы и методы. *На первом этапе проведён ретроспективный анализ протоколов вскрытия 166 пациентов (66% – мужчины), находившихся на стационарном лечении в Научном центре неврологии в 1976–2007 гг. На втором этапе изучены клинико-лабораторно-инструментальные данные 120 пациентов (59% – мужчины) с атеросклеротическим процессом брахиоцефальных артерий, которые были разделены на две подгруппы: основную – с поражением интракраниальных артерий в сочетании с экстракраниальным атеросклерозом (n = 60) и группу контроля – с поражением исключительно экстракраниальных артерий (n = 60).*

Результаты. *В результате патоморфологического исследования установлена высокая частота атеросклеротического поражения артерий каротидной системы как на экстра-, так и на интракраниальном уровне. У трети пациентов атеростеноз ⩾ 50% определялся в интракраниальных артериях в отсутствие выраженного атеростеноза экстракраниальных артерий. Наиболее значимыми независимыми клинико-лабораторными факторами, ассоциированными с интракраниальным атеросклерозом, по данным многофакторной логистической регрессии, стали ожирение – отношение шансов (ОШ = 3,22), мужской пол (ОШ = 6,17) и уровень липопротеидов низкой плотности (ОШ = 2,5).*

Заключение. *Интракраниальный атеросклероз является потенциально недооценённой причиной развития нарушений мозгового кровообращения. Общность неврологических и общесоматических проявлений изолированных интра- и экстракраниального атеросклероза может создавать обманчивое впечатление об истинной распространённости интракраниального атеросклероза.*

Ключевые слова: *интракраниальный атеросклероз; факторы риска; ишемический инсульт*

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Источник финансирования. Авторы заявляют об отсутствии внешних источников финансирования при проведении исследования.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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Introduction

Atherosclerosis (AS) is a complex pathophysiological process with a wide range of clinical manifestations. Decades of research on cerebral AS have provided robust evidence highlighting the significance of extracranial vascular lesions in the development and progression of a cerebrovascular event (CVE) [1, 2]. However, intracranial atherosclerosis (ICAS), which also represents a major global public health challenge due to the high risk of CVE [3], remains understudied. According to various studies, the rate of recurrent stroke in patients with ICAS ranges from 9.4% (short-term) to 15.1% (over 1 year) despite optimal preventive therapy [4], with a mortality rate of up to 13.2% within one year of a CVE [5]. Recent diagnostic advances have underscored the need for special attention to pharmacological treatment and prevention strategies, as well as the development of safe endovascular surgical techniques for intracranial artery stenosis. Current

research focuses on the variable prevalence of ICAS across different ethnic populations [6], the role of modifiable and non-modifiable risk factors, and the limitations of existing diagnostic protocols for patients with extracranial atherosclerosis (ECAS) and ICAS [7]. Multiple studies indicate that ECAS is predominantly associated with risk factors such as male sex, dyslipidemia, and diabetes, whereas ICAS is associated with hypertension and metabolic disorders, such as obesity and diabetes [8, 9].

The differential impact of risk factors on the development and progression of ICAS may be explained by morphological differences between intracranial and extracranial arteries [10]. Intracranial arteries have a stronger internal elastic lamina and sparse *vasa vasorum* (supporting the luminal diffusion theory and suggesting a more stable atherosclerotic plaque (AP) phenotype), absence of an external elastic lamina, and fewer smooth muscle cells. In addition, intracranial vessels have unique hemodynamics due to their tortuous anatomy, numerous perforating branches, and antioxidant defenses that provide long-term protection to the vessel wall. In contrast, extracranial arteries are elastic and musculoelastic vessels rich in collagen and elastin fibers in the medial layer and have an external elastic lamina [11].

Given the conflicting data on the prevalence of intracranial APs across different ethnic groups, we designed a two-phase study. The first phase involved morphological analysis, while the second phase focused on clinical and imaging comparisons in a cohort of patients with cerebral AS.

The **aim** was to evaluate manifestations of ICAS (patterns of intracranial artery lesions, including pathomorphological findings) and identify potential associations between known risk factors and ICAS in patients with cerebrovascular disease.

Materials and Methods

Phase 1

A retrospective analysis of autopsy protocols was conducted for 166 patients hospitalized at the Research Center of Neurology between 1976 and 2007. The study included 110 men and 56 women aged 38–89 years (mean age: 63.1 ± 11.0 years). The causes of death included cerebral infarction (n = 68), acute heart failure (n = 41), pulmonary embolism (n = 41), and other causes (n = 16). All evaluated cases revealed cerebral infarcts of varying size, location, and structure (1–15 infarcts per case, mean: 3.1 ± 2.4). Infarcts in the carotid artery system (CAS) or borderzone areas of the CAS and vertebrobasilar system were observed in 79.5% of cases (mean: 2.2 ± 1.6 infarcts per case): large/extensive infarcts (55 cases), medium infarcts (73), and small superficial/deep infarcts (51).

Cerebral artery macroscopy from autopsy reports was used to assess the prevalence of atherosclerotic lesions and the severity of stenosis in the CAS.

Statistica v. 13.0 (StatSoft) was used for statistical analysis. Student's t-test was used to evaluate differences. Results were considered statistically significant if $p < 0.05$.

Phase 2

The study included 120 patients with atherosclerotic lesions of the brachiocephalic arteries (confirmed by computed tomography (CT)/magnetic resonance (MR) angiography). Patients were divided into two subgroups: the main group (intracranial artery lesions with ECAS, n = 60) and the control group (extracranial artery lesions only, n = 60). The main group had a mean age of 64.8 ± 9.7 years [58; 71.5]; 43 patients (72%) were male. The control group had a mean age of 68.7 ± 6.99 years [63; 73]; 32 patients (53%) were male. Extracranial atherosclerosis involved stenoses of varying severity in the internal carotid and vertebral arteries. Exclusion criteria were recent (<6 months) acute CVEs, severe cognitive impairment, major medical/malignant/infectious disease, or allergy to iodine/gadolinium-based contrast agents.

All patients underwent detailed clinical and neurological examination, ultrasound, and angiographic imaging of the brachiocephalic arteries (CT angiography at extra-/intracranial levels for both groups; high-resolution MRI of the vessel wall for the main group).

Extra-/intracranial CT angiography was performed using a Siemens SOMATOM Definition AS scanner with intravenous iodine-based contrast (Omnipaque 350, 1–2 mL/kg; injection rate: 5 mL/s). High-resolution MRI of the vessel wall was performed using a Siemens Magnetom Prisma 3T scanner with gadolinium-based contrast (0.1 mmol/kg; injection rate: 4 mL/s).

R v.4.2.1 was used for statistical analysis. Quantitative parameters were compared using the Mann–Whitney U test. Qualitative parameters were compared using Pearson's χ^2 or Fisher's exact test. Univariate and multivariate regression analyses were performed. The resulting regression model was evaluated using ROC curve analysis with area under the curve (AUC) calculation. The sensitivity and specificity of the model were also evaluated. The null hypothesis was rejected at $p < 0.05$.

Results

Histopathology showed high prevalence of atherosclerotic lesions of CAS at both extracranial and intracranial levels (Figure 1): APs were detected in 96.4% of cases, with higher prevalence in intracranial (88.6%) than extracranial arteries (80.1%). Severe AS was most common in the internal carotid artery (ICA) sinus and middle cerebral artery (MCA, predominantly M1 segment), with comparable AP prevalence and the severity of stenosis (p = 0.66). APs were present in >75% of these arteries ($p < 0.0001$). Based on the incidence of APs and the severity of atherosclerotic stenosis, the intracranial ICA (the cerebral part and the carotid siphon) ranked second with APs detected in 68.7% of cases ($p < 0.0001$). Anterior cerebral artery atherosclerosis was less frequent (39.2% with APs).

Multiple atherosclerotic lesions were observed with 2–13 APs in 97.5% of CAS atherosclerosis (mean: 6.13 ± 2.84). Most patients (75%) had APs in both extracranial and intracranial arteries, predominantly (55.6% of cases) at three CAS system levels: common carotid artery/ICA sinus, intracranial ICA,

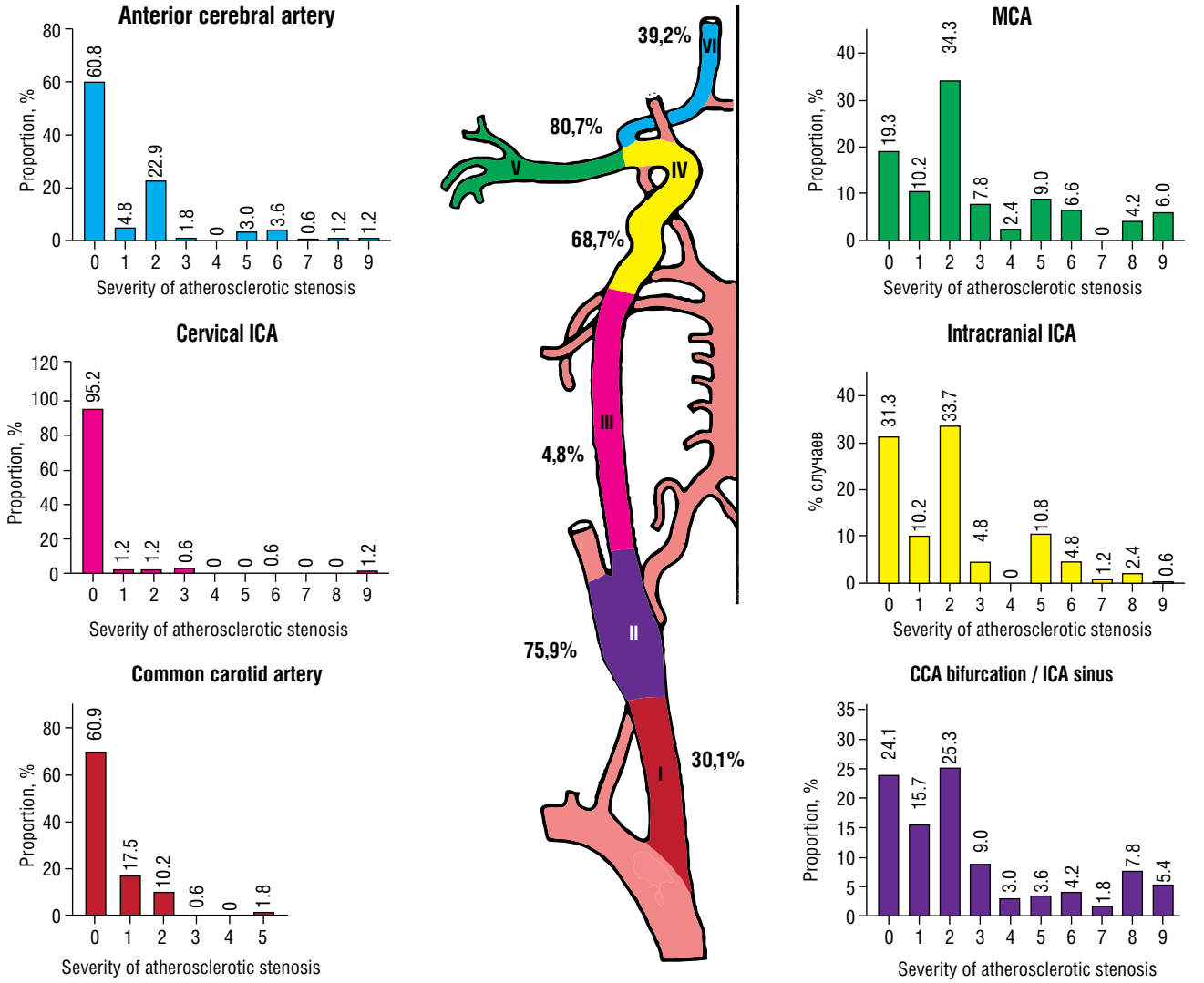

Fig. 1. Incidence of APs in the carotid system arteries (bilateral APs were considered; in the case of bilateral lesions, the most severe atherosclerotic stenosis was considered).
I – common carotid artery before bifurcation; II – CCA bifurcation with the ICA sinus; III – cervical ICA; IV – intracranial ICA; V – MCA; VI – anterior cerebral artery.
Severity of atherosclerosis: 0 – no APs; 1 – flat plaques; 2 – atherosclerotic stenosis < 20%; 3 – atherosclerotic stenosis ⩾ 20% and < 30%; 4 – atherosclerotic stenosis ⩾ 30% and < 40%; 5 – atherosclerotic stenosis ⩾ 40% and < 50%; 6 – atherosclerotic stenosis ⩾ 50% and < 60%; 7 – atherosclerotic stenosis ⩾ 60% and < 70%; 8 – atherosclerotic stenosis ⩾ 70% and < 80%; 9 – atherosclerotic stenosis ⩾ 80%.

and cerebral arteries (anterior cerebral artery and/or MCA). Multiple APs in intracranial arteries without extracranial lesions were observed in 14.4%, whereas isolated APs in extracranial arteries were rare.

APs were bilateral in 91% of cases, with 50% showing symmetrical CAS involvement. Bilateral lesions most frequently affected the ICA sinus/siphon and MCA (74–75% of cases). Multiple APs in the system of only one ICA (unilateral atherosclerotic lesion of the CAS) were rare (2.4%).

Most APs were flat or mildly stenotic (82%). APs with ⩾ 50% luminal stenosis were identified in 53.1% of cases (1.1 ± 1.6 APs per case). In 75% of these cases, 1 or 2 hemodynamically significant APs were detected. Severe stenoses (⩾ 50%) were primarily intracranial (40.6% of cases), predominantly isolated (30.6%), without significant extracranial atherosclerotic stenosis. Combined extra-/intracranial atherosclerotic stenosis ⩾ 50% was found in 6.3%. Multiple hemodynamically significant APs in intracranial arteries without ECAS were observed in 3.1%.

In patients with ICAS and ECAS (Table 1), hypertension was the major risk factor (100%), followed by male sex (73%), obesity (43%), and smoking (38%).

Most patients with ICAS had anterior circulation involvement: APs were most frequent in intracranial ICA segments (39%), followed by the MCA (23%) and anterior cerebral artery (4%).

Table 1. Characteristics of patients with ICAS and ECAS

Parameter	ICAS (*n* = 60)	ECAS (*n* = 60)	*p*
Age, years, Me [Q_1; Q_3]	66 [58; 72]	70 [63; 73]	0.027
Male, *n* (%)	44 (73%)	31 (52%)	0.014
Female, *n* (%)	16 (27%)	29 (48%)	0.014
Hypertension, *n* (%)	60 (100%)	55 (92%)	0.057
Systolic blood pressure, mm Hg, Me [Q_1; Q_3]	190 [180; 200]	180 [160; 190]	< 0.001
Diastolic blood pressure, mm Hg, Me [Q_1; Q_3]	100 [90; 110]	90 [90; 100]	0.005
Diabetes, *n* (%)	28 (47%)	19 (32%)	0.092
BMI, Me [Q_1; Q_3]	29.2 [27.4; 32.5]	27.6 [24.9; 29.0]	0.020
Obesity, *n* (%)	26 (43%)	12 (20%)	0.006
Smoking, *n* (%)	23 (38%)	38 (63%)	0.006
Cholesterol, Me [Q_1; Q_3]	4.40 [3.75; 5.80]	5.40 [4.55; 7.05]	0.002
Low-density lipoprotein, Me [Q_1; Q_3]	1.97 [1.42; 2.48]	1.88 [1.14; 2.49]	0.3
Family history, *n* (%)	27 (45%)	26 (43%)	0.9
Ischemic heart disease, *n* (%)	28 (47%)	25 (42%)	0.6
Lower limb atherosclerosis, *n* (%)	19 (32%)	24 (40%)	0.3
CVE, *n* (%)	41 (68%)	28 (47%)	0.016

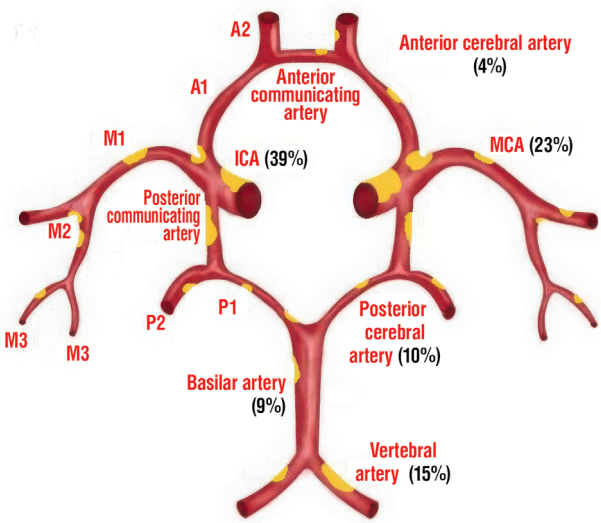

Fig. 2. Atherosclerotic burden on intracranial arteries.
M1–M3 – segments of the MCA; A1, A2 – segments of the ICA; P1, P2 – segments of the posterior cerebral artery.

Posterior circulation plaques were most commonly found in the V4 segments of the vertebral arteries (15%), followed by the posterior cerebral (10%) and basilar (9%) arteries. Figure 2 shows the atherosclerotic burden distribution across intracranial arteries (according to angiography data).

Among 60 patients with ICAS, stratification into subgroups based on the severity of ECAS revealed the following distribution: the largest subgroup comprised patients with ICAS without significant ECAS (stenosis < 50%), *n* = 30 (50%); subgroup with combined ICAS and hemodynamically significant ECAS (stenosis ⩾ 50%), *n* = 25 (42%); isolated ICAS (no ECAS): *n* = 5 (8%).

Comparative risk factor analysis between ICAS and ECAS groups demonstrated statistically significant differences:

- non-modifiable risk factors: younger age in ICAS group (66 years [58; 72] *vs* 70 years [63; 73]; *p* = 0.027); male predominance in ICAS group (73% [44/60] *vs* 52% [31/60]; *p* = 0.014);
- modifiable risk factors: higher systolic BP in ICAS group (190 mm Hg [180; 200] *vs* 180 mm Hg [160; 190]; *p* < 0.001); elevated diastolic BP in ICAS group (100 mm Hg [90; 110] *vs* 90 mm Hg [90; 100]; *p* = 0.005). Patients with ICAS more often had metabolic disorders and were characterized by higher body mass index (29.2 [27.4; 32.5] *vs* 27.6 [24.9; 29.0]; *p* = 0.020) and consequently greater prevalence of obesity (26 (43%) *vs* 12 (20%); *p* = 0.006). Conversely, prevalence of smoking (23 [38%] *vs* 38 [63%]; *p* = 0.006) and elevated total cholesterol levels (4.40 [3.75–5.80] *vs* 5.40 [4.55–7.05] mmol/L; *p* = 0.002) were significantly higher in controls.

No significant intergroup differences in type 2 diabetes rates were observed. However, ICAS patients with diabetes demonstrated higher rates of multifocal intracranial arterial involvement.

Univariate and multivariate analysis models were used to determine the significance of each risk factor in differentiating the level of atherosclerotic lesions of the cerebral arteries (Table 2). The multivariate logistic regression model identified independent predictors of ICAS such as age, sex, systolic blood pressure, lower total cholesterol levels, smoking, high low-density lipoprotein levels, and obesity.

Table 2. Univariate and multivariate logistic regression models of association of clinical and laboratory factors with ICAS

Risk factor	Univariate logistic regression model		
	odds ratio	95% CI	*p*
Age	0.95	0.90–0.99	0.015
Male	2.57	1.21–5.62	0.015
Systolic blood pressure, mm Hg	1.05	1.03–1.08	< 0.001
Diastolic blood pressure, mm Hg	1.05	1.02–1.10	0.007
Type 2 diabetes	1.89	0.90–4.02	0.094
Total cholesterol	0.68	0.52–0.86	0.002
Smoking	0.36	0.17–0.75	0.007
Chronic lower limb ischemia	0.70	0.33–1.47	0.3
Family history	1.07	0.52–2.21	0.9
Ischemic heart disease	1.23	0.60–2.53	0.6
Low-density lipoprotein	1.21	0.79–1.86	0.4
Obesity	3.06	1.38–7.09	0.007
BMI	1.10	1.00–1.22	0.053
History of stroke	2.47	1.18–5.26	0.017

Table 3. Characteristics of post-stroke patients with ICAS and ECAS, *n* (%)

Characteristic		ICAS (*n* = 60)	ECAS (*n* = 60)	*p*
CVE		41 (68%)	28 (47%)	0.016
Recurrent CVE		23 (56%)	5 (18%)	0.0024
Localization of a symptomatic AP (circulation system)	anterior	29 (71%)	23 (82%)	0.395
	posterior	12 (29%)	5 (18%)	0.395
Clinical presentation of the stroke	motor deficit	30 (73%)	21 (75%)	0.167
	sensory deficit	21 (51%)	14 (50%)	0.098
	vestibular ataxia	34 (83%)	23 (82%)	0.086
	cognitive dysfunction	28 (68%)	22 (79%)	0.954
	speech disorders	16 (39%)	19 (68%)	0.027
Severity of stroke, Me [Q_1; Q_3]	Rankin score	3 [2; 3]	2 [2; 3]	0.036
	Rivermead Mobility Index	11 [11; 13]	12 [10; 12]	0.043

ROC analysis evaluated the potential of this model as a predictor for verifying ICAS. Optimal threshold values were selected using the maximum Youden's index. The AUC was 0.882 (95% CI 0.826–0.939), indicating excellent predictive value. Sensitivity and specificity were 78 and 80%, respectively.

To further evaluate features of CVEs in patients with ICAS, we compared the characteristic localization of a symptomatic AP, clinical presentation of ischemic injury, and stroke severity between two patient groups (Table 3). The number of CVEs (41 (68%) *vs.* 28 (47%); $p = 0.016$) and recurrent ischemic events (23 (56%) *vs.* 5 (18%); $p = 0.0024$) was significantly higher in the ICAS group. Symptomatic APs in both ICAS and ECAS patients were more commonly located in the carotid (anterior) circulation (29 (71%) *vs.* 12 (29%); $p = 0.395$). Ischemic stroke severity, evaluated using the Modified Rankin Scale (3 [2; 3] *vs.* 2 [2; 3]; $p = 0.036$) and the Rivermead Mobility Index (11 [11; 13] *vs.* 12 [10; 12]; $p = 0.043$), differed significantly between ICAS and ECAS groups. No significant differences were observed in the clinical presentation of CVEs between two groups, except for speech disorders (16 (39%) *vs.* 19 (68%); $p = 0.027$).

Discussion

The presented analysis of morphological data demonstrates a high rate of atherosclerotic CAS involvement, with a slight predominance of intracranial lesions. This further highlights

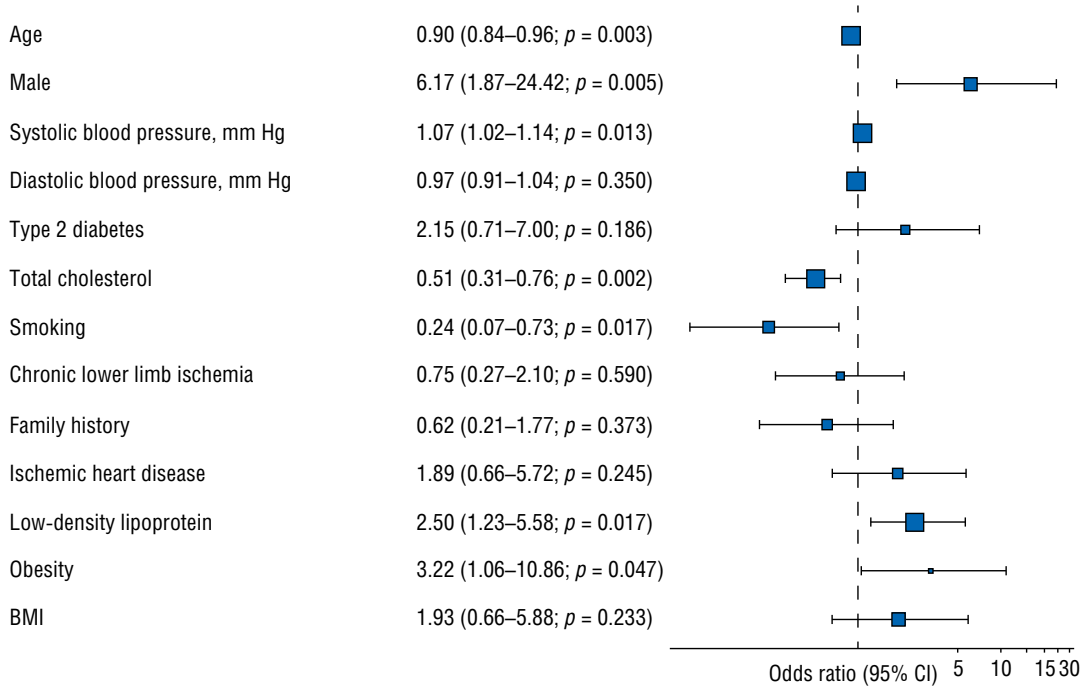

Fig. 3. Multivariate logistic regression models of association of clinical and laboratory factors with ICAS.

the clinical significance of ICAS and the need to improve conventional angiographic techniques, which currently fail to identify its true prevalence. It should be noted that 82% of APs were either flat or mildly stenotic, suggesting that lumen-oriented techniques such as digital subtraction angiography and CT angiography may overlook abnormalities in the intracranial arterial segments. This observation aligns with findings from H. Yan et al., who reported a higher prevalence of positive AP remodeling in the vertebrobasilar system and similarly cautioned against underestimation of such changes during angiographic evaluation [12].

Differences in risk factor profiles between ICAS and ECAS remain poorly understood. A 1995–2018 systematic review and meta-analysis of 31 risk factors for ICAS identified elderly age, metabolic syndrome, type 2 diabetes, and hypertension as the most significant contributors [13].

In our study, hypertension demonstrated a statistically significant role in both the initiation and progression of ICAS compared to ECAS. Most ICAS patients had grade 3 hypertension with hypertensive crises. These findings support previous hypotheses linking ethnic differences in ICAS prevalence (higher in Asian, Hispanic, and European populations) to differences in hypertension incidence, severity, and genetic risk factors in these groups [14]. In addition, metabolic disorders, such as obesity and diabetes, were also shown to contribute substantially to ICAS development and progression. While prior studies confirmed an association between diabetes and ICAS severity and progression [15], our analysis did not identify diabetes as an independent predictor of intracranial involvement. In contrast, obesity was a significant factor, more than tripling the potential for ICAS diagnosis. This difference may be attributed to the high prevalence of diabetes in both study groups.

As noted above, anatomical and physiological differences between extracranial and intracranial arteries tend to underlie differences in atherosclerotic lesion patterns. However, these processes are closely related: the presence of extracranial carotid APs independently predicts ICAS progression [16].

Our study found that patients with ICAS had recurrent CVEs more frequently than those with ECAS, aligning with global trends advocating aggressive management of ICAS [17]. Notably, strokes in the ICAS group predominantly localized to the anterior circulation, whereas Korean studies reported higher rates of posterior circulation stroke [18]. The lack of distinct clinical features between ICAS- and ECAS-related CVEs may contribute to underdiagnosis of ICAS in routine clinical practice. However, our findings suggest that a history of recurrent CVEs and increased stroke severity, both more common in ICAS, should prompt detailed angiographic and neuroimaging examinations.

Identifying associations between ICAS and established risk factors may increase neurologists' clinical alertness for this condition in patients with a history of CVE. To our knowledge, this is the first Russian study to identify independent risk factors for atherosclerosis at two anatomical levels (extra- and intracranial). Risk factors associated with ICAS should be considered when adjusting patient management strategies, given the high rate of recurrent CVE in this cohort. These findings may aid in developing personalized diagnostic and therapeutic algorithms and inform ICAS monitoring

programs. Further research and statistical analysis of these results may also allow the creation of specific scales for ICAS identification and risk stratification of recurrent CVE in ICAS patients.

A limitation of this study is its small sample size; larger cohort studies are needed to validate the significance of the identified ICAS risk factors.

Conclusion

ICAS represents a potentially underestimated cause of CVE. Isolated intracranial artery atherosclerotic stenosis (⩾ 50%) without significant extracranial atherosclerotic stenosis was observed in one-third of patients.

This study provides the first comprehensive clinical and pathological analysis of the structure, risk factors, and progression patterns of cerebral atherosclerosis. Our findings demonstrate that ICAS is not a part of extracranial vascular pathology but a distinct disease entity requiring personalized treatment and prevention strategies. The most significant independent clinical and laboratory factors associated with ICAS in our cohort were obesity, male sex, and elevated low-density lipoprotein levels.

Overlapping neurological and generalized manifestations of isolated ICAS and ECAS may obscure the true prevalence and etiological role of ICAS in cerebrovascular disease. We identified specific factors such as a history of recurrent CVE with severe symptoms, that may help clinicians suspect intracranial involvement during initial evaluation and prioritize advanced angioneuroimaging.

A deeper analysis of risk factors and improvement of diagnostic algorithms may lead to a better understanding of the ICAS pathophysiology and ultimately to optimal therapeutic and preventive strategies for this patient population.

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Delirium in Acute Ischemic Stroke: Risk Factors, Sequelae, and Pathogenetic Treatment

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Abstract

Introduction. *Delirium, or an acute confusional state, is a common complication affecting between 10 and 48% of acute stroke patients. In the acute phase of stroke, delirium contributes to prolonged hospital stays, higher treatment costs and in-hospital and long-term mortality, increased risk of disability, and reduced potential for post-stroke rehabilitation.*

*The **aim** of this study is to identify delirium risk factors in acute stroke patients, to study the effects of delirium on mortality rates, post-stroke cognitive functioning, and to assess treatment options.*

Materials and methods. *One hundred and thirty-eight patients (93 males and 45 females) with a mean age of 71 [69.0; 74.8] years were enrolled in the study. Delirium was assessed using the Confusion Assessment Method (CAM); for initial assessment and repeated measurements of delirium severity, the Delirium Rating Scale (DRS) was used. Pre-stroke cognitive decline was assessed retrospectively using the Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE). Neuropsychological testing and assessment of caregiver burden using the Caregiver Burden Scale (CBS) were performed at 3, 6, and 18 months post-stroke onset.*

Results. *An IQCODE score of > 91 is a risk factor for severe delirium in acute stroke patients ($p < 0.001$). Severe delirium in acute stroke was associated with greater cognitive deficits ($p < 0.05$) and greater caregiver burden ($p < 0.01$) at 3 and 6 months post-stroke. DRS score > 15 and delirium duration > 10 days were found to be death risk factors at 18 months after stroke onset (OR = 3.58; 95% CI 1.4–9.19; $p = 0.008$ and OR = 2.56; 95% CI 1.03–6.38; $p < 0.044$, respectively). Central acetylcholinesterase inhibitors reduced the delirium duration ($p = 0.015$), improved cognitive function at 3, 6, and 18 months post-stroke ($p < 0.01$), and decreased caregiver burden at 3 and 6 months post-stroke ($p < 0.05$).*

Conclusion. *Delirium in the acute phase of stroke contributes to post-stroke cognitive decline in the patients and greater burden for their caregivers. Central acetylcholinesterase inhibitors can improve the post-stroke patient's condition and decrease the strain for caregivers.*

Keywords: *delirium, ischemic stroke; risk factors; cognitive decline; caregiver burden; central acetylcholinesterase inhibitors*

Ethics approval. The research protocol was approved by the Ethics Committee of the S.M. Kirov Military Medical Academy (protocol No. 94, July 07, 2009).

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Спутанность сознания в остром периоде ишемического инсульта: факторы риска, последствия, патогенетическая коррекция

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Аннотация

Введение. *Спутанность сознания (СпС)– частое осложнение острых нарушений мозгового кровообращения, возникающее в остром периоде инсульта в 10–48% случаев. Развитие СпС в остром периоде ишемического инсульта (ИИ) способствует увеличению продолжительности госпитализации и стоимости лечения, частоты внутригоспитальных и отсроченных летальных исходов, вероятности инвалидизации, снижению реабилитационного потенциала.*

Цель *исследования – установить факторы риска СпС в остром периоде ИИ, её влияние на вероятность летального исхода, постинсультную динамику когнитивных функций, а также возможности медикаментозной коррекции СпС.*

Материалы и методы. *В исследование были включены 138 пациентов – 93 мужчины и 45 женщин в возрасте 71 [69,0; 74,8] год. Спутанность сознания оценивали по шкале CAM, степень выраженности спутанности сознания и её динамику – по шкале DRS. Доинсультный уровень когнитивных нарушений ретроспективно оценивали по анкете состояния когнитивных функций у пожилого пациента IQCODE. Нейропсихологическое тестирование пациентов и оценку нагрузки на родственников пациента по шкале CBS выполняли через 3, 6 и 18 мес после дебюта ИИ.*

Результаты. *Доинсультные когнитивные нарушения более 91 балла по анкете IQCODE являются фактором риска выраженной СпС в остром периоде ИИ ($p < 0,001$). Развитие выраженной СпС сопровождалось большим когнитивным дефицитом у пациента ($p < 0,05$), а также большей нагрузкой на родственников пациента ($p < 0,01$) через 3 и 6 мес. Факторами риска летального исхода в течение 18 мес после дебюта инсульта явились выраженность СпС более 15 баллов по шкале DRS (ОШ = 3,58; 95% ДИ 1,4–9,19; $p = 0,008$) и продолжительность СпС более 10 дней (ОШ = 2,56; 95% ДИ 1,03–6,38; $p < 0,044$). Применение центрального ингибитора холинэстеразы способствовало уменьшению продолжительности СпС ($p = 0,015$), улучшению когнитивных функций через 3, 6 и 18 мес ($p < 0,01$), уменьшению нагрузки на родственников пациента через 3 и 6 мес после дебюта инсульта ($p < 0,05$).*

Заключение. *СпС в остром периоде ИИ способствует развитию постинсультных когнитивных нарушений и увеличивает нагрузку на родственников пациента. Когнитивные нарушения могут быть скорректированы применением центрального ингибитора холинэстеразы.*

Ключевые слова: *спутанность сознания; ишемический инсульт; факторы риска; когнитивные нарушения; нагрузка на родственников пациента; центральный ингибитор холинэстеразы*

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Introduction

In the International Classification of Diseases (ICD-10), stroke-associated delirium is classified as “Delirium not induced by alcohol and other psychoactive substances” (F05), and in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-V), as “Delirium Not Otherwise Specified” (780.09) and is defined as an etiologically nonspecific organic cerebral syndrome characterized by concurrent disturbances of consciousness and attention, perception, thinking, memory, psychomotor behavior, emotion, and the sleep-wake cycle [1, 2]. Delirium is a common complication of acute stroke

with an incidence estimated to be between 10 and 48% in the acute phase [3–6]. This wide range of delirium incidence might be explained by considerably different diagnostic approaches and algorithms.

Delirium in the acute phase of stroke is associated with longer hospital stays and increased costs-of-illness, higher in-hospital and long-term mortality, increased risk for disability, and reduced potential for post-stroke rehabilitation [5, 7–10]. There are data indicating that acute stroke patients with delirium are at higher risk of post-stroke cognitive decline [11, 12]. Stroke sequelae have a negative impact on the health of caregivers, reducing their labor potential and compromising social and economic participation in the life of their communities [13].

Delirium is associated with predisposing – anamnestic and social – factors, and precipitating factors, or triggers, which occur during a hospital stay. In neurological patients, the most relevant predisposing factors are advanced age, neurodegenerative diseases, and substance use disorders. The most relevant precipitating factors are meningitis, acute renal failure, or intracranial hemorrhage [14]. Stroke, as an independent risk factor for delirium, exacerbates the effects of the other factors [15–17]. The modern approach to delirium prevention and management suggests a focus on reducing precipitating risk factors, creating comfortable hospital conditions, and pharmacological treatment. To date, central cholinergic deficiency is the leading hypothesized mechanism for delirium [18]. The acetylcholinesterase inhibitor rivastigmine has been shown to be effective [19, 20] and safe [21, 22] in the treatment of stroke patients with delirium.

The **aim** of this study is to identify delirium risk factors in acute stroke patients, to study the possible effects of delirium on mortality rates and post-stroke cognitive functioning changes, and to review treatment options for this condition.

Materials and methods

This prospective study included 138 acute stroke patients (93 males and 45 females) with a mean age of 71 [69.0; 74.8] years and was conducted in three multispecialty hospitals from 2009 to 2024.

Inclusion criteria:
- age > 18 years;
- hyper-acute stroke;
- normal level of consciousness at screening.

Non-inclusion criteria:
- speech impairment, decreased consciousness level, decreased muscle strength, and apraxia, preventing neuropsychological testing;
- history data indicating that the delirium might be a result of alcohol abuse or medication withdrawal;
- reperfusion therapy for acute ischemic stroke;
- previous neurometabolic therapy (including cholinergic agents)
- concomitant disease with life expectancy of less than 1 year;
- previously diagnosed mental illness;
- previously diagnosed brain tumor,
- demyelinating disease of the central nervous system, or epilepsy;
- history of head injury or neurosurgery in the past three years;
- pregnancy;
- clinically significant anxiety or depression;
- for the combined therapy subgroup: contraindications to rivastigmine.

Forty-four non-delirious stroke patients (30 males and 14 females with a mean age of 70 [68.8; 73.2] years) comprised the control group.

Delirium was diagnosed using the Confusion Assessment Method (CAM) [23], an accurate diagnostic algorithm for detecting delirium based on four features:
1) acute onset and fluctuating course;
2) inattention;
3) disorganized thinking;
4) altered level of consciousness.

A diagnosis of delirium requires the presence of features 1, 2, and either 3 or 4. The Delirium Rating Scale (DRS) was used for initial assessment and repeated measurements of delirium severity [24]. Severe delirium (DRS score > 11) in the acute phase of stroke persisting for at least 24 hours was used as a cut-off criterion. In all of the patients, the diagnosis of delirium was verified by a psychiatrist. Ninety-four patients (63 males and 31 females with a mean age of 72 [69; 76] years) with severe delirium were included in the confusion group.

All of the patients received a standard therapy for stroke. The confusion group was divided into two subgroups depending on the therapy administered:
- the standard therapy subgroup (n = 55): delirious stroke patients receiving standard therapy;
- the combined therapy subgroup (n = 39): delirious stroke patients receiving standard therapy + rivastigmine (central acetylcholinesterase inhibitor).

Rivastigmine solution was administered at a target dose of 9–12 mg/day (the route of administration was subsequently switched to transdermal therapeutic system) for 14–25 days (until the hospital discharge).

If the patient became too agitated, haloperidol was administered at a dose of 0.75–5.00 mg/day orally, intravenously, or intramuscularly. The haloperidol dose was reviewed daily by an attending psychiatrist, taking into account the patient's age and severity of delirium.

Pre-stroke cognitive decline was retrospectively assessed using the IQCODE score [25] with the following cut-offs:
- ⩽ 78 – no data indicating pre-stroke cognitive decline;
- 79–103 – moderate pre-stroke cognitive decline;
- ⩾ 104 – pre-stroke cognitive decline up to dementia.

Neuropsychological tests, including the mini–mental state examination (MMSE), the clock drawing test, and the frontal assessment battery (FAB) to screen for frontotemporal

dementia, the 5-word memory test, were performed during the hospital stay and at 3, 6, and 18 months post-stroke onset. The caregiver burden was assessed using CBS (Caregiver Burden Scale) [26] at 3, 6, and 18 months post-stroke onset.

The CBS comprises five factors: general strain, social isolation, frustration, disappointment, emotional involvement, and environment. Depending on the sum score, the results are evaluated as follows:
- 0–21: no to mild burden;
- 21–40: mild to moderate burden;
- 41–60: moderate to severe burden;
- 61–88: severe burden.

The data were analyzed using R.v.4.3.0 and Statistica for Windows v. 10.0 software. The cut-off values were determined using the decision tree analysis. The effects of the calculated cut-offs were determined using an odds ratio. The quantitative variables were compared using the Wilcoxon T-test, and the binary variables were compared using the Fisher's exact test. For contingency tables larger than 2 × 2, a Monte Carlo approximation to Fisher's exact test was used. The false discovery rate (FDR) in multiple comparisons was adjusted with the Benjamini and Yekutieli procedure. Descriptive group statistics are represented as a mean (standard deviation) and a median [Q_{25}; Q_{75}] for the quantitative variables and 95% Clopper-Pearson confidence intervals for binary variables. Inter-group correlations were analyzed using a Spearman's ρ coefficient. The effects of some baseline parameters on the delirium progression, duration, and mortality rates were assessed using logistic regression analysis. Statistical significance of the inter-group differences was determined by the presence of either a 0 (for a difference) or a 1 (for a ratio) in the CI, or by comparing the calculated *p*-value with a cut-off $p = 0.05$.

Results

There were no statistically significant differences in the frequency of stroke subtypes according to the Trial of ORG 10172 in Acute Stroke Treatment (TOAST) classification between patients in the control, standard therapy, and combined therapy groups.

Logistic regression analysis revealed no correlation between the patient's sex and a stroke subtype, and the likelihood to develop delirium. No significant correlation was found between the incidence of delirium and the location and volume of brain infarction.

The IQCODE scores for retrospective assessment of pre-stroke cognitive decline in the confusion group were higher than those in the control group: 93 [89; 99.8] vs 86 [83; 91.2], respectively ($p < 0.001$). The IQCODE cut-off value of 91 for the likelihood of severe delirium in the acute phase of stroke was determined using the decision tree analysis. The odds ratio for the scores > 91 was 5.06 (95% CI 2.72–11.26; $p < 0.001$). We also detected a significant correlation between IQCODE scores and duration of severe delirium in the acute phase of stroke (Spearman's $\rho = 0.24$; $p = 0.018$).

In the combined therapy and standard therapy subgroups, IQCODE scores (93 [86.0; 95.0] and 93 [90.0; 100.0], respectively) and DRS scores 14 [13; 14] and 13 [12; 14], respectively) were similar ($p > 0.05$). At the same time, the duration of delirium in the combined therapy subgroup was less than that in the standard therapy subgroup: 7 [4; 9] and 8 [5; 12] days, respectively ($p = 0.015$).

Neuropsychological assessment at 3 and 6 months revealed greater cognitive decline in post-stroke patients with severe delirium in the acute phase of stroke ($p < 0.05$). Compared with the standard therapy subgroup, neuropsychological tests in the combined therapy subgroup yielded greater MMSE and FAB scores at 3 and 6 months and greater FAB scores at 18 months ($p < 0.01$) (Figures 1 and 2).

Analysis of the CBS scores showed greater caregiver burden at 3 and 6 months post-stroke onset in the confusion group compared with the control group ($p < 0.01$), and less caregiver burden in the combined therapy subgroup compared with the standard therapy subgroup ($p < 0.05$; Figure 3).

Mortality rates during the follow-up period were 30.8% (12 [39]) in the combined therapy subgroup, 45.5% (25 [55]) in the standard therapy subgroup, and 27.3% (12 [44]) in the control group ($p > 0.05$). The cut-offs for increased likelihood of death at 18 months post-stroke onset determined by the decision tree analysis are DRS > 15 and duration of delirium > 10 days. The odds ratio for death likelihood at 18 months post-stroke with a DRS > 15 was 3.58 (95% CI 1.40–9.19; $p = 0.008$), with a duration of delirium > 10 days was 2.56 (95% CI 1.03–6.38; $p < 0.044$).

Frequency of stroke subtypes based on the TOAST classification, *n* (%)

IS subtype	All patients (*n* = 138)	Standard therapy group (*n* = 55)	Combined therapy group (*n* = 39)	Control group (*n* = 44)
Atherothrombotic	38 (27.5%)	17 (30.9%)	9 (23.1%)	12 (27.3%)
Cardioembolic	49 (35.5%)	22 (40.0%)	15 (38.5%)	12 (27.3%)
Lacunar	5 (3.6%)	1 (1.8%)	1 (2.6%)	3 (6.8%)
Other specified etiology	7 (5.1%)	1 (1.8%)	2 (5.1%)	4 (9.1%)
Unspecified etiology	39 (28.3%)	14 (25.5%)	12 (30.8%)	13 (29.5%)

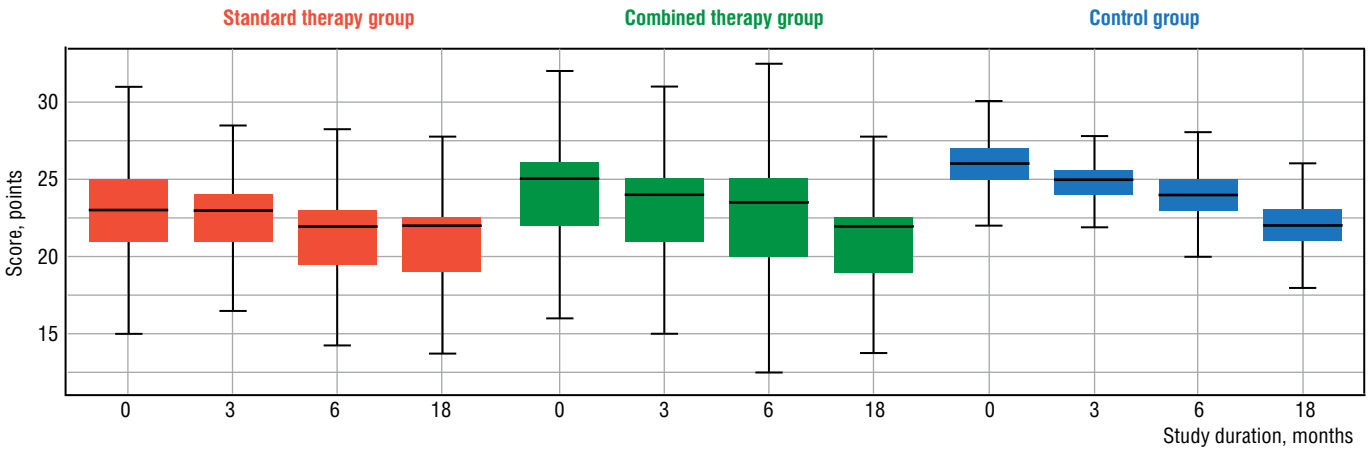

Fig. 1. MMSE scores.

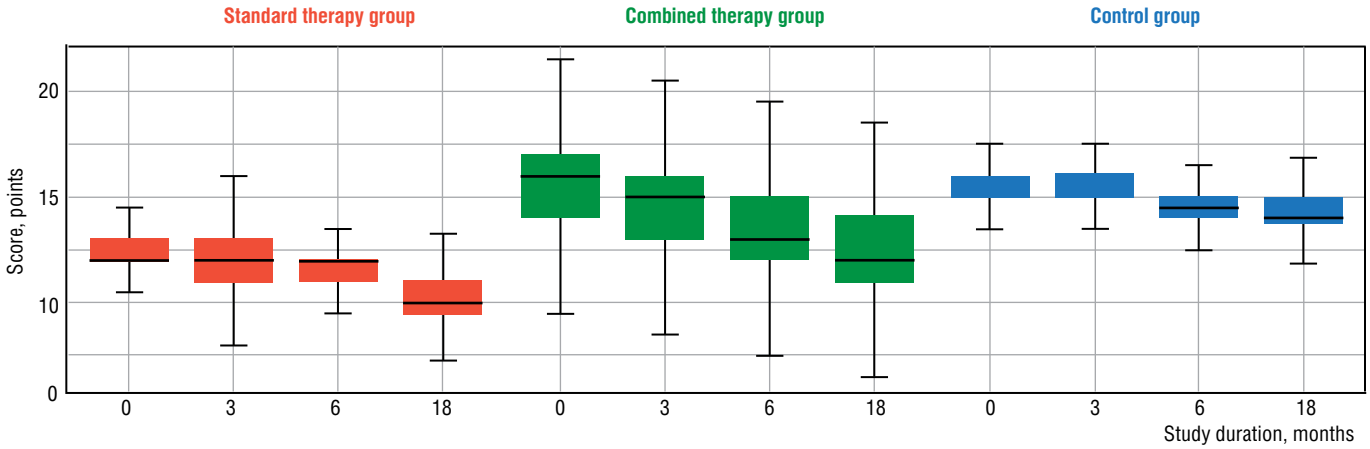

Fig. 2. FAB scores.

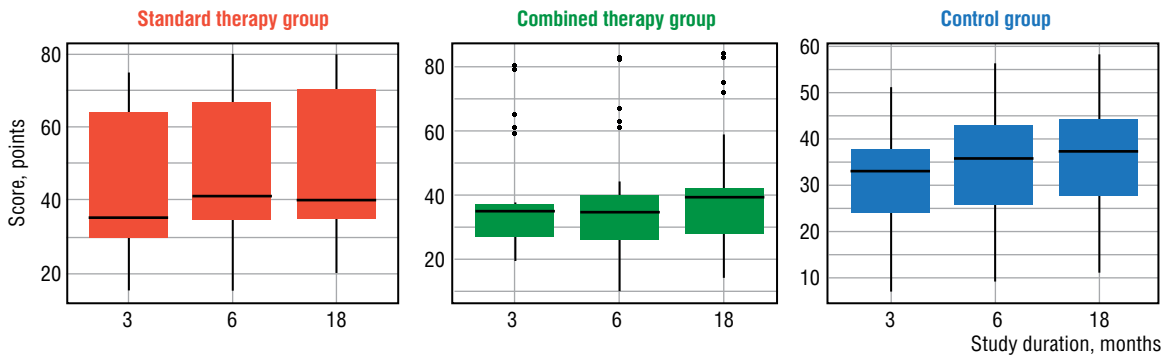

Fig. 3. CBS scores.

Discussion

This study allowed us to evaluate the significance of pre-stroke cognitive decline as a predisposing factor for severe delirium, which also leads to a longer duration of delirium in acute stroke patients. Neither patient's sex nor stroke subtype correlated with the likelihood of delirium onset.

Severe delirium was associated with greater cognitive decline in patients ($p < 0.05$) and greater caregiver burden at 3 and 6 months ($p < 0.01$). DRS > 15 and duration of delirium > 10 days were risk factors for the likelihood of death within 18 months post-stroke.

Treatment with central acetylcholinesterase inhibitor rivastigmine resulted in decreased duration of delirium ($p = 0.015$),

preserved cognitive function at 3, 6, and 18 months post-stroke ($p < 0.01$), and milder caregiver burden at 3 and 6 months post-stroke ($p < 0.05$). The available evidence on the clinical use of rivastigmine in patients with dementia of various etiologies, including those with cognitive decline in the early post-stroke period, suggests that rivastigmine therapy is appropriate for post-stroke patients, especially those with delirium in acute stroke.

Conclusion

Delirium in the acute phase of stroke contributes to post-stroke cognitive decline and increases the caregiver burden. This condition can be managed with central acetylcholinesterase inhibitors. The study results demonstrate the importance of timely diagnosis and appropriate treatment of both the acute stroke delirium and post-stroke cognitive decline.

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Metabolic Predictors of Ischemic Stroke in Young Adults

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Abstract

Introduction. *Ischemic stroke (IS) has a tendency towards younger age of onset among working-age adults, with an increasing role of obesity in IS development. New prognostic markers affecting stroke severity and early outcomes are being sought.*

Aim*: to investigate etiology and risk factors of cryptogenic IS in working-age patients (18–50 years) and evaluate the significance of metabolic markers of obesity and hemostasis in predicting immediate disease outcomes.*

Materials and methods. *We retrospectively analyzed 343 medical records of acute stroke patients aged 18–50 years using clinical, laboratory, and imaging findings and calculated risk levels.*

Results. *Obesity was observed in more than half (51.3%) of the patients. Early atherosclerotic changes in the vessel wall were detected in 62.26% of the cases. Worse immediate stroke outcomes were associated with all obesity parameters: body mass index (r = 0.48), waist circumference (WC) (r = 0.43), waist-to-hip ratio (WHR) (r = 0.52), levels of glucose (r = 0.47), C-reactive protein (r = 0.34), hematocrit (r = 0.41), high-density lipoproteins (r = –0.32), von Willebrand factor (r = 0.58), fibrinogen (r = 0.66), FVIII (r = 0.50), D-dimer (r = 0.50), and ADP-induced platelet aggregation (r = 0.41). Stroke severity was found to correlate with levels of triglycerides (r = 0.57), low-density lipoproteins (r = 0.35), von Willebrand factor (r = 0.55), fibrinogen (r = 0.46), coagulation factor VIII (r = 0.63), D-dimer (r = 0.39), antithrombin III (r = 0.39), and WHR (r = 0.53). Receiver operating characteristic curves revealed triglyceride-glucose index to be a predictor of worse early outcome (area under the curve, 0.66; threshold, 4.7), including in terms of WC (0.68 and 497.6, respectively) or stroke severity (0.63 and 4.7, respectively).*

Conclusion. *IS in young adults is accompanied by impaired metabolism, affecting the disease outcome. Indices including glucose, triglycerides, and the obesity status can play a role in predicting the stroke severity in young adults, among others.*

Keywords: *stroke; working age; obesity; prothrombogenic state; metabolic indices*

Ethics approval. The study was conducted with the informed consent of the patients. The research protocol was approved by the Ethics Committee of the Novosibirsk State Medical University (protocol No. 09/23, September 04, 2023).

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Метаболические предикторы течения ишемического инсульта у молодых

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Аннотация

Введение. *Ишемический инсульт имеет тенденцию к омоложению у лиц трудоспособного возраста, при этом возрастает роль ожирения, способствующего реализации ИИ. Ведётся поиск новых прогностических маркеров, влияющих на тяжесть течения инсульта и ранние исходы.*

Цель *исследования: изучить этиологию и факторы риска ишемического инсульта неустановленной этиологии у пациентов трудоспособного возраста (18–50 лет), оценить прогностическую значимость метаболических маркеров, отражающих статус ожирения, гемостаза на ближайшие исходы заболевания.*

Материалы и методы. *Проведён ретроспективный анализ 343 историй болезни пациентов в возрасте 18–50 лет в острейшем периоде нарушения мозгового кровообращения с использованием клинико-лабораторных, инструментальных показателей, с вычислением уровня риска.*

Результаты. *Ожирение встречается более чем у половины (51,3%) пациентов. У 62,26% выявлены начальные атеросклеротические изменения стенки сосуда. Худшие ближайшие исходы инсульта были ассоциированы со всеми показателями статуса ожирения: индексом массы тела (r = 0,48), объёмом талии (ОТ; r = 0,43), соотношением ОТ и объёма бёдер (r = 0,52), уровнями гликемии (r = 0,47), С-реактивного белка (r = 0,34), гематокрита (r = 0,41), липопротеидов высокой плотности (r = –0,32), фактором фон Виллебранда (r = 0,58), фибриногеном (r = 0,66), FVIII (r = 0,50), D-димером (r = 0,50), агрегацией тромбоцитов с АДФ (r = 0,41). В отношении тяжести инсульта выявлены зависимости от уровня триглицеридов (r = 0,57), липопротеидов низкой плотности (r = 0,35), фактора фон Виллебранда (r = 0,55), фибриногена (r = 0,46), фактора свёртывания VIII (r = 0,63), D-димера (r = 0,39), антитромбина III (r = 0,39), соотношения ОТ и объема бёдер (r = 0,53). По итогам построения ROC-кривых триглицерид-глюкозный индекс (ТГИ) определён как предиктор худшего раннего исхода (для ТГИ площадь под кривой 0,66, порог – 4,7), в том числе с учётом ОТ (0,68 и 497,6 соответственно) или тяжести течения (0,63 и 4,7 соответственно).*

Заключение. *Ишемические инсульты у лиц молодого возраста сопровождаются нарушением метаболического здоровья, которое влияет на исход заболевания. Индексы, включающие глюкозу, триглицериды и статус ожирения, могут иметь предиктивную роль при прогнозировании течения инсультов, в том числе у молодых.*

Ключевые слова: *инсульт; трудоспособный возраст; ожирение; протромбогенное состояние; метаболические индексы*

Этическое утверждение. Исследование проводилось при добровольном информированном согласии пациентов. Протокол исследования одобрен этическим комитетом Новосибирского государственного медицинского университета (протокол № 09/23 от 04.09.2023).

Источник финансирования. Авторы заявляют об отсутствии внешних источников финансирования при проведении исследования.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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Introduction

Stroke is one of the most critical medical and social issues today, resulting in severe disability and mortality among working-age adults in most high-income countries [1]. There is a current trend towards a decreasing number of cerebrovascular accidents in persons aged 65 to 84 years (–28.5%) and those aged 85 years or older (–22.1%); however, this trend contrasts with an increasing number of strokes among people aged 25 to 44 years (+43.8%) [1]. Statistically, adults aged 18 to 50 years account for 10% to 15% of all strokes worldwide. The working-age population accounts for 10% to 20% of all cases of ischemic stroke (IS) [2].

Rare diseases are usually linked to stroke in young adults; however, differential diagnosis and diagnostic workup do not always result in an identifiable cause. In such cases, thorough assessment of traditional risk factors and an increased alarm about risks they might pose are disregarded in favor of searching for unique, rare causes of stroke.

The traditional risk factors for cerebrovascular diseases, such as hypertension, atherosclerosis, dyslipidemia, and type 2 diabetes, are characteristic of patients older than 65 years. Nevertheless, recent studies demonstrated that these risk factors and their combinations tended to contribute to younger age at onset and premature age-related diseases in young adults [3]. Findings of C.A. Stack et al. show that in young patients, the most prevalent risk factors are hyperlipidemia (60%), smoking (44%), and hypertension (39%) [2].

Type 2 diabetes, hypertension, and dyslipidemia were found to be one of the most common comorbidities (82.7%) among IS patients younger than 50 years, with dyslipidemia being a potentially progressive risk factor [4, 5].

Risk factors for cardiovascular diseases become more common with each decade of life and are significantly associated with stroke risk by the third decade [6]. Traditional modifiable vascular risk factors can become even more significant due to young people's lifestyle [7].

The increasing percentage of obesity and overweight as confounding risk factors for cardiovascular diseases, mostly among young adults, is concerning. Over the past decades, obesity has risen to epidemic levels worldwide [3, 8, 9].

Total and visceral obesity were shown to be independent risk factors for cerebrovascular diseases after adjustment for hypertension, dyslipidemia, and hyperglycemia [10]; however, there are few studies highlighting relationships between obesity and IS in young persons [10, 11].

Both body mass index (BMI) and anthropometric measures, indicating central obesity, were found to be risk factors for stroke [12]. Waist circumference (WC), hip circumference (HC), and waist-to-hip ratio (WHR) are more strongly associated with stroke [10]. WC as a marker was found to have a significant association with a 28%–78% increase in the IS risk [13, 14]. The INTERSTROKE study revealed that elevated WHR increases the risk of all stroke types, including IS and hemorrhagic stroke, in young adults [15, 16]. The validity of WHR calculation has been shown in a group of patients with chronic cerebrovascular diseases compared with a control group with no history of these diseases [17].

The triglyceride-glucose index (TyG index) and combined TyG-related parameters (TyG-BMI and TyG-WC) have been suggested as simple and clinically useful surrogate markers for insulin resistance and metabolic health [18, 19, 20].

Combined with obesity indices, the TyG index becomes more informative for assessment of cerebrovascular accident risks and outcomes [21, 22]. The obesity impact on atherosclerosis is mediated by chronic inflammation, hyperlipidemia, and endothelial dysfunction [23]. In obese individuals, procoagulant and hypofibrinolytic factors were observed to predominate in the plasma [24], thus contributing to IS development. Increased prothrombotic state can remain latent for quite a long time and compensated due to the athrombogenic properties of the vessel wall [25].

Along with endothelial dysfunction, platelet activation and hypercoagulability are key processes in the development of a thrombotic cerebrovascular event [26].

Therefore, we hypothesized that obesity aggravates the development, severity, and immediate outcomes of IS, especially in cryptogenic cases.

The **aim** of the study was to investigate the etiology and risk factors of cryptogenic IS in working-age patients (18–50 years) and evaluate the prognostic significance of markers reflecting the status of obesity, hemostasis and insulin resistance indices on immediate disease outcomes.

Materials and Methods

We retrospectively analyzed 343 medical records of patients aged 18–50 (42.18 ± 5.20) years (of them, 180 men and 171 women) with acute IS (IS/transient ischemic attack) treated at the Regional Vascular Center No. 2 in 2021–2024 (Fig. 1).

Inclusion criteria for medical record analysis:
- age at admission (18–50 years);
- established and confirmed IS by clinical data, computed tomography (CT) and/or CT angiography and/or magnetic resonance imaging of the brain (I63.0–I64.9).

Exclusion criteria:
- established and confirmed intracranial hemorrhages of any etiology;
- venous thrombosis;
- age at onset over 50 years;
- established etiology of IS according to TOAST or SSS-TOAST classification.

We analyzed the following risk factors:
- smoking;
- regular alcohol consumption;
- elevated BMI;
- comorbidities: type 2 diabetes, hypertension.

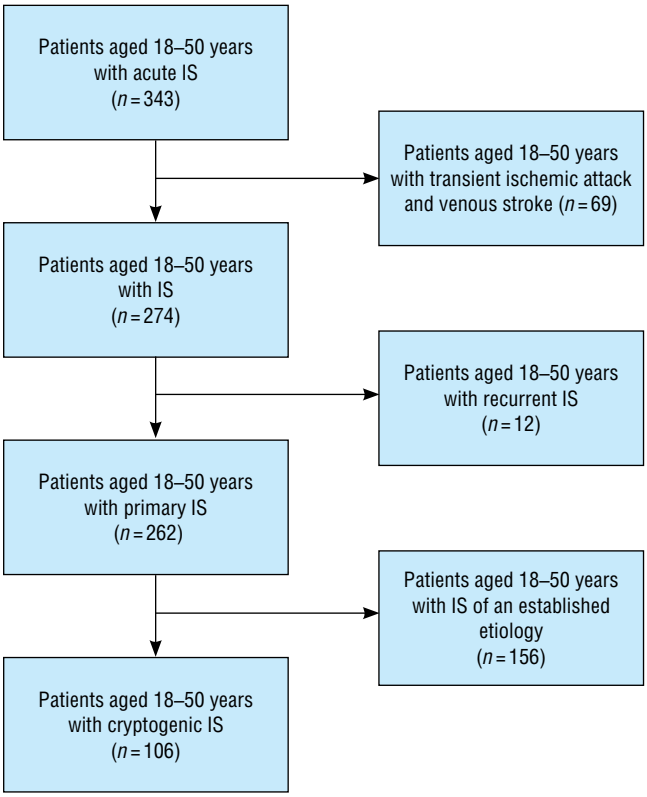

Fig. 1. Study design.

We studied biochemical analytes:
- fasting blood glucose;
- C-reactive protein;
- uric acid;
- total cholesterol;
- low-density lipoproteins;
- high-density lipoproteins;
- triglycerides;
- hemostasis (fibrinogen, ADP-induced platelet aggregation);
- D-dimer;
- Factor VIII;
- von Willebrand factor;
- Antithrombin III activity.

Doppler ultrasonography of brachiocephalic arteries was used to assess signs of atherosclerosis of the common and internal carotid arteries or hemodynamically significant stenosis.

Early outcomes were determined using the modified Rankin Scale (mRS) at discharge or 14 days after the disease onset. The National Institutes of Health Stroke Scale (NIHSS) was used to assess and group patients with neurologic deficits on admission: mild stroke (NIHSS $\leqslant$ 4) and moderate-to-severe stroke (NIHSS > 4).

Body weight, height, WC, and HC were measured without shoes and with light clothing.

BMI was calculated as follows:

$$\text{BMI} = \text{weight (kg)}/\text{height (m)}^2.$$

Data were interpreted according to World Health Organization recommendations:
- BMI $\geqslant$ 25, overweight;
- BMI $\geqslant$ 30, obesity [16].

Metabolic indices were calculated using the formulas:

$$\text{TyG index} = \text{Ln}\,[(\text{TG (mmol/L)} \times 88.495575 \times \text{FPG (mmol/L)} \times 18.018018)]/2;$$

where FPG is fasting plasma glucose;

$$\text{TyG index-BMI} = \text{TyG index} \times \text{BMI};$$

$$\text{TyG index-WC} = \text{TyG index} \times \text{WC}.$$

Statistical analysis was conducted using Prism v. 10 (GraphPad). Data are presented as mean (*M*) and standard deviation (*SD*). The Pearson test was used for normally distributed data, and the Spearman rank correlation coefficient was calculated for not normally distributed data. To assess the diagnostic accuracy of the model, a receiver operating characteristic curve (ROC curve) was drawn; the area under the curve (AUC) and standard deviation (*SD*) were calculated. The likelihood ratio (LR) test values for each point on the curve were also given. The optimal cutoff point (threshold) was selected based on the LR parameter. Additionally, the Youden index (J statistic) was used to validate a threshold.

Results

As per the aim of the study, we analyzed data from the patients with cryptogenic IS in terms of traditional vascular risk factors and obesity status (Table 1, Fig. 2).

More than half of the patients had obesity (74.4%) (including obesity in 51.3%); 41.5% of the participants had hypertension. Behavioral factors, such as smoking (16.0%) and alcohol consumption (8.5%), were less common. The proportion of patients with type 2 diabetes was 5.7%.

We correlated the obesity status, blood analytes, and acute stroke severity in the acute period (NIHSS, scores) and early outcome (mRS) by the end of acute IS in the studied group of the patients (Fig. 2). The severity of early functional impairment (mRS) significantly and directly correlated with the levels of glucose ($r = 0.47$), C-reactive protein ($r = 0.34$), hematocrit ($r = 0.41$), whereas an inverse relationship was found with the level of high-density lipoproteins ($r = -0.32$). Correlations between the IS severity (assessed using NIHSS) and the level of triglycerides ($r = 0.57$), low-density lipoproteins ($r = 0.35$) were revealed (Fig. 2).

Atherosclerotic changes in the vessel wall were detected in the majority of patients (62.26%); atherosclerotic plaques in the internal carotid artery ipsilateral to the lesion focus were found in 17.9% of the cases (Table 2).

During assessment of anthropometric measures and hemorheology and hemostasis parameters in the study sample, we found significant correlations between BMI and such parameters as the level of von Willebrand fac-

Table 1. General characteristics of the patients with cryptogenic IS

Parameter	Value
Mean age, years (*M* ± *SD*)	42.18 ± 5.20
Sex:	
male, *n* (%)	69 (65.09%)
female, *n* (%)	37 (34.9%)
Weight, kg (*M* ± *SD*) (min–max)	89.8 ± 21.6 (53–180)
BMI, kg/m^2 (*M* ± *SD*) (min–max)	30.19 ± 6.3 (19.00–53.17)
Normal weight, *n* (%)	27 (25.6%)
Overweight, *n* (%)	25 (23.1%)
Class 1–3 obesity, *n* (%)	54 (51.3%)
WC, cm (*M* ± *SD*)	103.8 ± 19.51
WHR (*M* ± *SD*)	1.011 ± 0.11
TyG index, *M* ± *SD*	4.76 ± 0.26
TyG index-BMI, *M* ± *SD*	140.8 ± 34.72
TyG index-WC, *M* ± *SD*	492.7 ± 108.2
NIHSS score ≤4 (minor stroke), *n* (%)	41 (38.7%)
NIHSS score > 4 (moderate or severe stroke), *n* (%)	65 (61.3%)
Presence of arterial hypertension, *n* (%)	44 (41.5%)
Type 2 diabetes mellitus, *n* (%)	6 (5.7%)
Tobacco smoking, *n* (%)	17 (16.0%)
Alcohol abuse, *n* (%)	9 (8.5%)

Table 2. Characteristics of laboratory and imaging findings

Analyte	Value
Glucose, mmol/L (*M* ± *SD*)	6.4 ± 2.0
C-reactive protein, mg/L (*M* ± *SD*)	7.4 ± 2.3
Uric acid, mmol/L (*M* ± *SD*)	338.2 ± 110.8
Cholesterol, mmol/L (*M* ± *SD*)	6.2 ± 1.4
Low-density lipoprotein cholesterol, mmol/L (*M* ± *SD*)	3.70 ± 1.14
High-density lipoprotein cholesterol, mmol/L (*M* ± *SD*)	1.18 ± 0.50
Triglycerides, mmol/L (*M* ± *SD*)	1.5 ± 0.6
Hematocrit, % (*M* ± *SD*)	41.0 ± 4.9
Fibrinogen, g/L (*M* ± *SD*)	416.5 ± 117.7
ADP-induced platelet aggregation, % (*M* ± *SD*)	82.7 ± 3.7
D-dimer, ng/mL (*M* ± *SD*)	262.8 ± 112.5
Factor VIII, % (*M* ± *SD*)	114.8 ± 51.6
von Willebrand factor, % (*M* ± *SD*)	159.40 ± 20.54
Antithrombin III activity, % (*M* ± *SD*)	99.3 ± 8.3
Atherosclerosis, primary changes in the vessel wall, % (*n*)	62.3% (*n* = 66)
Atherosclerosis, <50% brachiocephalic artery stenosis, % (*n*)	17.9% (*n* = 19)

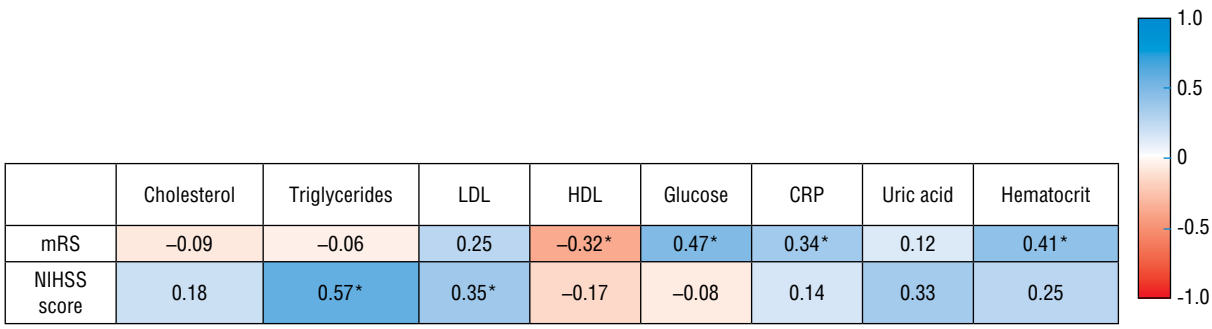

Fig. 2. Correlation between clinical and laboratory parameters.
*$p < 0.05$.

tor ($r = 0.51$) and fibrinogen ($r = 0.71$). WC has a significant correlation with fibrinogen levels ($r = 0.65$). WHR was directly correlated with the levels of factor VIII ($r = 0.35$), von Willebrand factor ($r = 0.36$), fibrinogen ($r = 0.58$), and antithrombin III ($r = 0.44$) (Fig. 3).

We found significant correlations between the stroke severity (NIHSS score) and the levels of factor VIII, von Willebrand factor, fibrinogen, antithrombin III, and D-dimer (Fig. 3). The mRS results were in direct and statistically significant correlation with the levels of factor VIII,

	Factor VIII	von Willebrand factor	Fibrinogen	Antithrombin III	D-dimer	ADP-induced platelet aggregation	BMI	WC	WHR	mRS	NIHSS score
BMI	0.19	0.51*	0.71*	0.16	0.05	0.22	1.00	0.65*	0.49*	0.48*	0.31
WC	0.05	0.26	0.65*	0.15	–0.04	0.07	0.65*	1.00	0.71*	0.43*	0.37
WHR	0.35*	0.36	0.58*	0.44*	–0.05	0.17	0.49*	0.71*	1.00	0.52*	0.53*
mRS	0.50*	0.58*	0.66*	0.28	0.50*	0.41*	0.48*	0.43*	0.52*	1.00	0.61*
NIHSS score	0.63*	0.55*	0.46*	0.39*	0.39*	0.23	0.31	0.37	0.53*	0.61*	1.00

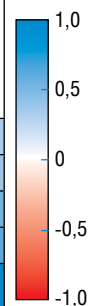

Fig. 3. Correlation between clinical and hemorheologic parameters.
*$p < 0.05$.

	TyG index	TyG index-BMI	TyG index-WC
mRS	0.44*	0.37*	0.43*
NIHSS score	0.33*	0.09	0.24

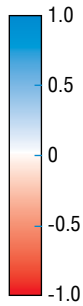

Fig. 4. Correlation of obesity status parameters and metabolic indices with early outcomes and cryptogenic IS.
*$p < 0.05$.

von Willebrand factor, fibrinogen, D-dimer, and ADP-induced platelet aggregation.

The results of early functional impairment were in direct correlation with BMI, WC, and WHR (Fig. 3). Significant correlations of the IS severity were found only with WHR.

The correlation between the stroke severity and early IS outcomes with metabolic indices was assessed. The TyG index as well as its combinations with anthropometric measures (TyG index-BMI, TyG index-WC) were found to be in a strong direct correlation with early outcomes (Fig. 4). Thus, they can be considered as a possible prognostic marker. In the case of the indices' impact on the IS severity, a direct relationship with TyG index was shown; however, TyG index-BMI and TyG index-WC did not have a strong correlation with the IS severity (Fig. 4).

The ROC analysis was performed to evaluate the prognostic role of the metabolic indices in relation to the stroke severity and outcomes (Table 4, Fig. 5).

According to the ROC curve analysis, AUCs over 0.5 were observed in relation to early neurological outcomes of IS (assessed using mRS) for all 3 indices, but only TyG index and TyG index-WC were statistically significant. TyG index >4.7 and TyG index-WC >497.6 can be considered predictors of more unfavorable outcomes in IS. TyG index-BMI with an AUC <0.8 and nonsignificant *P* value indicates its failure as a predictor of IS early outcomes. A predictor of more severe IS (assessed using NIHSS) is a TyG index >4.7.

Discussion

Stroke in working-age young adults is a serious medical and social issue.

Table 4. Characteristics of the ROC curve parameters

Model	mRS			NIHSS score		
	TyG index	TyG index-WC	TyG index-BMI	TyG index	TyG index-WC	TyG index-BMI
AUC	0.66	0.68	0.62	0.63	0.53	0.59
SD	0.075	0.06	0.095	0.065	0.095	0.09
95% CI	0.5165–0.8110	0.5641–0.7959	0.4383–0.8117	0.5016–0.7568	0.3483–0.7214	0.4102–0.7628
Threshold	4.7	497.6	144.8	4.7	489.4	138.6
Sensitivity, %	73.9	68.75	62.5	50	50.0	53.1
Specificity, %	64.4	62.07	75.9	73.08	76.9	69.2
LR	2.079	2.589	2.589	1.857	2.167	1.727
Youden index	0.38	0.31	0.38	0.23	0.27	0.22
P	0.03	0.05	0.17	0.04	0.7	0.36

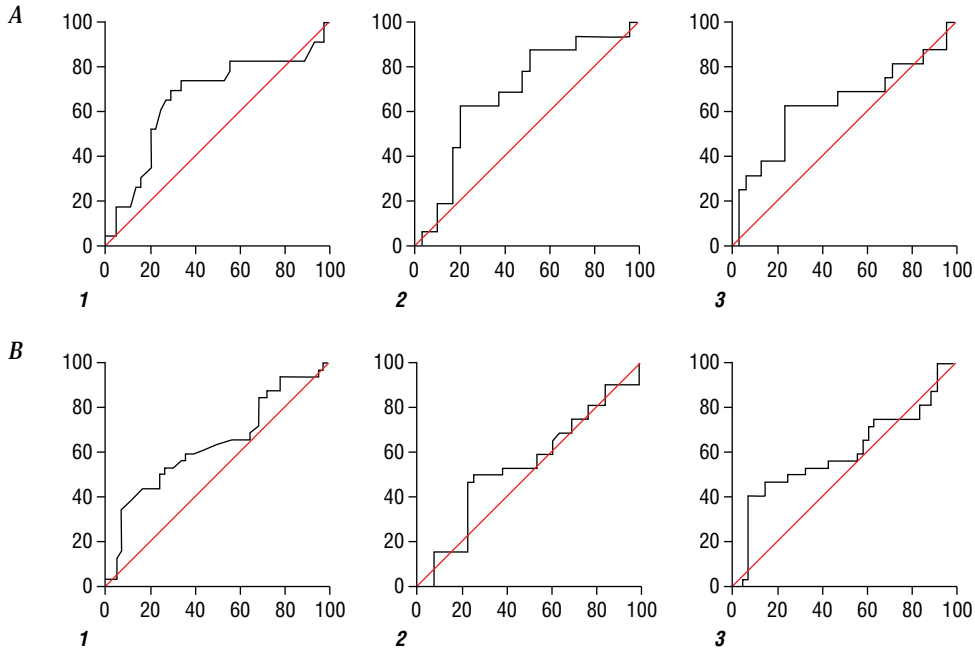

Fig. 5. ROC curves for TyG index, TyG index-WC, and TyG index-BMI as predictors of early outcomes (assessed using mRS) and IS severity (assessed using NIHSS).
A – early outcomes assessed using mRS; *B* – severity assessed using NIHSS.
x axes, 1 – specificity; y axes, sensitivity.
1 – TyG index; *2* – TyG index-WC; *3* – TyG index-BMI.

In the context of age, obesity is associated with an increased risk of stroke in all periods of life, but to the greatest extent in young patients [26].

The concept of cerebral metabolic health, concerning the unfavorable mutual influence of cerebrovascular disease and symptoms of metabolic syndrome, lays the foundation for consideration and differentiation of factors of cerebrovascular disease progression to prevent and target them [26–32].

Central obesity combined with metabolic disorders are considered markers of metabolic disorders: elevated WC in addition to hypertension, dyslipidemia, and hyperglycemia [17].

Our study in working-age individuals with an unspecified pathogenetic subtype of IS evidenced that more severe early disease outcomes had a strong association with BMI, WC, WHR, TyG index and its combinations with anthropometric measures. Furthermore, high levels of glucose, C-reactive protein, high-density lipoprotein, and hematocrit were statistically significant factors influencing early outcomes.

Systemic inflammation, including the one associated with obesity, triggers hemostasis system disorders: endothelial dysfunction and platelet hyperaggregation. Changes in the levels of markers of platelet activity and endothelial function (ADP-induced platelet aggregation, von Willebrand factor, factor VIII, fibrinogen, D-dimer) lead to increased blood thrombogenicity, aggravating cerebral ischemia, and consequently resulting in a more severe stroke and disability of the young patient [33].

In our study, the study sample was not homogeneous in terms of body weight and BMI. However, overweight and obesity in >70% of the IS patients, the obesity status values and their correlation with the hemostasis assessment results allow us to draw a conclusion about the significance of metabolic disorders associated with the adipose tissue accumulation in working-age individuals, which may elucidate the prothrombotic state of the blood. In turn, endothelial dysfunction predetermines the occurrence of structural changes in cerebral vessels and mediates the processes of atherogenesis: initial changes in the vessel wall and the development of atherosclerotic plaques.

Conclusion

Studies of various aspects of cerebral metabolic health show its significant contribution to changing the landscape of cerebrovascular disease and its age of onset. Metabolic indices can play a role in predicting the stroke severity in young adults, among others.

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Radiomics in the Differential Diagnosis of Glioblastoma under the Primary Neurooncoimaging Conditions

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Abstract

Introduction. *According to the 2021 WHO Classification of Tumors of the Central Nervous System (CNS) and the 2023 Clinical Practice Guidelines on the Drug Management of Primary CNS Cancers, the first step of molecular genetic testing to identify the morphological type and malignancy of adult-type diffuse gliomas is the detection of isocitrate dehydrogenase (IDH) mutation status. However, tumor tissue biopsy as the conventional diagnostic standard has a number of limitations that can potentially be mitigated by applying the principles of radiomics to the interpretation of magnetic resonance (MR) images.*

*The **aim** of our study is to develop a radiomics model for IDH mutation status prediction, which can be applied to primary diagnostic imaging in patients with suspected adult-type diffuse gliomas.*

Materials and methods. *We conducted a retrospective comparative statistical analysis of radiomic features extracted from 46 conventional brain MR images of the patients with adult-type diffuse gliomas and identified IDH mutation status using the Random Forest algorithm of machine learning in combination with various preprocessing methods of the source imaging data and a semi-automated LevelTracing tool used for segmentation of the regions of interest (ROI).*

Results. *The most effective combination of tools for preprocessing, segmentation, and classification was found to be ScaleIntensity, LevelTracing, and Random Forest, respectively. Using this combination, we verified the reliability of six radiomic predictors identified at the previous study stage. These features were all associated with IDH mutation status, and most of them capture texture heterogeneity in the ROIs at the voxel level. We were also able to improve the prognostic performance of our classification model up to AUC = 0.845 ± 0.089 ($p < 0.05$).*

Conclusion. *Based on a small, technically heterogeneous sample of routine MR imaging data, we developed a multiparametric model of IDH mutation status prediction in the patients with adult-type diffuse gliomas. Our conclusion is that relatively uniform preprocessing techniques based on uniform voxel intensity changes, which allow to preserve the structural detail, are feasible in clinical practice. The identified radiomic, likely voxel-based, features reflect the severity of perifocal vasogenic edema and the measure of intratumor morphological heterogeneity. We plan to assess the reproducibility of the study results using similar medical imaging data from open sources and to develop a color mapping technique for the ROIs to facilitate visual interpretation of quantitative radiomic data.*

Keywords: *adult-type diffuse gliomas; morphological heterogeneity; radiogenomics; radiomics; MRI; IDH mutation status*

Ethics approval. The study was approved by the local Ethics Committee of the Almazov National Medical Research Centre (Protocol No. 10-22, October 3, 2022).

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Радиомика в дифференциальной диагностике глиобластомы в условиях первичной нейроонковизуализации

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Аннотация

Введение. *Согласно классификации ВОЗ опухолей ЦНС 2021 г. и практическим рекомендациям по лекарственному лечению первичных опухолей ЦНС 2023 г., определение статуса изоцитратдегидрогеназы (IDH) является начальным этапом молекулярно-генетического тестирования при идентификации патоморфологических форм диффузных глиом взрослых. Однако традиционный диагностический стандарт, подразумевающий исследование биопсийного материала, обладает рядом ограничений, потенциально нивелируемых внедрением в алгоритм интерпретации традиционных магнитно-резонансных (МР) изображений принципов радиомики.*

Цель *исследования – разработка применимой в условиях первичных диагностических мероприятий радиомической модели прогнозирования IDH-статуса диффузных глиом взрослых.*

Материалы и методы. *Посредством применения метода машинного обучения Random Forest осуществляли ретроспективный сравнительный статистический анализ радиомических характеристик 46 традиционных МР-исследований головного мозга пациентов с диффузными глиомами взрослых и известным IDH-статусом в зависимости от вида предварительной обработки исходных данных визуализации с использованием полуавтоматизированного инструмента сегментации зон интереса LevelTracing.*

Результаты. *Установлена наиболее эффективная комбинация инструментов препроцессинга, сегментации и классификации – ScaleIntensity, LevelTracing и Random Forest соответственно. С её помощью верифицирована достоверность 6 выявленных на прошлом этапе исследования радиомических предикторов IDH-статуса, в большинстве являющихся характеристиками текстурной неоднородности зон интереса на воксельном уровне, а также увеличена прогностическая эффективность классификационной модели до AUC = 0.845 ± 0.089 ($p < 0.05$).*

Заключение. *Разработана мультипараметрическая предиктивная модель IDH-статуса при диффузных глиомах взрослых на основе рутинных данных МР-визуализации в условиях малой технически разнородной выборки. Сделан вывод о целесообразности использования относительно унифицированных методов предварительной обработки изображений, предполагающих равномерные изменения интенсивности вокселей с сохранной структурной детализацией. Выявленные радиомические характеристики, вероятно, на воксельном уровне иллюстрируют выраженность перифокального вазогенного отёка и феномена внутриопухолевой морфологической гетерогенности. Планируется оценка воспроизводимости полученных результатов на основе аналогичных данных медицинской визуализации из открытых источников, а также разработка методики цветового картирования зон интереса с целью привнесения элемента субъективного визуализационного анализа в процесс интерпретации количественных радиомических данных.*

Ключевые слова: *диффузные глиомы взрослых; морфологическая гетерогенность; радиогеномика; радиомика; магнитно-резонансная томография; IDH-статус*

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Introduction

Glioblastoma tends to be found in older adults, rarely < 55 yo, with the highest incidence rates among all primary CNS malignancies of 48.6% (annual incidence rate: 3.2–3.4 per 100,000 population) [1]. One-year survival rate does not exceed 13%, even in patients aged 20–44 years [2]. Glioblastoma is the most aggressive type of brain tumor. In most cases, a patient dies within 14–16 months, assuming chemotherapy and radiotherapy treatment [3].

Up to 2021, the grading of gliomas was primarily based on histological features [4]. Currently, various biomarkers are used to derive additional information valuable for the diagnosis and prognosis of the disease and impacting the treatment planning.

The first basic molecular genetic markers of primary CNS tumors are mutations in the *IDH1/IDH2* genes and 1p/19q codeletion. Detecting these markers helps narrow down the differential diagnosis options and make a definitive diagnosis [5].

According to the 2021 WHO Classification of Tumors of the CNS, IDH-wildtype (*IDH*-WT) glioblastoma, IDH-mutant astrocytoma, and IDH-mutant/1p/19q-codeleted oligodendroglioma belong to the adult-type diffuse glioma family [6, 7].

The grading of gliomas is no longer strictly histological. Astrocytomas are now graded as CNS WHO grade 2, 3, or 4; oligodendrogliomas as CNS WHO grade 2, 3; and glioblastomas are assigned to CNS WHO grade 4. In the previous classification, the grading of gliomas was based predominantly on histological features such as necrosis, microvascular proliferation, nuclear atypia, etc., while the current 2021 WHO classification of tumors of the CNS mandates genetic testing of tumor tissue and prioritizes the identified genetic features in the differential diagnosis [8].

In the adult-type diffuse glioma family, only glioblastoma is characterized by the absence of IDH mutations. Mutation in the IDH genes is the key feature of molecular diagnostics for grade 2–4 adult-type diffuse gliomas and secondary glioblastomas (grade 1 gliomas have no mutations in the *IDH* genes) [8].

The minimum scope of diagnostic assessments for a suspected glial tumor includes a 3-plane brain MRI with standard MRI pulse sequences [8]. In routine clinical practice, the diagnostic use of these data is often limited to the identification of tumor location and size. However, rapid advances in radiogenomics over the past 15 years enabled expanding the potential use of MRI data to non-invasive prediction of molecular genetic characteristics of detected neoplasms.

Radiogenomics links radiomics, an original methodology that extracts and classifies digitized, predominantly textural features of a medical image, to molecular genetics, revealing statistically significant correlation between radiomic features unavailable at routine visual interpretation and histological and molecular characteristics of the tumor [9].

If radiomics models can reach an adequate level of predictive performance, the current diagnostic paradigm requiring tissue biopsy analysis will potentially be inferior to the radiomics-based neuro-oncology imaging in a number of factors. Conventional molecular genetic testing is incomparably time-consuming, which can adversely affect patient triage. At present, stereotactic needle biopsy is the least invasive method of diffuse and deep-brain tumor verification. It is still associated with numerous complications, with intracranial hemorrhage (in 5.8% of patients) being the most frequent and life-threatening of them [10]. In the case of intratumor heterogeneity, multiple biopsies may be required, often worsening the patient's condition, whereas radiomics approach allows non-invasive evaluation of the tumor substrate.

This advantage is also of great value for the treatment planning in patients who are ineligible for radical resection of the tumor or stereotactic needle biopsy due to contraindications to surgery or anesthesia, or when the tumor is located close to functionally significant regions of the brain [11]. In addition, virtual reality diagnostic tools are cost-effective [12].

The importance of radiogenomics approach in oncology, particularly the IDH mutation status prediction in glial tumors, has significantly increased over the past decade, especially in international contexts [13, 14]. However, the high-tech neuroimaging techniques employed in many studies are not available at the initial evaluation of a patient with suspected glioma [15, 16]. Moreover, the research in this area also aims at improving the predictive performance of the developed models, not only by focusing on specialized medical image data but also by testing various methods of image preprocessing, tools for extracting radiomic features, and methods for their statistical processing. The current trend is to include clinical and anamnestic data significantly correlated with a particular tumor type (age, gender, Karnofsky Performance Status, etc.) and even the elements of the radiologist's subjective interpretation into the datasets for the predictive model training [17, 18].

The choice of datasets for model development obviously requires standardization. Otherwise, optimistic theoretical results will be unreproducible and of limited use in routine diagnostic practice.

In the four years since the last revision of the 2021 WHO classification of tumors of the CNS, a number of studies published abroad presented results showing promising performance of the predictive models. W. Rui et al. developed a model for IDH mutation status prediction using T2-FLAIR and T1FS-CE MRI pulse sequences. However, to improve the model accuracy, they included data on quantitative susceptibility mapping (QSM) into the analysis. The ROC AUC score for the model based on T2-FLAIR and for the combination of T2-FLAIR, T1FS-CE, and QSM was 0.69 and 0.88, respectively [19]. S. Zhong et al. focused on the analysis of routine MRI pulse sequence data (T1, T1FS-CE, T2). However, they also used natural language processing (NLP) models based on semantic analysis of MRI reports and other documented clinical and anamnestic information. These data were subsequently processed and incorporated into the training datasets in order to purposely increase the model predictive performance (AUC = 0.98 for IDH-mutation status

Table 1. Distribution of tumors by morphological type, malignancy grade, and IDH mutation status

Diagnosis		*n*	%
Morphological type, malignancy grade	Glioblastoma G4	24	52.2
	Oligodendroglioma G3	7	15
	Astrocytoma G2	2	4.3
	Astrocytoma G3	5	11
	Astrocytoma G4	5	11
	Oligodendroglioma G2	3	6.5
IDH mutation status	WT	24	52
	M	22	48

prediction). In our opinion, such an approach makes this model somewhat useless in the third-party medical facilities using another natural language [18].

The quality of the extracted radiomic features depends significantly on the MR image preprocessing techniques [20]. Thus, at this stage, there is a need to develop radiomics-based models to predict IDH mutation status in the patients with adult-type diffuse glioma and to standardize this process to pursue the potential applicability of the results when using these models in primary differential diagnosis.

Our study considers the results obtained during its previous stage and continues the search for the most effective preprocessing tool and an optimal classification model [21, 22]. Noteworthy, a predictive model for IDH mutation status based on the updated 2021 WHO classification of tumors of the CNS allows to rule out an entire morphologic type of tumor in a non-invasive manner already at the initial stage of the differential diagnosis: namely, primary glioblastoma IDH-WT, which is characterized by an almost twice worse prognosis compared to the IDH-M entity and by a poor response to radio- and chemotherapy [8].

The **aim** of our study is to develop a radiomics model for IDH mutation status prediction, which can be applied to primary diagnostic imaging in patients with suspected adult-type diffuse gliomas.

Materials and methods

We retrospectively analyzed primary brain MRI data yielded from 46 patients aged 18–84 years with adult-type diffuse gliomas and subsequently identified IDH mutation status. The data were retrieved from the archives of the V.A. Almazov National Medical Research Centre (n = 31) and the N.P. Napalkov Cancer Center (n = 15) for 2021–2023 (Table 1).

Inclusion criteria:
- verified primary glial tumor;
- identified IDH mutation status;
- T2-FLAIR pulse sequence data documented in the MRI report.

Non-inclusion criteria:
- a history of previous surgery in the ROI, chemo- and radiotherapy;
- brain malformations;
- artifacts compromising interpretation of tissue transformations in the ROI.

Table 1 shows that the majority of the neoplasms were grade 4 glioblastomas, since, according to the 2021 WHO classification of tumors of the CNS, only this morphological type of tumor is characterized by the absence of IDH mutations.

The MR scans were performed with different types of tomographs at 1.5 and 3 T, so the images had to be pre-processed. MRI parameters:
- pulse sequence, plane: T2FLAIR, ax;
- slice thickness: 2–6 mm;
- field of view: 186 × 230, 199 × 220, 201 × 230, 226 × 250;
- time of repeat (TR), ms: 4,800–11,000;
- time of echo (TE), ms: 61.00–365.27.

At this study stage, we compared the effectiveness of the following raw data preprocessing techniques (transforms):
1. Normalization of image intensity distribution: to bring it to the standard normal distribution where the mean is 0 and the standard deviation is 1.
2. Image scaling (ScaleIntensity): to bring image intensity values to the predefined range (from 0 to 1).
3. Image contrast adjustment via γ-correction (AdjustContrast): to highlight structures and details crucial for the analysis.
4. Histogram normalization: to redistribute voxel intensity values for a normal (Gaussian) distribution of frequencies throughout the entire range of values.

The transforms were performed with MONAI library generic interfaces [23]. All the normalization methods were applied to each image individually. Non-normalized data were used for comparison, allowing us to evaluate the effect of preprocessing on the study results.

ROIs were segmented by a radiology expert using LevelTracing, a semi-automatic segmentation tool in 3D Slicer open-source software. The choice of this tool, grounded by the

results of our comparative effectiveness research and its operation principle, has been described previously [22]. We also used neuro-fuzzy ensembles for brain tumor segmentation[1].

The ROIs traditionally covered the entire area of the tumor lesion with hyperintense MR signal on T2-FLAIR images, including cystic and/or necrotic, hemorrhagic, and calcified components of the tumor. Such coverage is intended to significantly speed up the segmentation of the primary MR image and to standardize it to some extent by eliminating potential discrepancies in the identification of the tumor structural components due to operator-dependent variations in image segmentation.

For each ROI, 851 radiomic features were extracted: 107 original features from 7 radiomic classes and additional data obtained by discrete wavelet transforms (DWT) with a wavelet filter computing eight decompositions (HHH, HHL, HLH, HLL, LHH, LHL, LLH, LLL) per segment[2].

To convincingly demonstrate the effect of various preprocessing methods on the performance of the developed predictive model, we have selected such radiomic features that showed the best results in the previous stage of our study [22], namely:

Sphericity – a measure of the roundness of the ROI shape relative to a sphere with the smallest possible surface area, which sphericity is equal to 1 (value range is 0–1; this parameter does not reflect textural features, so it cannot be filtered out by wavelet decompositions);

Dependence Entropy – a measure of dependence variance between voxel intensity values (computed with HHH wavelet decomposition);

Dependence Non-Uniformity Normalized – a measure of the dependence variance between different grey levels throughout the image (computed with HHH wavelet decomposition);

Dependence Variance – a measure of dependence variance between the gray levels throughout the image, which quantifies the difference between a voxel intensity value and the intensity value of its neighbors (computed using HHH- and HLN wavelet decompositions);

Small Area Emphasis – incidence of small zones with the same gray level. This feature reflects texture heterogeneity by highlighting frequently occurring small areas of equal intensity. High values of this feature may indicate a more homogeneous texture of the image, whereas low values indicate complex and heterogeneous structures (computed with a wavelet decomposition) [24].

To evaluate the classification performance of our model, we used the following metrics: accuracy, recall, precision, F1 score, and AUC score.

[1]Cardoso M.J., Li W., Brown R. et al. MONAI: an open-source framework for deep learning in healthcare. 2022. URL: https://arxiv.org/pdf/2211.02701v1
[2]Radiomic Features — pyradiomics 2.2.0.post35+g8da1db7 documentation. 2016. URL: https://pyradiomics.readthedocs.io/en/latest/features.html

The dataset (46 brain MRI reports) was divided into two groups: 31 reports were used as a training sample, and 15 reports were used as a test sample. To evaluate the predictive performance of the model and given the limited input data, we performed 5-fold cross-validation of the dataset. The developed model was evaluated by each feature individually using the AUC score. This approach ensured the reliability of the metrics obtained for assessing the stability and predictive performance of the model when applied to different subsets of the input data.

To train our classification model, we used Random Forest, a decision tree ensemble algorithm, which incorporates a bagging technique to aggregate predictions from different training sets. Random Forest trains each decision tree independently on random subsets of the input data, ensuring diversity of the ensemble models and considering non-linear correlation between the features. The number of decision trees was limited to 50 to balance the variance of the model with its stability.

The null hypothesis assumed that the selected image preprocessing techniques do not affect the accuracy of IDH mutation status classification. In other words, the difference between the mean AUC values for the features extracted using different preprocessing techniques and for the unprocessed data is statistically insignificant. The alternative hypothesis states that the preprocessing techniques do affect the accuracy of IDH mutation status classification, which is presented by statistically significant differences in the mean AUC values compared to the unprocessed data.

To evaluate the reliability of the model for the study results, we used Student's t-test, which allows us to compare the distribution of quality metrics for different features (AUC) and detect statistically significant differences for this parameter. The significance level was calculated for each feature individually.

Results

Using the test sample, we calculated predictive performance values for the ROI radiomic features, which significantly correlated with IDH mutation status, according to the applied methods of source image normalization (Table 2).

For the Sphericity feature, we found no statistically significant difference between the preprocessed and unprocessed data, suggesting that the effects of different preprocessing techniques may vary depending on the feature being analyzed.

Of particular note is an experimental predictive model based on a set of radiomic features. This model demonstrated a significant improvement in classification quality due to preprocessing techniques applied ($p < 0.05$).

In this experimental model, preprocessing with the ScaleIntensity transform considering the entire set of radiomic features yielded the best result. The highest scores of feature importance in this model had dependence variance (24.3%) and dependence entropy (22.0%), as the most significant for classification. They were followed by dependence non-uniformity normalized (19.3%) and small

Table 2. Effects of image preprocessing on accuracy of IDH mutation status prediction (AUC score), *M* ± *SD* (*p*)

Radiomic feature	Unprocessed	Image intensity normalization	ScaleIntensity	AdjustContrast	Histogram normalization
Sphericity	0.645 ± 0.197	0.635 ± 0.223 (0.695)	0.660 ± 0.194 (0.713)	0.65 ± 0.177 (0.464)	0.685 ± 0.235 (0.237)
Dependence Entropy_HHH	0.59 ± 0.142	**0.76 ± 0.141* (0.042)**	0.665 ± 0.144 (0.28)	0.725 ± 0.101 (0.101)	0.585 ± 0.161 (0.325)
Dependence Non-Uniformity Normalized_HHH	0.655 ± 0.16	**0.855 ± 0.093* (0.010)**	0.770 ± 0.109 (0.153)	**0.805 ± 0.128* (0.043)**	0.630 ± 0.119 (0.843)
Dependence Variance_HHH	0.68 ± 0.184	0.670 ± 0.107 (0.589)	**0.840 ± 0.119* (0.023)**	**0.795 ± 0.141* (0.036)**	0.56 ± 0.142 (0.358)
Dependence Variance_HHH	0.355 ± 0.126	0.650 ± 0.094 (0.566)	0.535 ± 0.211 (0.572)	0.66 ± 0.051 (0.687)	**0.825 ± 0.087* (0.013)**
Small Area Emphasis_LHL	0.48 ± 0.081	0.725 ± 0.094 (0.089)	0.650 ± 0.157 (0.532)	0.665 ± 0.111 (0.536)	0.695 ± 0.187 (0.753)
All features	0.63 ± 0.088	**0.815 ± 0.058* (0.020)**	**0.845 ± 0.089* (0.005)**	**0.805 ± 0.09* (0.037)**	**0.82 ± 0.127* (0.027)**

Note. $p < 0.05$ compared to the unprocessed data.

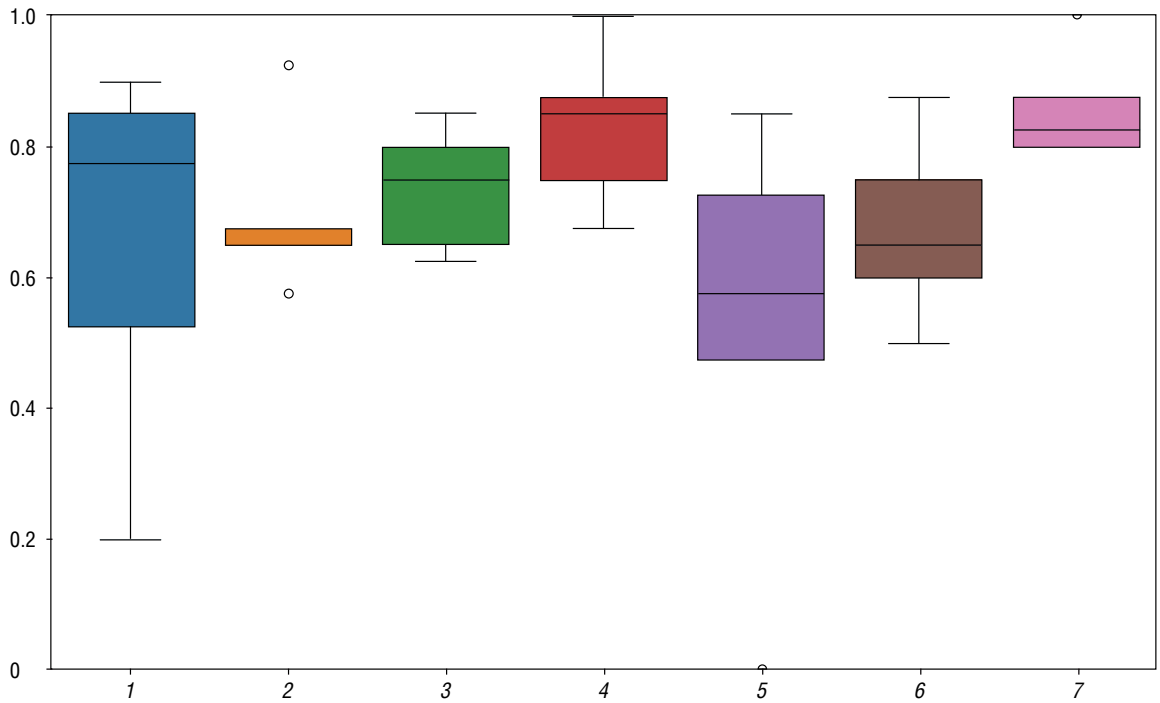

Fig. 1. Box plot displaying the results of 5-fold cross-validation of image preprocessing with the ScaleIntensity transform.
1 – Sphericity; *2* – Dependence Entropy_HHH; *3* – Dependence Non-uniformity Normalized_HHH; *4* – Dependence Variance_HHHH; *5* –Dependence Variance_HLH; *6* – Small Area Emphasis_LHL; *7* – all features.

area emphasis (18.5%). Sphericity and dependence variance had lower scores (8.2% and 7.7%, respectively); nevertheless, they contribute to classification improvement when combined with other features.

The box plot in Fig. 1 presents the spread of prognostic values for radiomic features extracted with the Scaleintensity transform (as the most effective preprocessing technique) and statistical characteristics of each subset. Classification

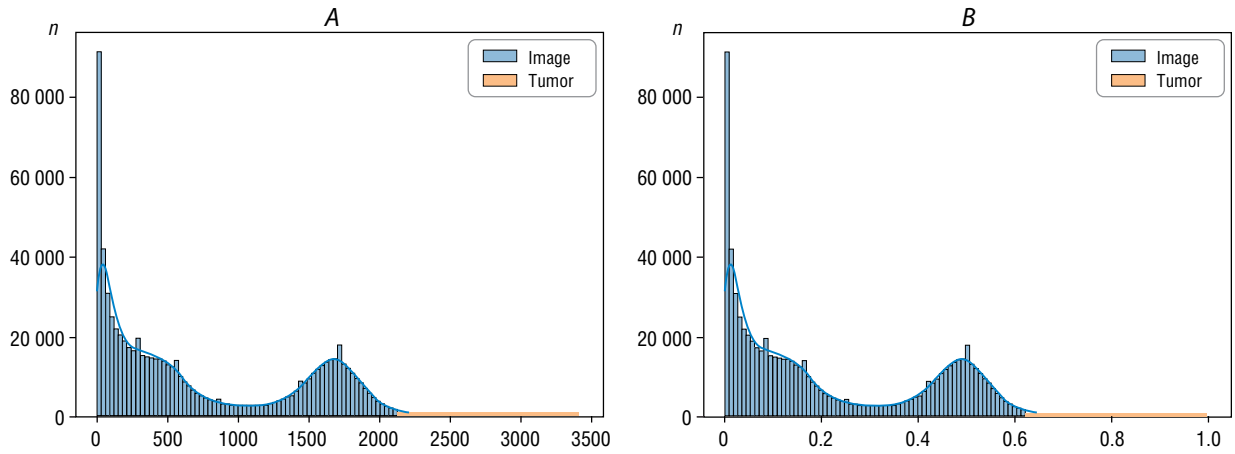

Fig. 2. Quantitative distribution of voxels with specific gray levels throughout all dataset images.
A — raw data; *B* — ScaleIntensity transformed data.

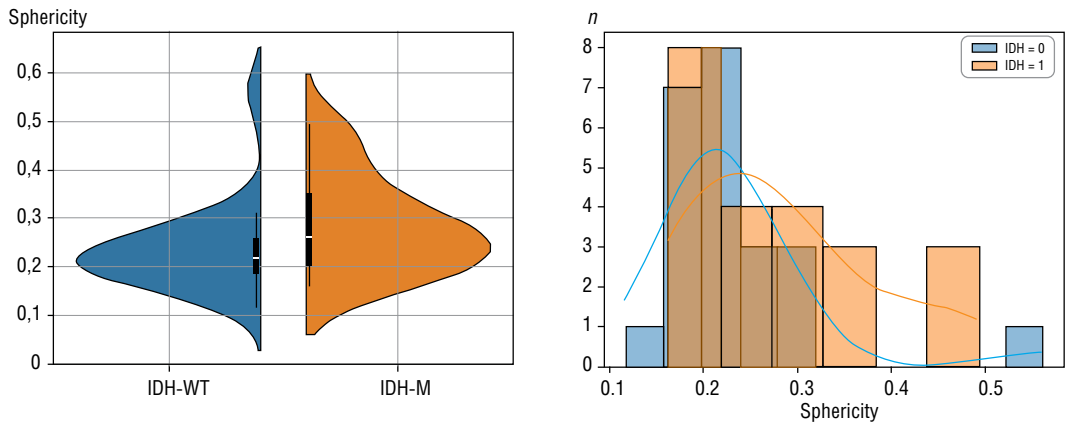

Fig. 3. Violin plot outlining sphericity values.

models considering the entire set of features demonstrate the highest predictive performance.

The ScaleIntensity transform adjusts the image intensity in the predefined range by applying the linear transformation to each element of the array. This approach allows comparing images acquired with different scanning methods.

The quantitative distribution of voxels with different gray levels across all dataset images prior to and after applying the ScaleIntensity transform is shown in Fig. 2.

Noteworthy, normalization had no effect on the sphericity value, as the sphericity formula uses only the volume and area of the segmentation zone. Differences in the AUC score for this feature can be explained by 5-fold cross-validation of the training sample, so the model was tested on five different subsamples. Distribution of the sphericity values within the entire dataset is presented in Fig. 3. A greater number of tumors with relatively high sphericity values were found in the IDH-M subgroup. The tumor with the highest sphericity value in the sample had no mutation in the IDH gene.

Figure 4 shows the ROC curve and confusion matrix for the IDH mutation status predictive model in adult-type diffuse gliomas based on the above-mentioned six radiomic features and trained with Random Forest classification, where the source images were normalized via the ScaleIntensity transform. The AUC score for the developed model is 0.845 ± 0.089, and the key metrics are accuracy 0.866; precision 0.875; recall 0.875; F1 score 0.874. According to the confusion matrix, the model produced 1 false-positive result and 1 false-negative result from the test sample of 15 reports. In other 13 cases, IDH-M and IDH-WT mutation statuses were classified correctly.

Discussion

At the previous study stage, we evaluated the predictive performance of six individual IDH mutation status predictors, extracted from ROIs in the MR scans, which were preprocessed by histogram matching and the ScaleIntensity transform, the latter yielding the best results [22]. At the current study stage, the evaluation of the predictive performance of the combined model incorporating four different preprocessing techniques showed a similar trend. Of those four tools, only AdjustContrast does not bring the signal characteristics of images to uniform ranges of predefined or mean values, as it aims to emphasize texture differences with γ-correction by augmenting or reducing the general contrast of the image.

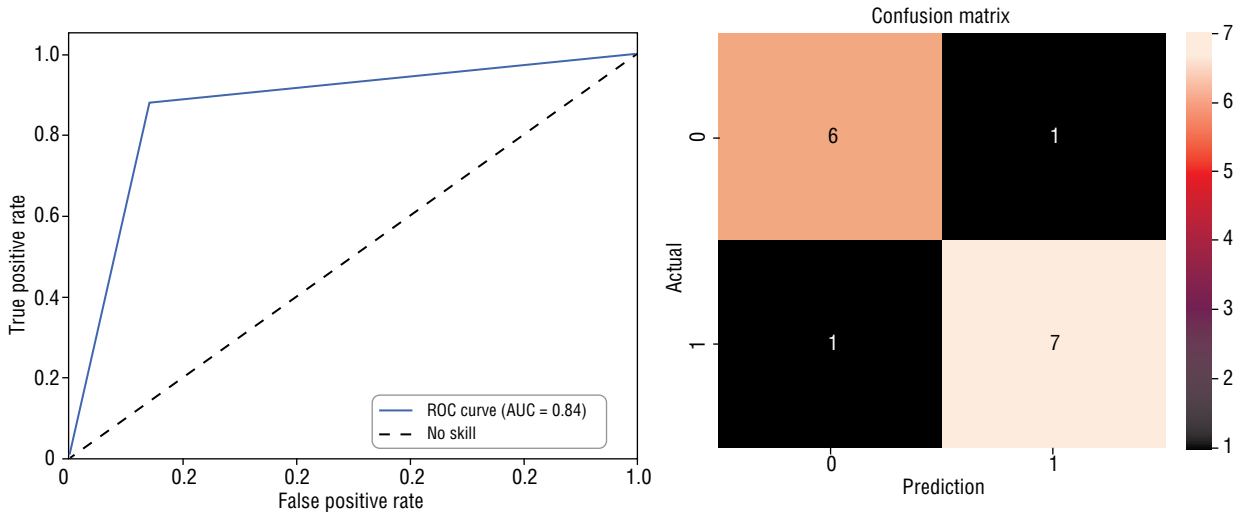

Fig. 4. ROC curve and confusion matrix of predictive model for IDH mutation status (test sample).

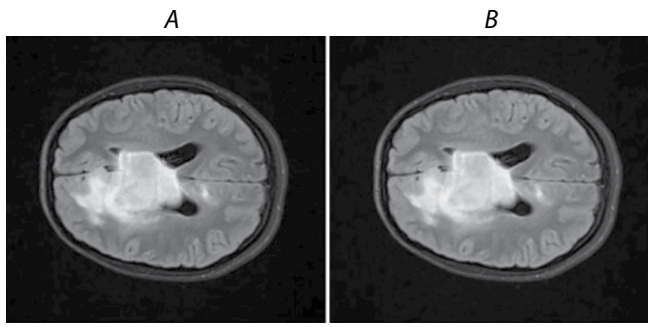

Fig. 5. Diffuse glioma (T2-FLAIR, ax).
A — raw data; *B* — AdjustContrast transformed (γ = 0.9).

At the previous study stage, we found that the majority of the radiomic predictors characterize ROI heterogeneity by gray level intensity of the voxels. Hence, the higher performance of the model built using the method of the current study stage can be associated with the contrast adjustment. Gamma (γ) value adjusts the contrast as a function: $\gamma < 1$ reduces contrast, and $\gamma > 1$ augments contrast. At the current study stage, γ was > 0.9, which means that the image contrast was slightly reduced (Fig. 5).

AdjustContrast is a crucial preprocessing technique used, for example, for computer vision tasks. Contrast adjustment improves the overall sharpness of the image, thereby enhancing the differentiation of its structural elements. This tool is primarily used for low-contrast images, where details are challenging to discern due to the insufficient difference between relatively light and dark regions[3].

The obtained result indicates that the preprocessing of source data from routine MRI based on contrast adjustment significantly improves the predictive performance of the developed model by reliably highlighting the key areas of altered MR signal, which is essential for the qualitative analysis of the MR image (see Table 2). Adjustment, almost imperceptible to the human eye, increased the model predictive performance by 17.5% compared with the model based on the raw data. Thus, γ-correction is not only critical for high-quality presentation of images and videos in different media formats (which is important considering the human-dependent perception of the image) but also a promising tool for standardization of raw medical imaging data preprocessing. However, we noticed slightly pronounced but significantly higher effectiveness of other normalization methods, which apply averaging over the signal amplitudes or bring them to a predefined range, for instance, the ScaleIntensity transform, which demonstrated the highest AUC score.

Let us compare these image preprocessing techniques. The ScaleIntensity transform is meant to uniformly increase the brightness of an image by adjusting the values of all its voxels. As a rule, the voxel values are scaled to the predefined value range by applying the linear transformation. For example, the ScaleIntensity transform can scale voxel values, which were initially in the range of 0–255, to a predefined range, often improving the quality of image interpretation without significant change of voxel-to-voxel ratio. In other words, ScaleIntensity allows us to augment

[3]Contrast Adjustment — MATLAB & Simulink.
URL: https://www.mathworks.com/help/images/contrast-adjustment.html

Table 3. ScaleIntensity vs AdjustContrast: a brief comparison of two medical imaging preprocessing techniques.

Preprocessing technique	ScaleIntensity	AdjustContrast
Effect	Uniform brightness enhancement	Greater differentiation between light and dark areas
Risk of detail loss	Low: image details are visible at any brightness level	Medium: risk of detail loss with aggressive contrast adjustment
Noise management	May reduce noise levels	May cause higher noise levels
Application	More suitable for comparative analysis	Limited to specific imaging data
Risk of clipping	Low: dynamic range preserved	High: risk of detail loss due to distorted color characteristics in too light/too light areas

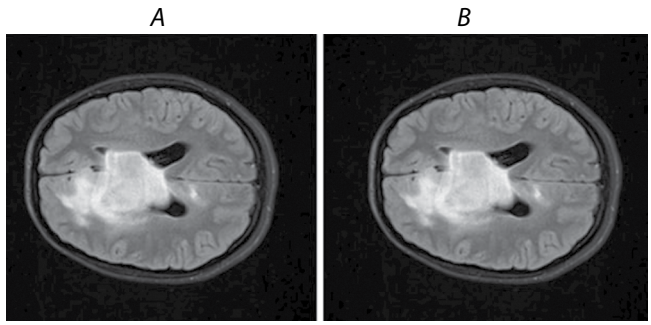

Fig. 6. Diffuse glioma (T2-FLAIR, ax).
A – raw data; *B* – ScaleIntensity transformed data.

the brightness of the image, preserving the texture of dark and light areas (Fig. 6). Uniform adjustment mitigates the risk of information loss due to critically excessive voxel intensity values.

This method also helps denoise MR images and facilitates the image structure interpretation by avoiding artifacts, which may appear with more aggressive contrast adjustment. In comparative analysis, ScaleIntensity brings a certain consistency to images with different noise levels, which is crucial for scientific imaging data analysis[4]. In turn, contrast adjustment modifies the difference between the darkest and brightest parts of the image by expanding or shrinking the range of voxel values, which improves visibility within the range by making dim areas darker and bright areas brighter. While AdjustContrast can help improve blurred image details and textures, it can also cause the risk of the detail loss in the clipping areas with too bright or dark colors. Unlike ScaleIntensity, which transforms all voxels uniformly, AdjustContrast improves the image non-uniformly by highlighting certain areas while shadowing texture in other areas, which may complicate the interpretation of the image[5].

Table 3 summarizes the main arguments, which may explain the significant difference between the predictive performance of models built using these two preprocessing techniques.

[4]Transforms — MONAI 1.4.0 Documentation. 2024.
URL: https://docs.monai.io/en/stable/transforms.html#scaleintensity
[5]Transforms — MONAI 1.4.0 Documentation. 2024.
URL: https://docs.monai.io/en/stable/transforms.html#adjustcontrast

Li et al. also demonstrated that although intensity normalization methods applied to source brain MRI images cannot completely remove the scanner effects at the radiomic feature level, they can make the neuroimaging data comparable for the subsequent analysis and increase the reliability of radiomic predictors [25]. Moreover, these methods appear to have a wider range of clinical applications, where the implementation of ComBat, a well-known image preprocessing technique, requires more computing power associated with a decrease in processing speed due to larger datasets and correspondingly more sophisticated predictive models trained on these datasets [26].

A higher number of ROIs with relatively high sphericity values in the IDH-M subgroup might be explained by less extensive areas of perifocal vasogenic edema typical for these tumor types. Such edema spreads along the gyri, giving the segmentation zones an irregular star-like shape [27, 28]. As our test sample included all morphological types of adult-type diffuse gliomas of all malignancy grades, relatively low sphericity values more often indicated more aggressive tumors, mostly represented by IDH-WT glioblastomas. The obtained result is indirectly consistent with the study of Y. Li et al., who showed that spherical disproportion (a radiomic feature characterized by minimal values for the ideal sphere) was the only independent prognostic factor positively correlated with Ki-67 proliferation index expression in lower grade gliomas [29].

The cumulative predictive performance of the model based on all six radiomic features is higher than that of the model based

on a single parameter. Therefore, our classification model based on the presence/absence of IDH mutation considers not only the severity of perifocal edema but also probably the measure of intratumor morphological heterogeneity presented by significantly higher textural ROI heterogeneity at the voxel level. We provided more detailed grounds for this assumption in one of our previous articles [22].

Given that morphological heterogeneity determines the glioma malignancy grading, our classification model, by measuring morphological heterogeneity, reflects most probably a lower or higher malignancy grade of the tumor. In its turn, numerous morphologic characteristics of high malignancy mentioned above are associated with the absence of IDH mutation, allowing the use of radiomics-based markers for indirect prediction of the IDH mutation status.

Numerous studies indicate that an astrocytic tumor, which does not fully meet the morphological criteria for higher malignancy grade but is IDH-M-free and has other specific molecular genetic characteristics, is defined as a grade 4 IDH-WT glioblastoma and should be treated according to the corresponding clinical guidelines [33–35]. Since the study samples covered tumors classified according to the updated WHO criteria, it is highly probable that some of the 24 studied IDH-WT gliomas also initially showed morphological characteristics of malignancy grade 3, which further was increased due to the IDH-M absence. So, given the diversity of adult-type diffuse gliomas in the dataset, the developed model learned to detect IDH-WT entities using the cases with less pronounced morphological characteristics of malignancy.

In many recent studies, training samples included clinical cases classified according to the 2016 WHO classification with a different IDH mutation status attributed to grade II, III diffuse astrocytomas, and grade IV glioblastomas [36]. Therefore, in some cases, the dataset was limited to specific morphologic types and malignancy grades so that the distribution of radiomic features indirectly depended only on the target variable, i.e., IDH mutation status [37, 38]. Thus, predictive models developed with this approach are of limited use for primary differential diagnosis, as they are often trained to classify IDH mutation status within a single morphological type, which is unknown to the radiologist at the time of the patient's initial examination.

Some studies disregarded IDH mutation status in the differential diagnosis of low-grade and high-grade gliomas, but in this case the radiomics-based differences between the IDH-WT and IDH-M tumor subgroups are largely associated with the minor phenotypic features, the same as in the morphologic analysis, i.e., radiomics supports the conventional methods used by radiologists [39, 40].

Conclusion

Our results demonstrate a significant impact of different MR image preprocessing methods on the accuracy of the radiomics-based IDH mutation status prediction in patients with adult-type diffuse glioma.

Based on the analysis of various tool combinations tested in routine neuroimaging and a small dataset, the combination with the highest prognostic value for pre-processing, segmentation, and classification of neuro-oncology images was found to be ScaleIntensity, LevelTracing, and Random Forest, respectively. The possible reason is that the ScaleIntensity technique can better preserve the detail and uniformity of images, which is particularly useful for comparative analysis. On the other hand, the AdjustContrast technique can improve the quality of visual interpretation, but at the cost of the structure detail loss due to non-uniformity of adjustment and the risk of clipping. At the same time, all four preprocessing techniques (ScaleIntensity, LevelTracing, Random Forest, and AdjustContrast) demonstrated similar predictive performance; hence, we should use larger datasets to train models based on these techniques and evaluate the reproducibility of the yielded results for alternative datasets.

The predictive performance of the presented model based on all six radiomic features reached the AUC score of 0.845 ± 0.089, which we will use in the further studies.

The sphericity value of the ROIs, including perifocal tissue transformations, is significantly lower in IDH-WT gliomas, as all of them have malignancy grade 4. This grade positively correlates with the severity of vasogenic edema, which is responsible for a typical irregular shape of the structural changes in tumor-associated areas.

Based on the criteria of the 2021 WHO classification of tumors of the CNS, the optimal models of IDH mutation status prediction should not be trained on samples presenting only one morphological type or a malignancy grade but rather on a sample covering various tumor types and grades to expand the model's applicability to the primary diagnostic imaging in patients with suspected adult-type diffuse glioma. Furthermore, this approach allows non-invasive exclusion of primary IDH WT glioblastoma at the initial stages of differential diagnosis.

We plan to evaluate the reproducibility of the presented model using an open-source brain MRI dataset with all types of adult-type diffuse gliomas with identified IDH mutation status and malignancy grade according to the 2021 WHO classification of tumors of the CNS. We also intend to develop a technique for color mapping of ROIs in order to facilitate visual interpretation of quantitative radiomic data.

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Polymorphisms in the *SNCA* Gene and the Risk of Synucleopathy

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Abstract

Introduction. *Synucleinopathies are mostly sporadic and multifactorial neurodegenerative disorders, which determines the involvement of various risk factors in their development. The polymorphic variants of the SNCA gene are considered as one of the predisposing genetic factors.*

Study aim*: to evaluate the effect of the 16 single nucleotide polymorphisms (SNP) located in various regulatory regions of the SNCA gene on the risk of developing three main forms of synucleinopathy – PD, DBL, and MSA – in Russian cohort of patients.*

Materials and methods. *The study included 73 PD patients, 46 MSA patients, 10 DLB patients, and 62 healthy volunteers. Genotyping of 16 SNPs of the SNCA gene was performed by direct Sanger sequencing on a capillary genetic analyzer. The Benjamini–Hochberg procedure was applied for multiple pairwise comparisons.*

Results. *A comparative case-control study showed that only one (rs11931074) of the 16 SNP analyzed was associated with PD: the minor T allele, located in the 3'-UTR region of the SNCA gene, increased the risk of PD (OR = 5.19; $p < 0.05$ (Benjamini–Hochberg adjusted $p = 0.6$)). An association with MSA was found for 11 of 16 SNP. The minor allele of 5 SNP (rs2619364, rs2619363, rs2619362, rs2619361, rs181489) reduced the risk of the disease, while for 6 SNP (rs7687945, rs2301134, rs2301135, rs3756063, rs2736990, rs11931074) increased the risk. The Benjamini–Hochberg procedure neutralized the significance of only one of these associations (rs181489).*

Conclusion. *This study is the first to genotype a large group of polymorphisms located in various regulatory regions of the SNCA gene and to establish significant associations with the risk of developing one of the forms of synucleinopathies, MSA, in the Russian population.*

Keywords: *synucleinopathies; SNCA gene single nucleotide polymorphisms; SNP*

Ethics approval. The study was conducted with the voluntary informed consent of the patients. The study protocol was approved by the Ethics Committee of the Research Center of Neurology (Protocol No. 2-8/24, 18 March, 2024).

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Conflict of interest. The authors declare that they have no apparent or potential conflicts of interest related to the publication of this article.

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Полиморфные варианты гена *SNCA* и риск развития синуклеинопатий

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Аннотация

Введение. *Большинство форм синуклеинопатий являются спорадическими и имеют многофакторную природу, что определяет участие различных факторов риска в их развитии. Как один из таких предрасполагающих генетических факторов рассматривается участие различных полиморфных вариантов гена SNCA.*

Цель *исследования: изучение влияния 16 однонуклеотидных полиморфных вариантов, локализованных в различных регуляторных областях гена SNCA, на риск развития в когорте пациентов российской популяции трёх основных форм синуклеинопатий: болезни Паркинсона (БП), деменции с тельцами Леви (ДТЛ) и мультисистемной атрофии (МСА).*

Материалы и методы. *В исследование были включены 73 пациента с БП, 46 с МСА, 10 с ДТЛ и 62 неврологически здоровых добровольца. Генотипирование 16 однонуклеотидных полиморфных вариантов (SNP) гена SNCA проводили методом прямого секвенирования по Сэнгеру на капиллярном генетическом анализаторе. Для коррекции ошибки при множественном попарном сравнении использовали поправку Беньямини–Хохберга.*

Результаты. *По результатам сравнительного анализа «диагноз–контроль» только 1 из 16 протестированных SNP (rs11931074), локализованный в области 3'-UTR гена SNCA, продемонстрировал связь с БП: минорный аллель T проявил тенденцию к увеличению риска развития БП (ОШ = 5,19; p < 0,05 (с поправкой Беньямини–Хохберга p = 0,6)). Для 11 из 16 SNP выявлена ассоциация с МСА. Минорный аллель 5 SNP из них (rs2619364, rs2619363, rs2619362, rs2619361, rs181489) снижал риск заболевания, а для 6 SNP (rs7687945, rs2301134, rs2301135, rs3756063, rs2736990, rs11931074) – повышал. Применение поправки Беньямини–Хохберга нивелировало значимость только одной из этих ассоциаций (rs181489).*

Заключение. *В результате проведённого исследования впервые генотипирована большая группа полиморфных вариантов, расположенных в различных регуляторных областях гена SNCA, и установлены значимые ассоциации с риском развития одной из форм синуклеинопатий – МСА – в российской популяции.*

Ключевые слова: *синуклеинопатии; однонуклеотидные полиморфные варианты; ген SNCA*

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Introduction

Synucleinopathies are a group of neurodegenerative disorders characterized by the aberrant accumulation of α-synuclein aggregates in neurons, nerve fibers, or glial cells. It is estimated that α-synucleinopathies affect over 10 million people worldwide [1].

Alpha-synuclein is a small, 140 amino acid protein [2, 3], widely represented in the human brain, primarily in presynaptic terminals. Normally, α-synuclein, found in the cell as a tetramer, presumably regulates vesicular transport processes and dopamine neurotransmission [4], and is crucial for normal brain functioning [5]. α-Synuclein is a type of protein that lacks a stable secondary structure in solution and tends to aggregate. Increased accumulation of α-synuclein in solution leads to the formation of insoluble fibrils and discrete spherical structures causing cell death [6].

Parkinson's disease (PD) is the most common phenotype form of synucleinopathies, with a prevalence of 100–200 cases per 100,000 people [7]. Less frequent is multiple system atrophy (MSA), with 2–5 cases per 100,000 people [8], and dementia with Lewy bodies (DLB), accounting for 4.2–5.0% of

all dementias [9]. Historically, the distinction of PD, MSA, and DLB was based on clinical manifestations and neuropathological signs. Subsequently, an active study of molecular-genetic mechanisms underlying neurodegenerative processes in this group revealed structural features of protein aggregates for different forms of synucleinopathy. Such filaments from patients with MSA are straight or twisted compared with the mostly straight filaments from PD patients [10]. MSA is associated with predominantly oligodendroglial α-synuclein insertions, whereas α-synuclein aggregates predominantly accumulate in neurons of PD patients [11].

Many questions regarding the pathogenesis of this disease remain unanswered. The mechanisms that trigger α-synuclein aggregation and subsequent neurodegeneration remain to be elucidated. These include genetic variants, epigenetic, and transcriptional processes. Most forms of synucleinopathy are sporadic and multifactorial in origin, and only 10% of cases have an aggravated family history. Several recent genome-wide association studies (GWAS) in PD have identified an association of the disease with single nucleotide polymorphisms (SNP) located in different regulatory regions of the *SNCA* gene encoding α-synuclein [12–14]. However, the contribution of most of these SNPs in the regulation of *SNCA* gene expression has not been studied. Two PD associated significant linkage disequilibrium blocks were identified: one covers the promoter region, while the other affects the 4th intron and the 3'-UTR regulatory region of the *SNCA* gene [15, 16]. Polymorphisms located in the 5'-region of the *SNCA* gene may affect the gene's transcriptional activity. For example, specific SNP (rs3756063) has been shown to be associated with hypomethylation in PD patients [17]. Polymorphisms located in the regulatory 3'-region may, in turn, play a role in the stability of mRNA translation by affecting its binding to the corresponding microRNA, as well as in alternative splicing [12, 13, 18].

Individual SNPs may have varying effects on the regulation of *SNCA* gene expression, leading to both down-regulation and up-regulation. In particular, a "protective" function of 2 SNPs genotypic combination was shown to be strongly correlated with low levels of mRNA [19]. In contrast, another study demonstrated a significant association of the SNP rs356168 minor allele with an increased *SNCA* gene expression due to enhancer activation and subsequently increased gene transcription [20, 21].

The impact of the *SNCA* gene polymorphisms on the synucleinopathy pathogenesis has not yet been sufficiently studied. A number of SNP for some forms of synucleinopathy has not been studied in the Russian population. Other populations have been studied to a limited extent. Hence, **the aim** of the study was to assess the effect of 16 SNP in the *SNCA* gene on the risk of synucleinopathy (PD, DLB, and MSA) developing in a cohort of Russian patients.

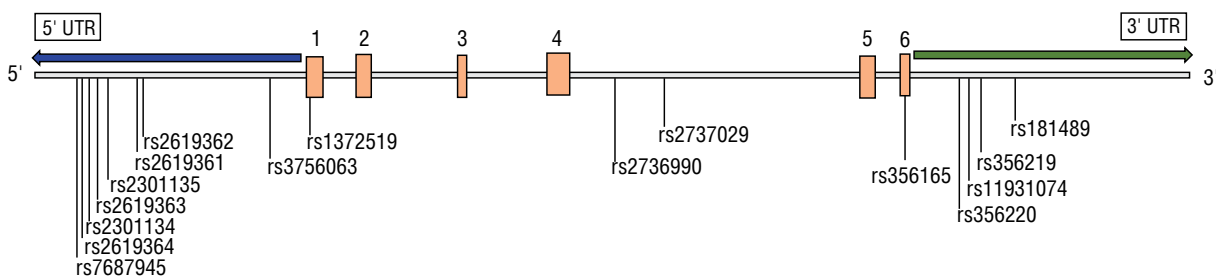
Materials and methods

The study was conducted at the Research Center of Neurology and included 73 PD patients diagnosed according to the MDS UPDRS criteria [22]; 46 patients with MSA: 17 patients with cerebellar type (MSA-C) and 29 patients with Parkinsonian type (MSA-P) [23]; and 10 patients with DLB diagnosed according to the relevant diagnostic criteria [9]. The Montreal Cognitive Assessment Scale (MoCA) was used to assess the cognitive status. The control group included 62 volunteers with no neurological disorders. The groups were mainly represented by people of Slavic ethnicity living in the European part of the Russian Federation. All participants were familiarized with the upcoming procedures and signed an informed consent before the study. The study protocol was approved by the Ethics Committee of the Research Center of Neurology (Protocol No. 2-8/24 dated 18 March, 2024).

Based on a meta-analysis of several available PD GWAS datasets for different populations [24], we selected 16 SNP identified as significantly associated with PD progression and localized in different regions of the *SNCA* gene: 9 in the 5'-UTR and promoter region; 2 in the 4th intron region; and 5 in the 3'-UTR region (Figure).

Genomic DNA samples were extracted from whole blood using the DNA-extran-1 reagent kit (Syntol, Moscow, Russia). 16 SNP of the *SNCA* gene were genotyped by direct Sanger sequencing on the Nanofor 05 capillary genetic analyzer (Institute for Analytical Instrumentation, Russian Academy of Sciences, Moscow, Russia).

The statistical analysis was carried out using the SPSS Statistics, version 26.0. (IBM). We used the Benjamini–Hochberg (BH) procedure for multiple pairwise comparisons. The null hypothesis was rejected at an adjusted significance level of $p_{adj} < 0.05$ based on FDR scores. Mean and standard deviation were used to describe quantitative variables, while absolute and relative frequencies were used to describe categorical variables. Categorical variables in the groups were compared using Pearson's χ^2 test; if there were limitations to its applica-



Structure of the SNCA gene and location of the 16 SNPs analyzed.

tion, Fisher's exact test was used. The odds ratio (OR) and the 95% confidence interval (CI) were calculated to quantify the relationship between the disease development and specific genotypes or alleles.

Results

The study groups were characterized according to clinical and demographic parameters (Table 1). The mixed form of PD was observed in 60 (82.2%) patients, while the akinetic-rigid form was observed in 13 (17.8%) patients. Patients were divided into groups according to the disease stage, as determined by the Hoehn–Yahr scale. Thus, 41 (56.2%) patients were diagnosed with stage 3, 23 (31.4%) with stage 2, 8 (11.0%) with stage 1, and 1 (1.4%) with stage 4. Late onset was found in 57 (78.1%) patients (⩾ 45 years), and 16 (21.9%) patients had early onset (21–44 years) of disease.

Table 2 summarizes the results of minor allele frequencies for the 3 phenotype groups of the synucleinopathy. Genetic screening showed variations in individual SNP minor allele frequencies in our sample compared to the European population in the international Genome Aggregation Database (GnomAD). According to the population databases, the prevalence of minor alleles for rs7687945, rs2301134, rs2301135 and rs2736990 was higher in our control group than in the European population. Accordingly, a comparative analysis of the incidence was conducted for the risk allele identified in our sample.

A comparative case-control study showed that only one (rs11931074) of 16 SNPs analyzed was associated with PD: the minor allele increased the risk of the disease. However, the BH correction leveled the significance of this correlation.

Eleven out of 16 SNPs showed correlation with MSA. For 5 of them (rs2619364, rs2619363, rs2619363, rs2619362, rs2619361, and rs181489), the minor allele reduced the risk of disease, while for 6 others (rs7687945, rs2301134, rs2301135, rs3756063, rs2736990, and rs11931074) it increased the risk. The BH correction leveled the significance of only one of these associations (rs181489).

Five out of 16 SNPs showed correlation with DLB. For 4 of them (rs2619364, rs2619363, rs2619362, rs2619361) the minor allele reduced the risk of disease, while for rs1372519 the risk was increased. However, the BH correction leveled the significance of all of these associations.

Quantification of the association between disease presence and genotype status was performed for the 11 SNPs that showed a significant association with MSA. For this purpose, we calculated the ORs for 2 genetic models: dominant and recessive (Table 3). Analyses were performed for both the total MSA group and for the 2 major subtypes: MSA-C and MSA-P.

Five SNPs (rs7687945, rs2301134, rs2301135, rs3756063, rs11931074) significantly increased disease risk for MSA patients for the minor allele in homozygous and heterozygous genotypes in the dominant model. Five other SNPs (rs2619364, rs2619363, rs2619363, rs2619362, rs2619361, rs181489) reduced disease risk for the minor allele in homozygous and heterozygous genotypes in the dominant model. For 4 of these SNPs (rs2619364, rs2619363, rs2619362, rs2619361), the recessive model was significantly less represented of homozygotes for the minor allele among patients with MSA (namely the absence of such).

During the MSA-P analysis, the risk of disease was significantly higher for minor allele of 5 SNPs (rs7687945, rs2301134, rs2301135, rs3756063, rs11931074) for homozygotes and heterozygotes compared to homozygotes for the more common allele in the dominant model. Moreover, the risk was significantly reduced only for minor allele of 2 SNPs (rs2619362, rs2619361) in homozygotes and heterozygotes compared to homozygotes for the more common allele in the dominant model. The recessive model did not show significant differences in the representation of homozygotes for the minor allele between the groups for any of the SNPs.

The MSA-C analysis did not show significant associations between genotypes and the disease in any of the models. This may be attributed to the low power of analysis, as the volume of the study group is small (n = 17).

Discussion

Disease-associated polymorphic genes are considered risk factors for multifactorial diseases. We performed genetic screening of 16 polymorphisms of the *SNCA* gene across three groups with different forms of synucleinopathy, where we relied on a large meta-analysis of the association between *SNCA* polymorphisms and the risk of PD, focusing on the most significant SNPs [24].

A number of studies have shown the greatest association with the risk of idiopathic PD for rs11931074 located in the

Table 1. Clinical and demographic characteristics of patients and control subjects, *M* ± *SD*

Parameter	PD (n = 73)	MSA (n = 46)		DLB (n = 10)	Control (n = 62)
		MSA-P (n = 29)	MSA-C (n = 17)		
Sex (M/F)	38/35	11/18	8/9	9/1	44/18
Age, years	59.0 ± 11.6	64.1 ± 8.4	56.1 ± 6.6	70.2 ± 4.1	53.4 ± 9.8
Onset age, years	54.0 ± 11.4	59.7 ± 8.4	53.2 ± 7.2	66.0 ± 5.0	–
MoCA score	23.9 ± 3.7	24.4 ± 3.6	24.3 ± 1.8	15.4 ± 3.8	–

Table 2. Association analysis of minor SNP alleles of the *SNCA* gene with the presence of SP

SNP	Allele	Group	Allele frequency	OR (95% CI)	*p*	p_{adj}
		GnomAD	0.52			
		Control	0.41			
rs7687945	*T*	PD	0.47	1.296 (0.795–2.115)	0.321	1.000
		MSA	0.60*	2.194 (1.252–3.845)	0.008	0.020
		DLB	0.31	0.659 (0.215–2.015)	0.590	1.000
		GnomAD	0.24			
		Control	0.41			
rs2619364	*G*	PD	0.38	0.891 (0.546–1.452)	0.708	1.000
		MSA	0.18*	0.311 (0.160–0.604)	< 0.001	0.005
		DLB	0.10*	0.159 (0.035–0.716)	0.011	0.258
		GnomAD	0.24			
		Control	0.40			
rs2619363	*T*	PD	0.36	0.872 (0.533–1.429)	0.616	1.000
		MSA	0.18*	0.331 (0.173–0.634)	0.001	0.006
		DLB	0.15*	0.270 (0.075–0.971)	0.044	0.301
		GnomAD	0.52			
		Control	0.39			
rs2301134	*G*	PD	0.48	1.458 (0.897–2.370)	0.141	1.000
		MSA	0.58*	2.167 (1.247–3.766)	0.008	0.021
		DLB	0.40	1.056 (0.402–2.770)	1.000	1.000
		GnomAD	0.52			
		Control	0.40			
rs2301135	*C*	PD	0.48	1.360 (0.836–2.213)	0.219	1.000
		MSA	0.60*	2.241 (1.283–3.914)	0.005	0.016
		DLB	0.45	1.211 (0.468–3.135)	0.807	1.000
		GnomAD	0.23			
		Control	0.40			
rs2619362	*T*	PD	0.40	1.026 (0.627–1.679)	1.000	1.000
		MSA	0.17*	0.322 (0.169–0.616)	0.001	0.005
		DLB	0.15*	0.270 (0.075–0.971)	0.044	0.263
		GnomAD	0.27			
		Control	0.40			
rs2619361	*A*	PD	0.40	1.026 (0.627–1.679)	1.000	1.000
		MSA	0.17*	0.322 (0.169–0.616)	0.001	0.004
		DLB	0.15*	0.270 (0.075–0.971)	0.044	0.234
		GnomAD	0.40			
		Control	0.40			
rs3756063	*C*	PD	0.44	1.190 (0.731–1.939)	0.535	1.000
		MSA	0.62*	2.493 (1.433–4.337)	0.002	0.007
		DLB	0.40	1.020 (0.389–2.677)	1.000	1.000

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SNP	Allele	Group	Allele frequency	OR (95% CI)	*p*	p_{adj}
		GnomAD	0.21			
		Control	0.21			
rs1372519	*A*	PD	0.17	0.738 (0.399–1.368)	0.349	1.000
		MSA	0.21	0.961 (0.494–1.869)	1.000	1.000
		DLB	0.45*	3.021 (1.132–8.063)	0.046	0.221
		GnomAD	0.40			
		Control	0.47			
rs2737029	*C*	PD	0.48	1.051 (0.647–1.708)	0.902	1.000
		MSA	0.37	0.670 (0.385–1.167)	0.164	0.225
		DLB	0.28	0.440 (0.147–1.310)	0.203	0.648
		GnomAD	0.54			
		Control	0.42			
rs2736990	*A*	PD	0.44	1.064 (0.640–1.769)	0.897	1.000
		MSA	0.60*	2.045 (1.152–3.630)	0.015	0.034
		DLB	0.61	2.143 (0.769–5.969)	0.199	0.683
		GnomAD	0.36			
		Control	0.46			
rs356165	*G*	PD	0.54	1.393 (0.858–2.263)	0.218	1.000
		MSA	0.39	0.765 (0.434–1.347)	0.390	0.468
		DLB	0.40	0.788 (0.300–2.066)	0.809	1.000
		GnomAD	0.36			
		Control	0.45			
rs356220	*T*	PD	0.52	1.327 (0.817–2.154)	0.269	1.000
		MSA	0.38	0.743 (0.419–1.318)	0.315	0.388
		DLB	0.40	0.815 (0.311–2.137)	0.809	1.000
		GnomAD	0.07			
		Control	0.02			
rs11931074	*T*	PD	0.08*	5.194 (1.139–23.688)	0.025	0.595
		MSA	0.16*	10.973 (2.424–49.672)	< 0.001	0.008
		DLB	0.05	3.053 (0.264–35.339)	0.377	0.953
		GnomAD	0.36			
		Control	0.47			
rs356219	*G*	PD	0.53	1.285 (0.786–2.100)	0.381	
		MSA	0.37	0.672 (0.369–1.225)	0.228	1.000
		DLB	0.40	0.759 (0.290–1.985)	0.635	0.296
		GnomAD	0.29			
		Control	0.37			
rs181489	*T*	PD	0.40	1.135 (0.681–1.890)	0.697	1.000
		MSA	0.23*	0.495 (0.263–0.930)	0.031	0.059
		DLB	0.39	1.071 (0.385–2.980)	1.000	1.000

Note. *Significant differences from the control.

Table 3. SNP genotype association analysis for SNCA gene with the presence of MSA (n = 46), MSA-P (n = 29), and MSA-C (n = 17) compared with healthy volunteers (n = 62)

SNP	MSA type	The dominant model		The recessive model	
		OR (95% CI)	p_{adj}	OR (95% CI)	p_{adj}
rs7687945	MSA	6.833* (1.882–24.807)	0.007	2.125 (0.805–5.610)	0.215
	MSA-P	13.500* (1.709–106.648)	0.029	1.545 (0.491–4.869)	0.727
	MSA-C	3.500 (0.724–16.922)	0.207	3.400 (0.988–11.697)	0.166
rs2619364	MSA	0.265* (0.116–0.604)	0.008	NA	0.025
	MSA-P	0.298 (0.115–0.772)	0.054	NA	0.111
	MSA-C	0.216 (0.066–0.707)	0.128	NA	0.235
rs2619363	MSA	0.283* (0.126–0.633)	0.010	NA	0.043
	MSA-P	0.313 (0.125–0.782)	0.054	NA	0.124
	MSA-C	0.233 (0.071–0.758)	0.083	NA	0.234
rs2301134	MSA	6.045* (1.916–19.067)	0.008	1.905 (0.715–5.079)	0.289
	MSA-P	7.962* (1.731–36.619)	0.038	1.536 (0.490–4.818)	0.710
	MSA-C	4.128 (0.860–19.816)	0.164	2.677 (0.751–9.546)	0.209
rs2301135	MSA	7.000* (1.937–25.299)	0.006	2.208 (0.838–5.821)	0.215
	MSA-P	13.317* (1.688–105.050)	0.024	1.683 (0.533–5.314)	0.618
	MSA-C	3.841 (0.802–18.397)	0.201	3.212 (0.948–10.882)	0.157
rs2619362	MSA	0.273* (0.122–0.610)	0.007	NA	0.041
	MSA-P	0.313* (0.125–0.782)	0.050	NA	0.118
	MSA-C	0.213 (0.066–0.686)	0.108	NA	0.247
rs2619361	MSA	0.273* (0.122–0.610)	0.006	NA	0.040
	MSA-P	0.313* (0.125–0.782)	0.047	NA	0.112
	MSA-C	0.213 (0.066–0.686)	0.090	NA	0.241
rs3756063	MSA	7.883* (2.190–28.376)	0.007	2.576 (1.001–6.631)	0.104
	MSA-P	15.400* (1.960–120.995)	0.022	1.874 (0.620–5.662)	0.600
	MSA-C	4.125 (0.863–19.718)	0.160	4.122 (1.246–13.642)	0.100
rs2736990	MSA	2.449 (0.943–6.358)	0.116	2.550 (1.020–6.372)	0.117
	MSA-P	1.664 (0.597–4.635)	0.658	2.567 (0.927–7.110)	0.213
	MSA-C	7.941 (0.969–65.065)	0.093	2.520 (0.740–8.576)	0.238
rs11931074	MSA	11.952* (2.533–56.401)	0.006	NA	0.500
	MSA-P	13.500* (2.678–68.064)	0.022	NA	NA
	MSA-C	9.500 (1.558–57.929)	0.081	NA	0.250
rs181489	MSA	0.313* (0.135–0.723)	0.021	NA	1.000
	MSA-P	0.348 (0.133–0.910)	0.121	NA	1.000
	MSA-C	0.263 (0.084–0.816)	0.082	NA	1.000

Note. NA: OR estimate is impossible (one variant of compared genotypes / their combinations is missing in at least one of the groups) or significance level estimate is impossible (one variant of compared genotypes / their combinations is missing in both groups).

* Significant differences compared to the control.

3'-UTR region of the *SNCA* gene [12–14, 25]. It was predicted that rs11931074 would affect mRNA stability and translation efficiency [12, 13, 18, 25, 26]. The results of our study also showed that the rs11931074 minor allele (*T*) tended to increase the risk of PD (OR = 5.19; $p < 0.05$ ($p_{adj} = 0.6$).

Our sample demonstrated no associations with PD risk for the other 15 SNPs. It is likely explained by the heterogeneity of the PD group (both the clinical picture and the rate of disease progression), which precluded observing significant associations with specific disease forms. To assess the association of *SNCA* polymorphisms with PD, the PD groups should be specified by clinical, temporal, and demographic characteristics, as well as by the age at disease onset. Furthermore, the role of individual SNPs in the regulation of *SNCA* gene expression is population-dependent. For example, in the East Asian group, rs11931074, rs2736990, and rs356219 are associated with an increased risk of PD, while in the European group, rs11931074, rs356219, rs181489, rs2737029, and rs356165 are associated with an increased risk of PD [24, 27–30]. There are only rarely studies about the role of individual polymorphisms in *SNCA* contributing to the risk of PD in the Russian population. Thus, one study demonstrated an increase in α-synuclein levels in $CD45^+$ blood cells in the Russian PD group, which was significantly associated with rs356168 and rs356219 [31].

In the MSA group, 11 out of 16 analyzed *SNCA* polymorphisms were statistically significant ($p < 0.05$) for the risk. Our study focused on polymorphisms located in the promoter and 5'-UTR regions of the *SNCA* gene that affect the gene's transcriptional activity. Eight out of 9 SNPs in this region were associated with the risk of MSA in our patient cohort. Of these, an increased risk of MSA was observed for 4 SNPs (rs7687945, rs2301134, rs2301135, and rs3756063), which was confirmed only for patients with P-MSA in a dominant inheritance model, where a minor allele in both homozygous and heterozygous states influences the development of the disease. According to Y. Wei et al., a *G>C* nucleotide substitution in the rs3756063 polymorphism results in a CpG site in the promoter region, and affecting the DNA methylation status of *SNCA* may increase the risk of PD [32]. Another multi-center study found an association between rs7687945 and the age of PD onset [33]. In our study, another 4 SNPs (rs2619361, rs2619362, rs2619363, rs2619364), for which the significance of association with an increased PD risk has been confirmed in several studies [24, 34], showed a protective role of the minor allele of these SNPs against MSA ($p < 0.05$).

Of the two analyzed SNPs located in intron 4 (rs2737029, rs2736990), only the rs2736990 minor *A* allele showed a significant association ($p = 0.034$) with an increased risk of MSA. In the Asian population, rs2736990 is associated with PD risk but not with MSA risk [35]. In the literature, this SNP is linked with the enrichment of one of the *SNCA* splicing variants, called *SNCA-112*, which lacks exon 5 [36–39].

In our study, 5 SNPs (rs356165, rs356220, rs11931074, rs356219, rs181489) are located in the 3'-UTR region of the *SNCA* gene, of which only rs11931074 was statistically significant for MSA: the minor *T* allele was a risk factor for the disease progression (OR = 10.973; 95% CI 2.424–49.672; $p < 0.001$). Genotype analysis for this SNP showed an increased risk only for the P-MSA in the dominant inheritance model (OR = 13.5; 95% CI 2.678–68.064; $p = 0.022$). In multi-center studies in a European population, this SNP also showed a high risk of MSA: OR (recessive model) = 6.2 (95% CI 3.4–11.2; $p = 5.5 \times 10^{-12}$) [40] and OR (recessive model) = 9.32 (95% CI 4.03–21.55; $p < 0.00001$) [41]. However, it should be noted that in one GWAS study including 1,030 European patients with MSA, the risk of rs11931074 was not confirmed [42]. The authors attribute it to potential intrapopulation differences in the European group. No association between MSA and rs11931074 was found in the Asian population [27]. In addition, we found a trend toward a decreased risk of MSA with rs181489 (OR = 0.495; 95% CI 0.263–0.930; $p = 0.031$).

Despite the limited DLB sample in our study, we found a trend towards a decreased risk for 4 SNPs (rs2619364, rs2619363, rs2619362, rs2619361) and an association of rs1372519 with an increased risk of the disease.

Thus, we were the first to analyze a large group of polymorphisms in various regulatory regions of the *SNCA* gene for their association with the risk of developing three forms (PD, MSA, and DLB) in the Russian population. Our study had several limitations. First, the small sample size and individual groups may have reduced the power of the analysis, increasing the likelihood of missing significant associations. Second, some significant associations may have been diminished by strict adjustment for multiple comparisons. The results of risk assessment and its significance also depended on the population characteristics of the control group [40–42].

Considering the above-mentioned limitations, we established significant associations between *SNCA* polymorphisms and different forms of synucleinopathy based on the study findings. This study should be continued in larger, carefully defined cohorts in comprehensive research examining the role of genetic and epigenetic factors in the regulation of *SNCA* gene expression, taking into account population-specific features.

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Mechanisms of Neuromuscular Junction Dysfunction in Amyotrophic Lateral Sclerosis

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Abstract

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder characterized by the death of upper and lower motor neurons. Numerous studies show that structural and functional impairments of neuromuscular junctions (NMJ) occur as early as the presymptomatic stage of ALS. NMJ involvement is independent and one of the primary events in ALS pathogenesis. Aim: *to review the data on characteristics and mechanisms of NMJ dysfunction at pre- and postsynaptic levels in ALS patients and a transgenic animal model of the disease. Furthermore, we report on the dysfunction of perisynaptic Schwann cells and impaired mechanisms of motor neuron and skeletal muscle interaction in ALS, with a focus on reviewed publications on targeting of molecular mechanisms underlying NMJ dysfunction and disruption in ALS. The NMJ may be a potential target for novel therapeutic approaches for ALS.*

Keywords: *amyotrophic lateral sclerosis; neuromuscular junction; nerve terminal; skeletal muscle; motor unit*

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Механизмы развития дисфункции нервно-мышечных синапсов при боковом амиотрофическом склерозе

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Аннотация

Боковой амиотрофический склероз (БАС) является прогрессирующим нейродегенеративным заболеванием, характеризующимся гибелью верхних и нижних мотонейронов. В многочисленных исследованиях показано, что структурно-функциональные нарушения нервно-мышечных синапсов (НМС) при БАС развиваются уже на досимптомной стадии болезни. Поражение НМС является самостоятельным и одним из первичных патогенетических процессов при БАС. Цель *обзора – анализ научных данных о характере поражения и механизмах нарушения функционирования НМС на пре- и постсинаптическом уровне при БАС у пациентов и в модели данного заболевания на трансгенных животных. Кроме того, представлены сведения о дисфункции перисинаптических шванновских клеток и о нарушении механизмов взаимодействия мотонейрона и скелетной мышцы при БАС. Особое внимание уделено анализу научных работ, связанных с коррекцией молекулярных механизмов, лежащих в основе дисфункции и деструкции НМС при БАС. Сделан вывод о том, что НМС может выступать потенциальной мишенью для разработки новых терапевтических подходов при БАС.*

Ключевые слова: *боковой амиотрофический склероз; нервно-мышечный синапс; нервное окончание; скелетная мышца; двигательная единица*

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Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal, progressive neurodegenerative disorder characterized by the death of upper and lower motor neurons [1]. ALS mostly begins with focal muscle weakness and hypotrophy that spread to adjacent myotomes along the cerebrospinal axis. Approximately one third of ALS patients start with the onset of bulbar symptoms, while two-thirds of patients have limb-onset disease [2, 3]. As ALS progresses, it leads to atrophy and paralysis of skeletal muscles, including the diaphragm. Average survival rate from the first diagnosis is 3 years [4]. Classic ALS is characterized by simultaneous upper and lower motor neuron involvement at one or more levels of the cerebrospinal axis, whereas atypical forms, such as primary lateral sclerosis, predominantly involve either upper or lower motor neurons [2, 5].

ALS is considered a multifactorial disease, with genetic, environmental, and age-dependent risk factors underlying its onset and development [6–9].

There are several hypotheses for neurodegeneration development and spread in ALS. The dying-forward hypothesis suggests that hyperexcitability of upper motor neurons is one of the initial events leading to glutamate excitotoxicity and lower motor neuron involvement [10–12]. Through the lens of the dying-back hypothesis the neuromuscular junction (NMJ), skeletal muscle, and distal axon play a crucial role in neurodegeneration initiation and development [9, 13, 14]. Alternatively, some investigators propose that upper and lower motor neuron degeneration proceeds independently [15, 16]. The involvement is thought to be independent and one of the primary events in ALS pathogenesis [9, 17, 18].

Aim: to review the data on characteristics and mechanisms of NMJ dysfunction at pre- and postsynaptic levels in ALS patients and a transgenic animal model of the disease.

NMJ Structure

The NMJ is a specialized synapse that connects the distal axon of a motor neuron with a skeletal muscle fiber. Perisynaptic Schwann cells (PSCs) cap the NMJ and regulate its structure and function. All the 3 elements of the NMJ (nerve terminal, postsynaptic membrane, and Schwann cell) are thought to be involved in ALS pathogenesis [9, 11].

A motor neuron and its innervated muscle fibers form a functional unit known as a motor unit (MU). Based on their contraction velocity and fatigability, MUs are categorized into slow (S), fast resistant to fatigue (FR), and fast fatigable (FF) [19, 20]. FR and FF fibers are innervated by fast motor neurons, whereas S fibers, by slow ones. Fast motor neurons have a larger soma size and axon diameter, a more branched dendritic tree, lower excitability, a higher rate of action potential generation, and faster axon conduction [21–24]. Experiments using mouse models of ALS revealed that FF MU involvement can be detected as early as the presymptomatic stage [25]; signs of FR MU involvement can be observed at symptom onset, while S MUs are affected at the late stage of the disease [26].

Involvement of NMJs and MUs in Patients

There is considerable evidence that the NMJ is affected at early stages of the disease in both ALS patients and multiple ALS models. Examinations of muscle biopsy specimens from ALS patients revealed pronounced fragmentation of end plates and their denervation [27]. Electron microscopy in ALS patients demonstrated a decrease in pre- and postsynaptic areas as well as in the percentage of nerve terminal mitochondria [28]. Expression of acetylcholine receptor subunits within the postsynaptic membrane is also reduced in ALS patients [29]. The study of muscle biopsy specimens revealed an increased proportion of slow muscle fibers, indicating selective vulnerability of fast MUs [30]. Electrophysiological studies also confirm that fast MUs are predominantly affected [31], except for extraocular muscles, which are spared in ALS [32].

In ALS patients, NMJ denervation and axon retraction may precede motor neuron degeneration and occur while spinal motor neurons and ventral roots remain intact [33]. Electrophysiological techniques confirm the NMJ involvement in ALS patients: amplitudes of miniature end-plate potentials and quantal content of end-plate potentials were shown to be decreased in muscle biopsy specimens of ALS patients at the early stages of the disease [34].

Transgenic Animal Models of ALS

Animal models significantly expanded possibilities of studying pathogenesis mechanisms and developing ALS therapies. Transgenic mouse lines expressing ALS-associated mutant human genes are usually used as model animals. Thus, a number of transgenic mouse models with ALS-linked gene mutations were developed to study the disease: *SOD1*, *FUS*, *C9orf72*, and *TARDBP* [9, 35]. These ALS models replicate clinical features and key pathogenesis mechanisms quite well, serving as an effective tool for studying the disease.

A mutation in the *SOD1* gene encoding superoxide dismutase 1 was the first identified genetic cause of ALS [36]. The first transgenic mouse model of ALS was a line of mice expressing the human *SOD1* with a *G93A* mutation [37]. This model is one of the most studied; it is actively used for preclinical studies and contributed to the introduction of riluzole and edaravone in ALS therapy [35]. The *SOD1*(*G93A*) model reproduces most mechanisms of ALS pathogenesis and demonstrates progressive motor neuron degeneration leading to paralysis and death in transgenic mice at 4–5 months of age[37].

Transgenic ALS model associated with the expression of a mutant *FUS* (fused in sarcoma) gene is widely used. *FUS* gene encodes a nuclear RNA/DNA-binding protein FUS [38]. The first transgenic models based on the FUS expression appeared in the early 2010s [39–41]. FUS transgenic mice reproduce such pathological processes in human ALS as accumulation of intracellular FUS aggregates, progressive death of motor neurons, skeletal muscle denervation with the development of paralysis and atrophy [42].

There is a transgenic model of ALS expressing a mutant *TARDBP* gene that encodes the DNA/RNA-binding protein TDP-43 [43]. Postmortem tissue changes in ALS patients include affected neurons and glia of the brain and spinal cord, characterized by the loss of nuclear TDP-43 and cytoplasmic accumulation of insoluble phosphorylated TDP-43[8]. Several TDP-43 models of ALS have been developed, and different phenotypes have been obtained [9, 44].

A *C9ORF72*-based genetic model of ALS was created. This gene encodes a protein found in neurons and other cells and involved in signaling in the nervous system [9]. Mouse models expressing the human *C9ORF72* repeats exhibit various pathological, functional, and behavioral characteristics of ALS [45].

Presynaptic NMJ Disorders

SOD1 Model

In SOD1 mice, NMJ involvement can be observed as early as the presymptomatic stage, preceding the first signs of ALS in motor neurons [46, 47]. Prior to obvious signs of NMJ denervation at the presymptomatic stage, one can observe altered nerve terminal morphology, as well as vacuolization and swelling of mitochondria with their decreased number in the presynaptic membrane [46, 47]. The changes primarily occur in the FF MUs [26, 46–48]. This model replicates several key mechanisms of ALS development, such as impaired axonal transport and mitochondrial dysfunction. Similar abnormalities and a decreased number of synaptic vesicles in the SOD1 model develop at the presymptomatic stage selectively in FF MUs, while FR and S MUs remain intact [26]. FR MU involvement becomes evident in the early symptomatic stage, while S MUs are affected in the late stage of the disease [26].

Impaired expression of synaptic proteins could be observed in this model. In SOD1 mice at the presymptomatic stage, we detected a significant decrease in the expression of presynaptic proteins, such as SNAP-25 and synapsin-1; after symptom onset we additionally observed a significant decrease in the synaptophysin expression [49]. Among the studied presynaptic proteins, SNAP-25 showed the most pronounced change: its expression reduced by ~50% compared with wild-type mice [49]. This vulnerability could be caused by SNAP-25 sensitivity to oxidative stress [50]. Oxidative stress in the presynaptic membrane also develops at the presymptomatic stage due to the decreased number of mitochondria and aberrant mitochondrial morphology [51]. Because of impaired axonal transport, the motor neuron cannot compensate for mitochondrial dysfunction in the nerve terminal [26].

The same ALS model was found to have impaired neuromuscular synaptic transmission. SOD1 mice have decreased quantal content of end-plate potentials and prolonged synaptic vesicle recycling both before and after symptoms onset [52]. Amplitude and frequency of miniature end-plate potentials was observed to be altered, with impaired synaptic transmission first becoming evident in FF MUs [25]. In this model, synaptic vesicle docking to the presynaptic membrane is also impaired [46, 47], which may result from impaired SNARE complex formation due to decreased SNAP-25 expression [49].

FUS Model

In a transgenic model overexpressing the human *FUS* (*hFUS*), NMJ denervation is accompanied by the preserved number of spinal motor neurons at the presymptomatic stage [53]. FUS aggregates are observed to accumulate in the presynaptic membrane of the NMJ [53]. Ultrastructural analysis in FUS mice revealed a decrease in the number of synaptic vesicles and nerve terminal mitochondria and their morphological abnormalities at the NMJ, while the postsynaptic membrane remains relatively intact [53]. However, another model, $FUS^{\Delta NLS/+}$, displayed reduced postsynaptic membrane area [54]. The selective vulnerability of fast MUs is also characteristic of FUS mice [55].

The FUS model is observed to have impaired expression of presynaptic proteins. Thus, we detected increased expression of synaptic proteins SNAP-25 and synapsin-1 in transgenic FUS(1-359) mice at the presymptomatic stage [17], whereas at the symptomatic stage a significant decrease in the expression of SNAP-25, synapsin-1, and synaptophysin was observed. The enhanced expression of some presynaptic proteins at the presymptomatic stage may be caused by messenger RNA stabilization due to FUS accumulation in the presynaptic membrane, which may affect local protein translation processes in the synapse [56, 57].

In the FUS model, impaired neuromuscular transmission is observed as early as the presymptomatic stage. In the FUS(1-359) model at the presymptomatic stage, we found a decrease in the amplitude of miniature (spontaneous) and evoked end-plate potentials, as well as in the rise time and half-decay time of miniature end-plate potentials compared with wild-type mice. Furthermore, there was a more significant decrease in the amplitude of end-plate potentials during high-frequency activity

(20 Hz) and a slower recovery of this amplitude after the stimulation in FUS(1-359) mice compared with wild-type mice. The FUS(1-359) mice also showed a decrease in the intensity of synaptic vesicle endocytosis induced by high-frequency synaptic stimulation (20 Hz) compared with wild-type mice [17].

Another study of FUS mice revealed a decrease in the amplitude of evoked motor responses that precedes morphological changes in pre- and postsynaptic membranes of the NMJ and axons, followed by loss of motor neurons [55].

Not all transgenic TDP-43 mice reliably reproduce the neuromuscular phenotype with muscle weakness, amyotrophy, and NMJ denervation. However, the TDP-43^{Q331K} model shows signs of impaired synaptic transmission at the presymptomatic stage (increased amplitude and decreased frequency of miniature end-plate potentials), as well as signs of NMJ polyinnervation [58].

Postsynaptic NMJ Disorders

Specific changes are observed in the postsynaptic membrane in ALS models. Transgenic SOD1 mice demonstrated morphologic changes: shortening of the end-plate folds [47]. In the SOD1 model, the expression of crucial postsynaptic structural proteins, such as nestin, dystrophin, LRP4, and rapsin, which are responsible for end-plate morphology and acetylcholine receptor clustering, is impaired at the symptomatic stage [59].

FUS mice have reduced postsynaptic membrane area, and these changes can be detected both at the presymptomatic stage [54] and only at the symptomatic stage [17]. Such changes are likely due to a direct effect of FUS on the expression of acetylcholine receptor subunits when FUS accumulates in the subsynaptic nuclei of skeletal muscle fibers [54].

Previously, the changes in skeletal muscles were thought to be secondary and solely the result of motor neuron degeneration. However, a number of studies suggest otherwise. Thus, in case of *SOD1* mutations, the accumulation of mutant superoxide dismutase 1 aggregates is observed in skeletal muscle at the presymptomatic stage [60] and leads to mitochondrial damage, resulting in impaired morphology and reduced number of mitochondria in the postsynaptic membrane at the presymptomatic stage, and oxidative stress [51]. The independent role of skeletal muscle in the ALS pathogenesis is also supported by the fact that, despite the prevention of spinal motor neuron death and their preserved number owing to p38 MAPK inhibitor, skeletal muscle denervation and atrophy still develop [46, 61].

Skeletal muscle can also act as a direct aggressor within the ALS pathogenesis. Selective overexpression of mutant *SOD1* results in NMJ involvement, distal axonopathy, and likely corticospinal tract damage, as evidenced by hyperreflexia and spasticity [62]. The impact of skeletal muscle may be mediated by the secretion of extracellular vesicles, which may exert neurotoxic effects by negatively affecting motor neuron survival and inhibiting axon growth [63, 64].

Skeletal muscle may also contribute to NMJ denervation by secreting Nogo-A factor, which is a chemorepellent, more properly a substance that repels the axon growth cone. This prevents effective reinnervation of the NMJ and contributes to progressive denervation of skeletal muscle [65]. The expression of this factor is elevated in ALS patients, with the level of expression correlating with the rate of disease progression [66]. Meanwhile, antibodies against Nogo-A notably delay disease progression in an ALS model [67].

Skeletal muscle metabolism is elevated in mSOD1 mice, leading to chronic energy deficits observed prior to amyotrophy and muscle denervation. Energy deficit and muscle hypermetabolism can lead to NMJ disruption, skeletal muscle denervation, and motor neuron death [68]. Dietary modification (a fat-enriched high-energy diet) extended life expectancy and motor neuron survival in a mouse model of ALS [69].

Involvement of PSCs in ALS

Apart from changes in the pre- and postsynaptic compartments of the NMJ, ALS patients also show pathological changes in the terminal PSCs [66]. The morphology of these cells is altered; outgrowth and intrusion in the synaptic cleft significantly reduce the available surface area of the postsynaptic membrane for neuromuscular transmission.

The SOD1(*G37R*) model showed that PSCs cannot produce an adequate response to NMJ degeneration (adoption of a phagocytic phenotype), nor can they guide nerve terminal sprouts. This impairs compensatory reinnervation and contributes to progressive denervation [70].

The SOD1(*G93A*) model revealed selective loss of PSCs and their macrophage infiltration in fast MUs at the presymptomatic stage [25, 71]. This observation also correlated with a reduced capacity of motor neurons innervating fast muscle fibers to reinnervate. The PSC involvement was also noted in the TDP43 model of ALS [72].

Moreover, the SOD1(*G93A*) model of ALS showed that PSCs in FF MUs are capable of *de novo* expression and secretion of the chemorepellent semaphorin 3A (Sema3A), which, like Nogo-A, repels the axon growth cone and leads to denervation, thereby contributing to the selective vulnerability of FF MUs [73]. R. Maimon et al. found that elevated Sema3A levels correlate with muscle denervation, with inhibition of Sema3A expression reducing the severity of NMJ and axon degeneration [74].

The role of PSCs in the ALS pathogenesis is also indirectly evidenced by the fact that masitinib administration in transgenic mice prevented loss of PSCs and delayed the disease [71]. At the same time, masitinib in combination with riluzole showed significant efficacy in ALS patients [75].

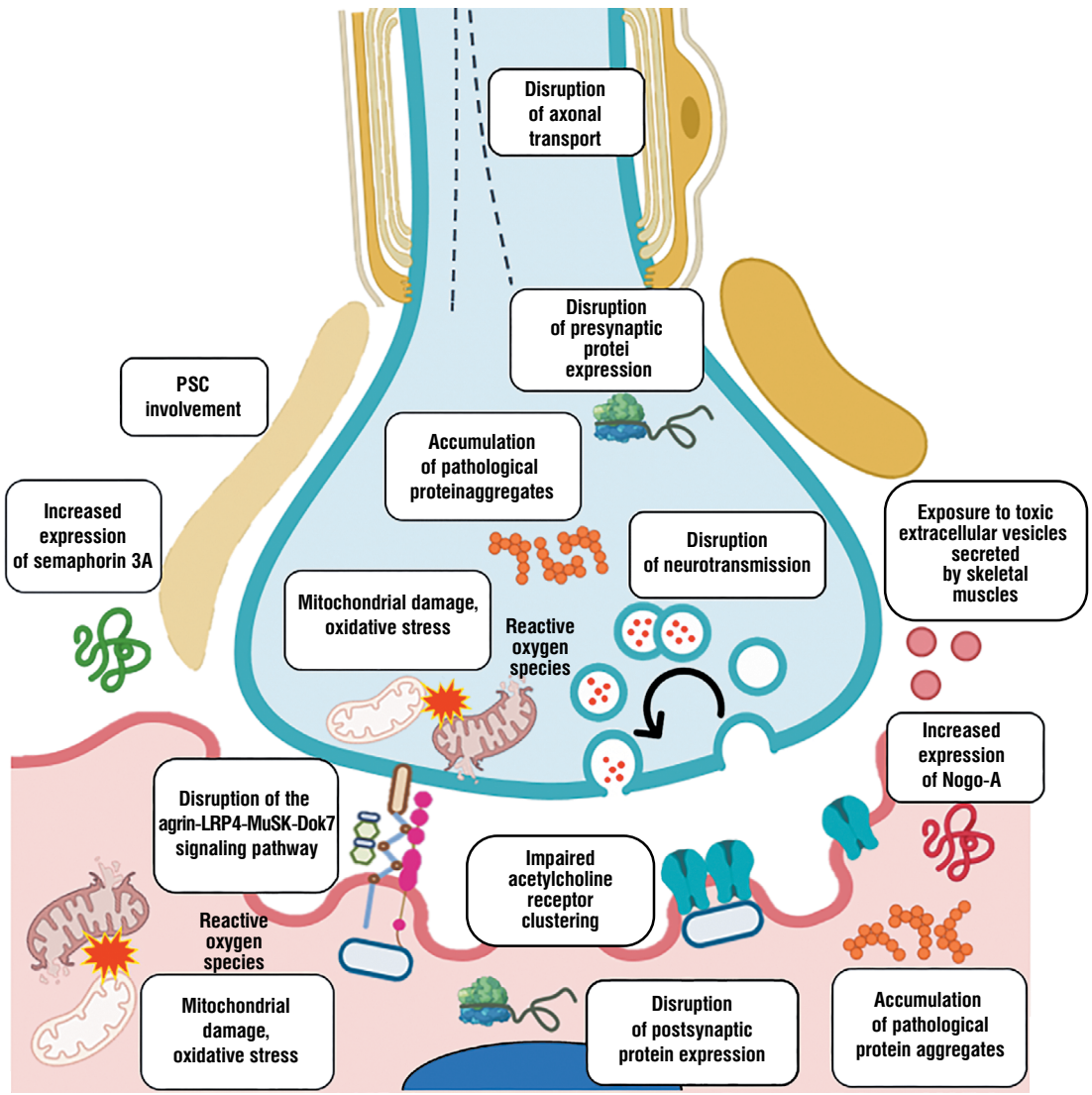
Impaired Mechanisms of Interaction Between Motor Neurons and Skeletal Muscles

In ALS, there are specific changes in each part of the NMJ: pre- and postsynaptic compartments, as well as the surrounding PSCs. Such changes inevitably lead to disruption of the motor neuron–skeletal muscle interaction, which in turn contributes

to further disease progression. Normally, when a motor neuron and a muscle fiber form a functional synapse, the formed MU begins to secrete a number of trophic and growth factors that ensure motor neuron survival, axon growth and regeneration, structural and functional stability of NMJs, differentiation and contractile properties of muscle fibers [76]. Such a secretome contains high concentrations of vascular endothelial growth factor, glial neurotrophic factor, brain-derived neurotrophic factor, neurotrophins-3 and -4, insulin-like growth factor-1, and insulin-like growth factor-3 binding protein. Innervated skeletal muscle was found to actively express the muscle-specific microRNA miR-206 [77]. miR-206 is thought to play a protective role by ensuring the survival of synaptic contacts and sprouting activity. High expression levels of miR-206 in ALS patients are associated with a slower rate of disease progression [78].

Disruption of the agrin-LRP4-MuSK signaling pathway may play a key part in NMJ involvement. Motor nerve terminals secrete agrin and low-density lipoprotein receptor-related protein-4 (LRP4), whereas skeletal muscle synthesizes rapsin, muscle-specific tyrosine kinase (MuSK), and the adaptor protein Dok-7. The interplay of these factors maintains the normal structure and functioning of the NMJ [79]. The signaling pathway regulates the acetylcholine receptor clustering on the postsynaptic membrane of the NMJ through a complex interaction of 3 proteins [80].

Internal processes in skeletal muscles may lead to disruption of the agrin-LRP4-MuSK signaling pathway. Thus, muscle fibers derived from induced pluripotent cells of ALS patients do not form functional NMJs with axons of healthy motor neurons, and there is no acetylcholine receptor clustering on the postsynaptic membrane in response to secreted agrin [30]. Disruption of MU functioning and integrity in such a case will inevitably lead to a deficiency of neurotrophic and growth factors, which will only contribute to



Pathogenic mechanisms of NMJ dysfunction in ALS and an ALS model.
The image was created with BioRender.com.

further disease progression [76]. The C9orf72 model of ALS demonstrated that poly(GA)-peptides formed as a result of the mutation inhibit the agrin-LRP4-MuSK signaling pathway, which leads to impaired neuromuscular transmission and damage to the pre- and postsynaptic membrane of the NMJs [81]. The SOD1(G93A) model revealed impaired MuSK transport into the postsynaptic membrane, resulting in NMJ involvement [82].

Activation and normalization of the agrin-LRP4-MuSK signaling pathway may have a positive effect on the ALS course. For instance, agrin overexpression in the TDP-43 model can prevent motor neuron death and preserve NMJs [83]. MuSK activation in this signaling pathway also has a beneficial effect by delaying denervation, promoting motor neuron survival, and increasing the lifespan of SOD1(G93A) transgenic mice [84–86]. Dok7 activation in the signaling pathway is also beneficial in terms of reducing the severity of NMJ degeneration and muscle atrophy, prolonging lifespan, and improving motor skills in the SOD1(G93A) transgenic model [87].

Conclusion

NMJ damage is an independent and early event in the ALS pathogenesis, as evidenced by the data from the studies in both transgenic animal models and ALS patients (Fig.). We should note that as early as the presymptomatic stage, a number of functional and structural disorders of the NMJ are observed in ALS models. All models with NMJ denervation demonstrated selective vulnerability of FF MUs in the early stages of the disease. In many models, the presynaptic compartment has been shown to be more vulnerable than the postsynaptic compartment. The identified functional disorders of the NMJ in ALS (according to the transgenic animal models data) indicate a decrease in the reliability of neuromuscular transmission both at low and high frequency. Structural abnormalities of NMJs in ALS include decreased area and fragmentation of synaptic contacts, altered expression of some synaptic proteins, etc.

Targeting molecular mechanisms underlying the dysfunction and destruction of NMJ in ALS garners a lot of interest. The NMJ may become a potential target for novel therapeutic approaches for ALS. We reviewed a number of quite successful attempts to modulate signaling pathways disrupted in the motor neuron–skeletal muscle system in ALS models [65–67, 73, 74, 77, 78].

Based on the findings obtained in transgenic animals, therapeutic methods aimed at increasing the agrin and miR-206 expression, activating MuSK, and suppressing the Sema3 and Nogo-A expression could potentially be quite effective in ALS. In addition to further study of the therapeutic potential of modulating the above-mentioned molecules, the possibility of their combination with drugs already in use (riluzole, edaravone) should be investigated to improve the efficacy of ALS therapy.

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Potential Biochemical Markers of Epilepsy

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Abstract

The diagnosis of epilepsy and assessment of the frequency and severity of seizures are essential for the treatment of patients. Epileptogenesis monitoring at different stages can be beneficial in assessing the efficacy of antiepileptic therapy. This approach relies on the concept of biomarkers. A subset of these biomarkers may possess not only diagnostic value but also prognostic value, which is defined as the ability to predict the nature of the epilepsy course and the probability of recurrent seizures.

Keywords: *epileptogenesis; biomarkers; epilepsy*

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Потенциальные биохимические маркеры эпилепсии

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Аннотация

Диагностика эпилепсии, оценка частоты и тяжести эпилептических приступов являются неотъемлемыми условиями лечения больных. Мониторинг эпилептогенеза на разных стадиях обеспечивает контроль эффективности противоэпилептической терапии. Базовым понятием такого подхода является категория биомаркеров, некоторые из них могут иметь, помимо диагностического, и прогностическое значение, которое заключается в возможности предсказать характер течения эпилепсии и вероятность возникновения повторных эпилептических приступов.

Ключевые слова: *эпилептогенез; биомаркеры; эпилепсия*

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Introduction

In a mechanistic context, epileptogenesis is defined as the process by which the brain's neural network undergoes reorganization, accompanied by increased susceptibility to seizures and the probability of spontaneous recurrent seizures [1].

Previously, epileptogenesis was considered to be represented by the latent period, the time between the epileptogenic insult (epileptogenesis had been studied mainly in models of post-traumatic or post-stroke epilepsy) and the appearance of the first unprovoked seizure [2].

Subsequent studies have shown that epileptogenesis is progressive process. The phenomenon of "kindling," defined as an escalation in neuronal activity triggered by repetitive electrical or chemical stimuli, underscores the notion that recurrent seizures may amplify the probability of subsequent seizures. This view is consistent with the theory that "seizures beget seizures" and further neuronal death proposed by W. Gower in 1885 [3]. Concurrently, some authors have critically reviewed existing data on the effects of recurrent seizures on brain neural networks. On the one hand, epileptic activity causes molecular, structural, and functional changes, including neuronal loss, circuitry reorganization, and metabolic changes that may contribute to disease progression. On the other hand, seizure remission in two thirds of epilepsy cases and various chronic epilepsy animal models oppose the theory. Experimental studies showed that seizures could induce neural changes that increase the seizure threshold and decrease the risk of a subsequent seizure [2].

At the cellular level, epileptogenesis includes:
- dramatic decrease in the number of neurons and synapses;
- astrogliosis;
- microglial activation;
- agiogenesis [4].

Recent decades have brought dramatic changes in the understanding of the molecular dysfunction cascade in epilepsy, to which various dynamic processes contribute: formation of excitatory synapses; ion homeostasis imbalance; compromised blood-brain barrier (BBB) integrity; glymphatic dysfunction; and accumulation of proinflammatory cytokines along with amyloid and phosphorylated tau proteins [4].

Epileptogenic factors are believed to initially lead to selective neuronal vulnerability. However, this theory can be refuted by a study in which hyperthermia-induced epilepsy is not associated with neuronal loss [2]. A key mechanism underlying epileptogenesis is the loss of inhibitory interneurons [5].

Activated astrocytes play a pivotal role in the dysfunction of the BBB and glymphatic system, mediated through the release of proinflammatory cytokines, and facilitate the abnormal neuronal excitability. Astrocytes release gliotransmitters, such as glutamate, which alter synaptic activity and further contribute to the neuronal imbalance between excitation and inhibition characteristic of epileptic circuits [6].

BBB disruption triggers albumin accumulation in the extracellular space. By activating transforming growth factor beta (TGF-β) receptors on astrocytes, albumin activates TGF-β signaling pathways, TGF-β formation, and increased astrocyte activity, which causes dysregulation of potassium and glutamate content in the cells and further enhancement of proinflammatory cytokines (interleukin-1β and -6) secretion. The expression of astrocytic inwardly rectifier potassium channels (mainly Kir4.1 subunits, which regulate inward passage of potassium ions) has been shown to decrease in epileptogenic regions during epileptogenesis, which causes neuronal hyperexcitability [7]. This condition is exacerbated by decreased levels of amino acid transporters. Matrix metalloproteinases that degrade tight junctions of the BBB (due to destruction of dystroglycan, a protein that anchors astrocyte endfeet to the vascular basement membrane, accompanied by leukocyte infiltration of brain tissue) and increase the release of proinflammatory cytokines also play a certain role in the BBB destruction [8, 9]. During epileptogenesis, the clearance of cytokines regulated by the glymphatic system is impaired due to dysfunction of perivascular spaces [4].

The vicious circle of epileptogenesis includes astrogliosis, which contributes to aquaporin-4 (AQP4) dysregulation. The fundamental role of AQP-4 is maintaining water homeostasis and contributing to potassium buffering in the brain, i.e. prevention of excitotoxicity. Dysregulated AQP-4 expression can lead to ion imbalances in the extracellular environment, promoting neuronal hyperexcitability and increasing the risk of recurrent seizures [10].

The mechanistic target of rapamycin (mTOR) signaling pathway is one of the potential metabolic pathways of epileptogenesis. Studies have shown that the mTOR pathway is involved in epileptogenesis in genetic epilepsies and tuberous sclerosis. The mTOR pathway regulates synaptic plasticity, ion channel expression, and programmed cell death. The mTOR dysfunction (impaired cell proliferation, synaptic plasticity, ion channel expression) that occurs during pathologic processes in brain tissue leads to epilepsy. Hyperactivation of the mTOR signaling pathway has been observed in genetic animal models of epilepsy [11].

According to the basic definition, a biomarker is a characteristic that can be objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention [4].
According to the purpose, all epilepsy biomarkers are subdivided into:
1) Biomarkers that define a group of patients at high risk of disease development (high predisposition to epilepsy).
2) Diagnostic biomarkers.
3) Monitoring biomarkers that characterize the disease severity and enable prediction of the progression probability and prognosis.
4) Biomarkers whose function is to evaluate the efficacy and safety of antiepileptic therapies and methods in animal experimental models.
5) Biomarkers used to optimize the selection of homogeneous groups of patients for clinical trials.

The study of biomarkers in epilepsy encompasses several domains, including risk stratification, the diagnostic process, the assessment of severity, and prognosis determination. It also covers clinical trials of new agents and medical technologies. Evaluation criteria for diagnostic tests include rapidity of performance, reliability of the results obtained, the ability to use diagnostic information to optimize treatment programs, ease of use, high sensitivity and specificity. To date, no epilepsy marker that satisfies all of the aforementioned criteria has been identified. Consequently, the prevailing approach involves the study of potential candidates using a multiparametric detection method [4].

Biochemical Markers in Epilepsy

Amphoterin

Amphoterin (high mobility group box-1, HMGB1) participates in the immune response through binding to Toll-like receptor 4 (TL4) and releases tumor necrosis factor-α (TNF-α), interleukins (IL)-1 and -6 by activating macrophages and endothelial cells. In addition, by stimulating TL4 and neutrophils HMGB1 induces oxidative stress. In the central nervous system (CNS), HMGB1 mediates microglial activation. HMGB1 serum levels increase within 3–4 h post-seizure [12]. Overexpression of P-glycoprotein under the influence of HMGB1 is associated with the development of pharmacoresistance [13].

MicroRNA

MicroRNAs (miRNA) are a group of non-coding, single-strand, endogenous molecules. miRNAs are involved in both physiological (cell division, cell cycle control, cell differentiation, apoptosis, angiogenesis) and pathological processes through regulation of homeostasis [14].

There is evidence that miRNAs are modulators of the immune response [15], are involved in the destruction of the BBB [16], and activate oxidative stress by enhancing the expression of enzymes that induce the formation of reactive oxygen species (ROS) [17].

Elevated levels of miRNA-23a, -34a, -132, and -146a have been found in epilepsy [18]. miRNA-4521 and -301a-3p have been reported as potential markers of pharmacoresistant epilepsy [19].

Aquaporins

Aquaporins are a group of membrane proteins involved in the transport of water and ions across the cell membrane. Through their function, aquaporins regulate water homeostasis, cell migration, and inflammation.

M.M. Salman et al. discovered high levels of AQP4 in brain tissue samples obtained during amygdalohippocampectomy [20].

Elevated AQP4 levels were found in resection samples of epileptic temporal cortex [21]. G.T. Manley et al. hypothesized a relationship between AQP4 and drug-resistant epilepsy. The water-electrolyte (especially potassium ion) imbalance in astrocytes leads to the release of potassium ions from the neuropils into the intercellular space, where they are uptaken and deposited by astrocytes. Osmotic swelling of astrocytes, which reduces the extracellular space volume, increases epileptiform activity.

Glial fibrillary acidic protein

Glial fibrillary acidic protein (GFAP) is a type III intermediate filament protein that is expressed by numerous cell types of the CNS, including astrocytes. GFAP is a marker of astrogliosis that develops in hippocampal sclerosis [22–24].

Matrix metalloproteinase

Matrix metalloproteinase-9 (MMP9) is a zinc-dependent endoprotease that is actively involved in extracellular matrix degradation, neuroinflammation, BBB function, and synaptic plasticity. During a seizure, high cytokine expression stimulates MMP9 activation, which is accompanied by the degradation of the extracellular matrix and the BBB disruption [25–27].

Cytokines

Cytokines are specific proteins produced by glial cells and neurons during neuroinflammation. Pro-inflammatory cytokines (IL-1β, -2, -6) have been found in negligible amounts in the CNS. In clinical studies, IL-1β, IL-6 and TNF-α levels have been shown to be significantly elevated in febrile convulsions [28]. Overexpression of IL-1β by microglia and astrocytes is believed to enhance glutamate accumulation and neuronal excitability.

Brain-derived neurotrophic factor

Brain-derived neurotrophic factor (BDNF) is the most sensitive indicator of neuroplasticity. BDNF synthesis, processing, or transport disorders can lead to a variety of neurological diseases, including Alzheimer's disease, Huntington's disease, and epilepsy. BDNF circulates in the blood, does not cross the BBB, and is deposited in platelets and leukocytes. Increased expression of BDNF and its receptor TrkB has been reported in temporal lobe epilepsy and hippocampal sclerosis [29].

Glial cell-derived neurotrophic factor

Glial cell-derived neurotrophic factor (GDNF) is involved in the development and maintenance of neurons and gliocytes. It is expressed in neurons and binds to GDNFα-1 receptors. GDNF/GDNFα-1 complex conducts signals to nigrostriatal dopaminergic neurons, motor and sensory neurons, supporting their survival. In epilepsy, GDNF is believed to be initially synthesized in activated astrocytes and microglia and subsequently detected in cerebrospinal fluid [30].

Biomarkers of neurodegeneration

The frequent hippocampus involvement in the epilepsy pathogenesis has prompted a substantial number of studies investigating the relationship between epilepsy and dementia [31, 32].

There is evidence that tau protein and beta-amyloid are not only products of neurodegeneration but are also involved in epileptogenesis [33]. The potential relationship between these diseases may be attributable to the glutamate neurotoxicity. Beta-amyloid promotes secretion and accumulation of glutamate in the synaptic cleft, leading to activation of intracellular calcium and phosphorylated tau protein production [34]. The dysregulation of calcium-mediated pathways has been demonstrated to increase neuronal excitability and accelerate neurodegeneration.

S100β

S100β is a glial-derived protein, a member of the S100 family of calcium-binding proteins. At low concentrations, S100β stimulates astrocyte proliferation and modulates functional rearrangement of synapses, exerting a neurotrophic effect. At high concentrations, S100β has a toxic effect on astrocytes, induces neuroinflammation, and promotes epileptogenesis [35]. There is strong evidence that elevated S100β levels are associated with the severity and prognosis of epilepsy [36, 37]. High S100β expression in the acute phase of stroke is a marker of post-stroke epilepsy [38].

Neuron-specific enolase

Neuron-specific enolase (NSE) is a dimeric glycolytic enzyme composed of 3 subunits and 5 isoenzymes (αα, ββ, γγ, αβ, and αγ). The αα isoenzyme is known to be found in glial cells, whereas γγ enolase is neuron-specific. NSE is a marker of neuronal death in stroke and hypoxia [39].

Elevated NSE levels have been found post-seizure and in status epilepticus [40, 41]. However, there is evidence that there is no association between temporal lobe epilepsy and NSE levels [42, 43].

NSE is also found in platelets and red blood cells; therefore, evaluating NSE levels in the blood may not be accurate in hemolysis.

Ubiquitin carboxy-terminal hydrolase L1

Ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) is an enzyme found in large quantities in neurons. UCH-L1 levels are associated with neuronal death and increased BBB permeability. UCH-L1 enters the bloodstream shortly after brain damage and therefore may be a potential biomarker of epilepsy. Despite the paucity of studies, there has been evidence of increased blood levels of this enzyme both in patients with a history of recurrent epileptic seizures [44] and post-seizure [45, 46].

Visinin-like protein

Visinin-like protein 1 (VILIP-1) is a neuron-specific calcium-binding protein. It was previously studied as a biomarker of stroke, Alzheimer's disease, and traumatic brain injury. In the study by M.A. Tikhonova et al. no association between VILIP-1 levels in hippocampal preparations and blood was observed; however, this study was performed on a small sample of patients [47]. On the contrary, Z. Tan et al. found that VILIP-1 levels were positively associated with severity of epilepsy [48].

Conclusion

The identification and validation of potential biochemical markers is of paramount importance for elucidating the pathogenesis of epilepsy and establishing laboratory methods for its diagnosis. Furthermore, this may also serve as a foundation for identifying targets for antiepileptic therapy.

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Pharmacological Functional MRI Technology: Potential for Use in Neurology

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Abstract

This review presents recent data on one of the most promising neuroimaging techniques, pharmacological functional magnetic resonance imaging (phFMRI). PhFMRI technologies are described as well as task-based approaches inducing neuronal activation in the areas of interest when evaluating the effects of neuroactive agents. We reviewed the potential use of phFMRI in various neurological disorders such as cerebrovascular disease and epilepsy, as well as in the management of metabolic disorders, cognitive impairment, pain syndrome, etc. Limitations of phFMRI and possible ways to address them in designing and conducting studies are presented. The potential uses of phFMRI for the objective assessment of the targeted effects of pharmacological agents are suggested.

Keywords: *pharmacological magnetic resonance imaging; functional magnetic resonance imaging; targeted effects; paradigm; personalized medicine*

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Технология фармакологической функциональной МРТ: потенциал использования в неврологии

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Аннотация

В обзоре представлены современные данные об одной из перспективных нейровизуализационных методик – фармакологической функциональной магнитно-резонансной томографии (фарм-фМРТ). Описаны технологии проведения фарм-фМРТ, варианты применения парадигмы в качестве триггера нейрональной активации зон интереса при изучении эффектов нейроактивных препаратов. Рассмотрены потенциальные возможности применения фарм-фМРТ при различных неврологических состояниях, таких как цереброваскулярные заболевания, эпилепсия, а также в отношении коррекции метаболических расстройств, когнитивных нарушений, болевого синдрома и др. Представлены ограничения применения фарм-фМРТ, возможные пути их преодоления при планировании и проведении исследований. Предложены перспективы применения фарм-фМРТ, которые позволят дать объективную оценку таргетного воздействия фармакологических агентов.

Ключевые слова: *фармакологическая магнитно-резонансная томография; функциональная магнитно-резонансная томография; таргетное воздействие; парадигма; персонифицированная медицина*

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Introduction

Due to the challenges of developing and evaluating agents that affect the central nervous system (CNS), techniques and approaches are needed to make a translational leap from preclinical models to predicting clinical effects in patients [1]. *In vitro* and *in vivo* laboratory studies characterize various pharmacological properties of study molecules, but efficacy data from such studies (especially for neuroactive agents) should be interpreted with particular caution in the context of the human population. There is currently no generally accepted reference for determining the effect of agents on the CNS, whether they are neuroprotective, antidepressant, antipsychotic, etc. [2]. Neuropharmacological functional magnetic resonance imaging (FMRI) of the brain may be one of the most promising techniques.

FMRI technologies using relative cerebral blood flow (rCBF), blood oxygen level dependent (BOLD) signal [4] or T1-weighted cerebral blood flow quantification [5] have revolutionized brain mapping [6]. All of these approaches are based on the interplay between neuronal activity, metabolism, and hemodynamics, parameters that are sensitive to changes in MR signal intensity. Although all of these techniques can be considered functional, the term "FMRI" traditionally refers to the assessment of the BOLD signal.

FMRI typically uses a specific paradigm (e.g., visual stimulation, finger movements, cognitive tasks, etc.) as a trigger for neural activation in the areas of interest (so-called task-based fMRI). However, a similar effect can be achieved using various types of pharmacological agents, both as a direct stimulant and as a mediator that modulates the brain response to another paradigm (e.g., cognitive). In 1997, Y.C. Chen et al. called this type of FMRI "pharmacological MRI" (phMRI) [7]. In mouse experiments, they evaluated the regional selectivity of dopamine ligands (although a conceptually similar approach was implemented by others at least since 1993 [8, 9]).

In early-phase clinical trials, FMRI techniques can demonstrate the functional effects of a pharmacological agent on the CNS in those regions of the brain that are etiologically and/or pathogenetically relevant to the biochemical mechanisms [10]. It should be noted that, technically, we are not talking about markers of target action (e.g., visualization of the agent binding to a corresponding site), but rather about indirect evidence of the effect between the fMRI response and the biological plausibility of the agent's action. Dose-dependent associations identified by FMRI may be valuable in planning further stages of research or clinical implementation of the agent [11].

In later-phase clinical trials, phFMRI findings will be likely used to demonstrate normalization of disease-related MR signal changes (e.g., activation/deactivation of specific brain regions in response to a paradigm or changes in functional connectivity). This may be potentially considered a more objective assessment of the change in CNS impairment.

PhFMRI can also be used to determine the cerebral targets of investigated pharmacological compounds, to clarify expected/unexpected mechanisms of action, to identify dose-dependent responses, and to provide valid markers of therapeutic response (including for clinical trials) [12]. Since the development of phFMRI, a rather wide range of neuroactive molecules has been studied in experimental and/or clinical models, both chemical compounds (nicotine, amphetamine, etc.) and therapeutic agents (neuropeptides, cholinergic, serotonergic and glutamatergic agents, cannabinoids, opioids, etc.) [14].

Several criteria should be met for phFMRI to be used as a relevant diagnostic and research tool (including in pharmaceutical development):

1) PhFMRI data should be reproducible and should change with the effects of a pharmacological agent.
2) Quantitative phFMRI characteristics (based on equipment) should be standardized.
3) Prior to study initiation, the specific features of phFMRI performance and analysis should be determined.
4) For further research, selected fMRI techniques should be available in multiple centers (e.g., type of pulse sequences, voxel sizes, slice thickness, temporal resolution, deflection angle, and selected paradigms should be similar for all MR examinations).
5) An MRI technician should be engaged in the FMRI process (e.g., in case of excessive head movement, a repeat scan should be performed).
6) Quality control should be performed at all stages (DICOM[1] verification of protocol compliance, artifact identification, etc.).

[1]DICOM (Digital Imaging and Communications in Medicine) is a medical industry standard for the creation, storage, transmission, and visualization of digital medical images and documents of examined patients.

The ability of pharmaceutical agents to induce short- and long-term changes in the FMRI signal is one of the most important reasons for the phFMRI investigation. Multiple studies published suggest that FMRI findings may be sensitive to both short- (i.e., after the first dose) and long-term (chronic, after multiple dosing) pharmacotherapy. For example, several studies show that the FMRI response of the amygdala increases when patients with depression are exposed to photographs of faces with negative emotions, while the use of clinically effective doses of antidepressants normalizes this response [15, 16]. Other groups of agents thought to produce changes in phFMRI signals include analgesics, antipsychotics, calcium channel blockers, cyclooxygenase-2 inhibitors, and immunotherapy.

Many targets for neuroactive molecules have been identified in previous experimental studies (e.g., positron emission tomography). The cumulative effect of phFMRI per examination can be classified as specific (i.e., directly related to receptor activation) and general or non-specific (related to side effects that may affect the FMRI signal intensity).

PhFMRI can identify single points of agent application. Similar patterns of FMRI activation can be observed when agents with the same indication (e.g., pain) but radically different mechanisms of action (e.g., non-steroidal anti-inflammatory agents, opioid analgesics, etc.) are used. In addition, the functional status (i.e., FMRI characteristics) of certain brain structures may serve as predictors of therapeutic response, as has been shown for pregabalin in the insula and inferior parietal lobule [17].

Technology from a current perspective

The echo-planar gradient echo sequenceis the most commonly used technique to obtain the BOLD signal. This sequence is sensitive to local magnetic field inhomogeneities, such as those caused by paramagnetic substances such as deoxyhemoglobin, in the presence of which the signal is attenuated [5]. When excitation occurs in a particular region of the brain, a local increase in blood flow to that area is observed, leading to increased oxyhemoglobin levels and decreased deoxyhemoglobin levels. As a result, a change (increase) in the signal is observed and recorded using the sequence described. Therefore, FMRI records the distribution of neuronal activity, which is indirectly assessed by the change in the signal as a function of the blood oxygenation level in the cerebral vessels. In each voxel, signal oscillations during the scanning time are recorded and then evaluated using various statistical tools with the subsequent possibility of data group presentation as well as between group and within group analysis (Fig. 1).

Signal oscillations occur not only during task performance (task-based FMRI), but also spontaneously at resting state. Due to internal neuronal activity, such oscillations are recorded during resting-state FMRI [18], i.e., when a patient is scanned without being presented with stimuli of any modality. When BOLD signal oscillations are similar and correlate between gray matter regions, there is a high probability that these regions are functionally related. Based on this claim, several resting-state brain networks have been described using different methods of mathematical analysis. This technique has some advantages over paradigm-based FMRI, including the ability to perform an examination even when a patient is unable to understand or perform a task, and the elimination of the need to use multiple devices to present stimuli, thus reducing labor and cost. However, processing such data is more complex and error-prone due to multiple physiological noises.

For phFMRI, both techniques are used [19, 20]. Administration of a pharmaceutical agent or other substrate that has the potential to alter functional brain activity may affect the BOLD signal at both the vascular and neuronal levels, impeding interpretation of the results. The signal changes associated with the substance administration are insignificant. This justifies studying its effects in the context of paradigm-based FMRI signal changes and comparing stimulus-related activation areas in subjects receiving a substance with activation areas and those not receiving a substance or receiving a placebo. In addition to being a simpler technique, resting-state FMRI has the advantage of evaluating the effect of the administered substance at the network level, even far from the expected area of maximum concentration of substance receptors or the expected area of activation/deactivation [10].

However, for a valid phFMRI, some pharmacokinetic and pharmacodynamic aspects of the administered substances should be considered, such as time to maximum blood concentration, half-life, and cumulative effect, in order to perform the examination when the maximum substance effect on the human body is achieved. In addition, the administration of other substances that may interact with the study substance or may alter the functional activity of the brain should be considered [14]. These data are used to calculate the time of examination after the study substance administration and the time of examination after the course of therapy.

PhFMRI in different areas of neurology

Cerebrovascular diseases

The available data on the use of phFMRI in cerebrovascular disorders are limited and mainly relate to chronic cerebrovascular diseases and neuroprotective agents. One of the pilot studies in this area was conducted by the Research Center of Neurology. For example, in a 2010 study, course treatment using an agent with a reported neuroprotective effect was associated with the expansion of existing activation areas and/or the development of new activation areas, primarily in the parietal occipital region, which was accompanied by improved performance in basic cognitive tests [21]. In contrast, a year later, a study of a neuropeptide showed a reduction in activation areas (especially in the temporal and frontal lobes) in response to an original cognitive paradigm developed at the Research Center of Neurology [22]. An indirect phFMRI comparison of several potential neuroprotective agents suggested the main targeted mechanisms of action and phFMRI patterns, such as cerebroactivating effect, improved microcirculation, reduced brain energy expenditure, and neurometabolic effect [23].

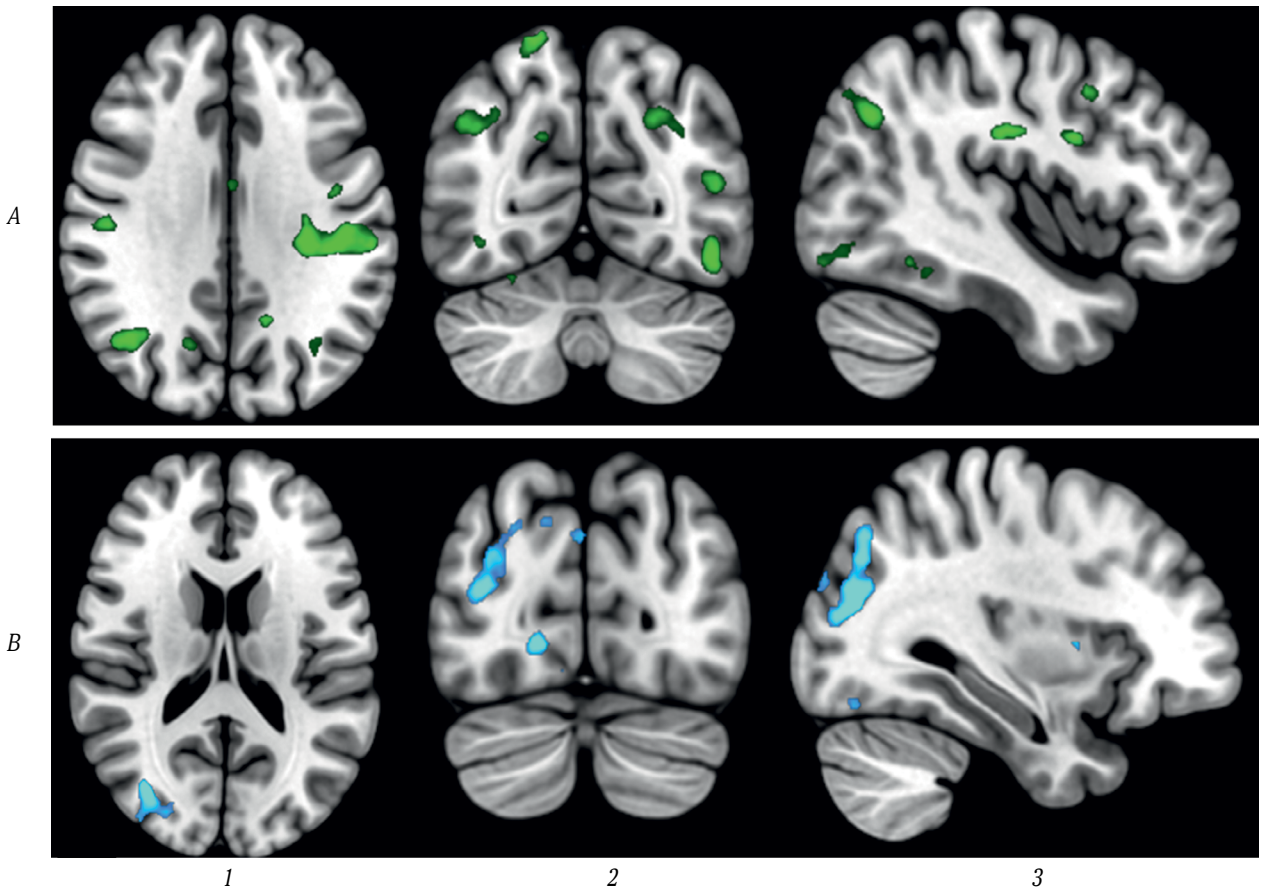


Fig. 1. Results of within groupcomparison of brain activation during a cognitive paradigm before and after treatment.
A – patients who received vascular and metabolic therapy for 10 days demonstrated a decrease in activation in the supramarginal and angular gyri and in the visual cortex; *B* – patients who received placebo demonstrated a decrease in activation only in the visual cortex.
1 – axial view; *2* – coronal view; *3* – sagittal view.

The phFMRI results for a domestic neuroprotective agent were intriguing; the treatment was followed by a decrease in the areas responsible for performing a cognitive task (in the supramarginal and angular gyri) and improved executive cerebral functions associated with processing language information (enhanced connection between the left dorsolateral prefrontal cortex and the superior temporal gyrus). Clinically, these neuroimaging changes were manifested by an increase in functional activity and optimization of executive functions, which is an important pathogenetic effect in patients with cerebrovascular diseases [24].

Pain syndromes

Some studies evaluated the analgesic properties of opioid agents on the activity of brain structures [25, 26]. The opioid analgesic nalbuphine increased BOLD signal intensity in 60 brain regions and decreased it in 9 regions, including the middle frontal cortex, inferior orbitofrontal cortex, postcentral parietal cortex, superior temporal pole, and cerebellum. However, after naloxone administration, the pattern of altered activation changed significantly: BOLD signal intensity increased in only 14 areas and decreased in 3 areas. Low doses of naloxone significantly blocked nalbuphine activity in the superior medial and middle frontal cortex, postcentral parietal cortex, occipital cortex (Rolandic fissure), caudate nucleus, pons (principal sensory nucleus of the trigeminal nerve), and cerebellum.

Currently, antidepressants and anticonvulsants play a special role in pain management, demonstrating their multimodal capabilities in the control of chronic pain syndromes. A.E. Edes et al. evaluated the effects of intravenous citalopram/placebo on the activity of the anterior cingulate cortex as a major structure involved in descending modulation and the emotional aspect of pain in 27 healthy volunteers and 6 patients with migraine without aura [27]. A significant difference in the temporal pattern of activation of the anterior cingulate cortex was found between healthy controls and patients with migraine without aura in response to even small increases in serotonin levels induced by citalopram.

Epilepsy

Research on the use of phFMRI in epilepsy is quite extensive. Considering the diversity of available antiepileptic agents

and the heterogeneity of epileptic syndromes in terms of the neural networks involved, FMRI-based biomarkers should be developed for early assessment of treatment efficacy and potential side effects [10]. For example, phFMRI showed that the use of a higher dose of valproic acid in patients with juvenile myoclonic epilepsy was associated with attenuation of abnormal coactivation of motor cortex with cognitive networks in a working memory study [28]. The use of another antiepileptic agent, levetiracetam, in patients with temporal lobe epilepsy was associated with restoration of the normal activation pattern, according to phFMRI findings [29]: for example, an increase in deactivation in response to a cognitive paradigm was observed in the affected temporal lobe during the treatment with the agent, and a dose-dependent effect was confirmed.

Studies with topiramate demonstrate the potential role of phFMRI in clarifying the cerebral mechanisms of adverse effects of neuropharmacological agents. During the use of topiramate (both in patients with epilepsy and migraine and in healthy volunteers), phFMRI with its multiple capabilities identified a pattern of reduced activation in language-dependent areas of the brain (inferior and middle frontal gyri, superior temporal gyrus of the dominant hemisphere) [30–32], as well as the absence of deactivation of paradigm-independent areas, including the default mode network [33, 34].

Cerebral metabolic health

The concept of cerebrometabolic health encompasses a wide range of syndemic neurological and metabolic disorders and suggests that it is relevant to evaluate the effects of different agents to address specific symptoms [35].

Currently, several modalities are available to investigate eating behavior, with neurocognitive testing using various questionnaires being the most important modality. FMRI allows real-time assessment of changes in activation of brain structures in response to different stimuli (e.g., a visual food paradigm). The main areas evaluated in obese patients are called the reward system and include the prefrontal cortex, insula, cingulate gyrus, and limbic system. A simple and reproducible visual FMRI paradigm was developed by the Research Center of Neurology to assess the system of eating behavior control [36], which was subsequently used in studies involving phFMRI patterns. For example, sibutramine (a centrally acting drug for the treatment of obesity, with the mechanism of action based on selective inhibition of serotonin and norepinephrine reuptake) was shown to produce a different pattern of signal changes in response to a eating paradigm in obese patients compared to healthy volunteers. The most significant changes in functional activity were found in the occipital lobes, insula, and middle and superior frontal gyri. It should be noted that before the initiation of pharmacotherapy, patients with obesity showed excessive activity in the occipital lobes compared to the control group (healthy volunteers), which indirectly indicates a more significant emotional response to the demonstration of high-calorie food in people with overweight [37].

The study by O.M. Farr et al. in 20 patients with type 2 diabetes showed the effect of liraglutide (a human long-lasting GLP-1 analogue) on the activation of brain areas (dorsolateral prefrontal cortex, midbrain, thalamic region) in response to food stimuli [38]. H. Cheng et al. demonstrated multimodal effects of liraglutide on cognitive function with increased activation in the hippocampal region, expanding the use of this class of agents in patients with type 2 diabetes and obesity [39].

The focus on the effects of various nutrients on the brain and human behavior has increased significantly in recent decades. Sugar and artificial sweeteners are widely used in modern nutrition science. Brain FMRI can evaluate these mechanisms with assessing neural activity over time in response to nutrient consumption. Glucose is known to activate reward systems in the brain (such as the dopamine system) associated with pleasure and motivation. Understanding how fast-digesting carbohydrates, such as sucrose, activate different areas of the brain in healthy people may provide clues to the mechanisms of overeating and addiction.

Sweeteners (aspartame, sucralose, stevia, erythritol) are offered as healthier alternatives to sucrose and fructose. However, their effects on brain activity and human behavior remain debatable. Some studies suggest that artificial sweeteners may not activate reward systems like carbohydrates, which could affect satiety and subsequent food consumption. In obesity, FMRI is used to assess functional neuronal activity involved in the regulation of energy exchange and metabolism. The Research Center of Neurology obtained pilot FMRI data comparing the effects of sucrose and an artificial sweetener, showing differences in activation in the supplementary motor area and dorsolateral prefrontal cortex in healthy volunteers (Fig. 2).

Cognitive disorders

PhFMRI may be a promising modality for identifying targets for the treatment of cognitive impairment. The differential effect of cholinergic therapy (galantamine) is convincingly demonstrated depending on the target patient cohort, such as mild cognitive impairment (activation of the posterior cingulate gyrus, left inferior parietal and anterior temporal lobes) or Alzheimer's disease (bilateral hippocampal activation) [40]. Such changes in response to cholinergic load may reflect a baseline difference in the functional status of the cholinergic system between two groups, which is consistent with clinical studies. In addition, differences in activation patterns were found after single and chronic administration of agents, highlighting the need for evaluating phFMRI as a dynamic modality.

Key areas for using phFMRI in neurology include:
- study of classical neuroprotectants in patients with cerebrovascular disease;
- study of antidepressants in neurological patients (post-stroke depression, chronic pain, neurodegeneration, etc.);
- resting-state assessment in patients with epilepsy depending on pharmacokinetics/pharmacodynamics of antiepileptic agents;
- management of acute/chronic pain;
- evaluation of FMRI correlates of neuroplasticity in post-stroke patients;

- cholinergic therapy in patients with cognitive disorders (vascular dementia, Alzheimer's disease, Parkinson's disease, etc.);
- dopaminergic therapy in patients with Parkinson's disease;
- patients with multiple sclerosis on pulse corticosteroid therapy.

Technological challenges of phFMRI and potential solutions

PhFMRI is a complex modality that requires a high level of logistical effort, as well as caution and a balanced approach to interpretation of the results.

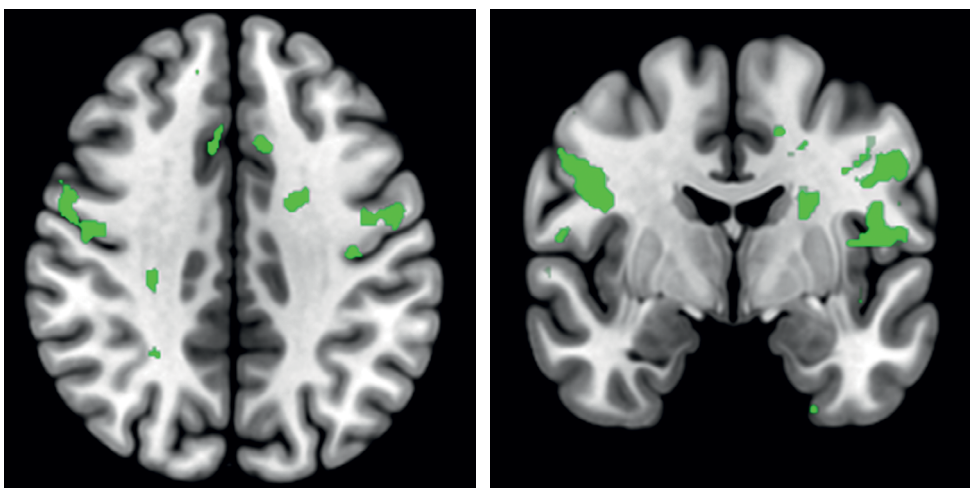
Some of the existing limitations of phFMRI are listed below:
- lack of an optimal set of settings for obtaining and processing MR images;
- limitations of generalized linear model as a basic approach to statistical analysis of FMRI data;
- lack of standardized paradigms for specific research tasks;
- inadequacy of current phFMRI data presentation standards for proper evaluation and interpretation;
- bias in conducting and publishing FMRI validation (repeated) studies;
- challenges in selecting and assessing potential covariates;
- the use of the BOLD signal as a surrogate indicator depending on the baseline level of neurovascular coupling, and its modulation by pharmacological agents is often difficult to predict;
- for most studies, small and highly heterogeneous sample size;
- high inter- and intra-individual variability of the FMRI signal [14, 41].

Understanding limitations and above-described characteristics of phFMRI may allow for (at least partial) modification of the methodology to obtain reproducible and meaningful results. For example, the selection of neuroactive molecules for an experiment should be based on clinical feasibility as well as pharmacokinetics/pharmacodynamics and target interaction. Time of onset and duration of the expected effect should also be considered to properly design the experiment. Since changes in the BOLD signal during FMRI may be attributed to systemic effects (heart rate, blood saturation level, etc.), it is recommended to include these routine parameters in the statistical analysis [42]. Normalization of baseline differences in cerebrovascular reactivity between patients and in the context of placebo/active agent use is recommended to overcome the limitations of the BOLD signal assessment method. This task requires assessment of the baseline level of cerebral perfusion (using arterial spin labeling) [43] and measurement of the cerebral metabolic rate of oxygen consumption [44].

When using different phFMRI paradigms, it is important to perform separate (several days/weeks apart) scans with placebo [45]. In addition, the study of pharmacological agents with predominantly subjective effects (e.g., sedative or energizing effects, mood modulation, etc.) should be supplemented with psychometric tests at pre-specified time intervals during and/or between scans (in case of suspected long-term effects) [46]. In some cases, resting-state FMRI should be used instead of or in addition to phFMRI, as resting-state FMRI can assess functional connectivity and identify potentially more stable markers of treatment response [47].

Conclusion

Brain phFMRI, as one of the multiple angio-neuroimaging subtypes, has significant potential for neuroscience research. With the proper study design, this modality is suitable for objective *in vivo* assessment of the target effect of a pharmacological agent. This is critical for several reasons:
1) Personalization of prescribed treatment (e.g., in the case of antiepileptic therapy adjustment).
2) Validation and/or discovery of new mechanisms of action of neuroactive agents (especially neuroprotectants).



A *B*

Fig. 2. Within group comparison of brain activation in healthy subjects during visualization of a food paradigm (images of foods that look tasty and foods that do not look tasty) after intake of sugar and a sweetener. Brain slices show areas of different activation. After sugar intake, higher activation was observed in the supplementary motor and dorsolateral prefrontal cortex bilaterally.
A – axial view; *B* – coronal view.

3) Reduced time to develop new agents due to direct visualization of the presence/absence of cerebral effects.
4) Clarification of the origin of adverse effects of neuroactive drugs.
5) Expansion of basic science tools and the ability to investigate specific receptors.

Collaboration between advanced phFMRI laboratories and the pharmaceutical industry can provide a competitive advantage for domestic research and developementand accelerate clinical implementation of experimental neuroscience findings. However, the modality has many limitations that require both the search for a solution and direct investigation of the issue.

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Late-Onset Pompe Disease in a Patient with Cerebellar Hemorrhage

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Abstract

Pompe disease (glycogen storage disease type II) is a rare autosomal recessive multisystem disorder characterized by the deposition of glycogen in skeletal muscles and internal organs. The late-onset form is characterized by slow progression with proximal muscle damage, respiratory failure, and less severe internal organ damage than the infantile form.

The article presents a case report of a 61-year-old female patient who underwent inpatient treatment. The patient had been having progressive muscle weakness for over 20 years and had a positive family history, but the reason for further evaluation and treatment was hemorrhage in the left cerebellar hemisphere. Laboratory and instrumental data are presented and clinical manifestations are discussed.

Keywords: *Pompe disease; glycogen storage disease type II; cerebellar hemorrhage; dilated cerebral arteriopathy; diaphragmatic ultrasound; magnetic resonance imaging of the thigh soft tissues*

Ethical statement. The publication of the clinical case was carried out with the voluntary informed consent of the patient.

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Болезнь Помпе с поздним началом у пациентки с кровоизлиянием в мозжечок

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Аннотация

Болезнь Помпе (гликогеноз II типа) – редкое аутосомно-рецессивное мультисистемное заболевание, для которого характерно отложение гликогена в скелетных мышцах и внутренних органах. Поздний дебют заболевания характеризуется медленным прогрессированием с поражением проксимальной мускулатуры, явлениями дыхательной недостаточности и менее выраженным, чем при инфантильной форме, поражением внутренних органов.

В статье представлено клиническое наблюдение пациентки 61 года, проходившей стационарное лечение. У неё на протяжении более 20 лет наблюдалась прогрессирующая мышечная слабость, выявлялся отягощённый наследственный анамнез, однако поводом для обследования и лечения стало развитие кровоизлияния в левую гемисферу мозжечка. Приведены данные лабораторно-инструментальных методов обследования, обсуждены особенности клинических проявлений.

Ключевые слова: *болезнь Помпе; гликогеноз II типа; кровоизлияние в мозжечок; дилатационная церебральная артериопатия; ультразвуковое исследование диафрагмы; магнитно-резонансная томография мягких тканей бёдер*

Этическое утверждение. Получено добровольное информированное согласие пациентки на публикацию клинического случая.

Источник финансирования. Авторы заявляют об отсутствии внешних источников финансирования при проведении исследования.

Конфликт интересов. Авторы заявляют об отсутствии явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи

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Introduction

Pompe disease (glycogen storage disease type II; PD) is a rare autosomal recessive disorder characterized by the glycogen accumulation in the lysosomes of various tissues. This disease is caused by mutations in the *GAA* gene (OMIM #606800) located on the long arm of chromosome 17 (17q25.2-25.3) and encoding acidic α-1,4-glucosidase, an enzyme involved in the breakdown of glycogen in cellular lysosomes [1, 2]. PD is a multisystem disease. Although skeletal muscle damage is the predominant clinical manifestation, glycogen metabolism disorders are also observed in cardiac muscle, liver, smooth muscle, and other organs and tissues [2, 3].

The clinical picture of PD depends on the age of onset, with earlier onset predisposing to more severe disease. This can be explained biochemically by the persistent residual activity of acid alpha-1,4-glucosidase in late-onset patients [4, 5].

PD is divided into infantile and late-onset forms. The infantile form is more severe due to the extremely low (<1%) activity of alpha-1,4-glucosidase. Symptoms develop at birth or in the first few months of life. This form is characterized by severe hypotonia (floppy infant syndrome), rapidly progressive myopathy with respiratory muscle dysfunction, hypertrophic cardiomyopathy, hepatomegaly, and a high risk of death [6, 7]. Some authors also identify a non-classical childhood-onset form. In this group of patients, clinical symptoms include delayed motor development, myopathic (predominantly proximal muscle damage) and diarrheal syndromes, respiratory failure due to respiratory muscle weakness and atrophy, elevated blood levels of creatine phosphokinase (CPK), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) [7].

Later-onset PD (after the age of 1 year) often develops in adulthood. Progressive weakness of the trunk and proximal muscles with predominant damage to the lower extremity muscles is observed. Respiratory failure occurs and progresses with damage to the diaphragm. Cardiomyopathy is less common. Clinical symptoms may include bulbar muscle damage manifested by tongue weakness with dysarthria and dysphagia, sleep apnea, cardiac arrhythmias, gastrointestinal dysfunction, lower urinary tract and anal sphincter involvement [5, 8–10]. Late-onset PD is characterized by a slowly progressive course. The patient's condition depends on the degree of damage to the skeletal muscles and internal organs.

Significant increases in CPK, lactate dehydrogenase, ALT, and AST levels are the most common laboratory changes. Electroneuromyography can confirm myopathy. Muscle biopsy is not always helpful in establishing the diagnosis because typical changes may not be present in a muscle biopsy sample [1, 11, 12].

If PD is suspected, determination of acid alpha-1,4-glucosidase activity in dried blood spots by tandem mass spectrometry is a gold standard diagnostic test, and if it is decreased, molecular genetic testing by direct *GAA* gene sequencing should be performed to identify different mutations [1, 6, 13].

Clinical case

Female patient A, 61 years old, underwent planned inpatient treatment in the Neurological Department of the I.I. Mechnikov North-Western State Medical University.

The patient presented with unsteady gait, skeletal muscle weakness, especially in the proximal limbs and back muscles, difficulty getting out of bed and standing up from a chair (using myopathic maneuvers), pain in the skeletal muscles when moving, and decreased voice volume.

She reported having the disease since the age of 20–30, when she first noticed weakness in her back muscles and difficulty maintaining an upright position. This was followed by a slow progression of the disease with abnormal gait and damage to the proximal muscle groups of the legs and arms. She did not seek medical attention for the above complaints.

The family history was clarified; the diagram is shown in Fig. 1. The patient's brother (deceased) had progressive muscle weakness since the age of 25, requiring respiratory support (artificial ventilation). The nephew (30 years old) had a 2–3 year history of slowly progressive skeletal muscle weakness. The nephew's son had progressive muscle weakness in early childhood, including respiratory muscles, requiring mechanical ventilation and resulting in death at the age of 4 years.

On the evening of 15 March 2024, the patient noticed a general deterioration, acute dizziness, a clicking sensation in her head followed by loss of consciousness, and a single episode of vomiting. She was urgently admitted to the Regional Vascular Center for further evaluation and was diagnosed with hemorrhagic stroke with intracerebral hematoma in

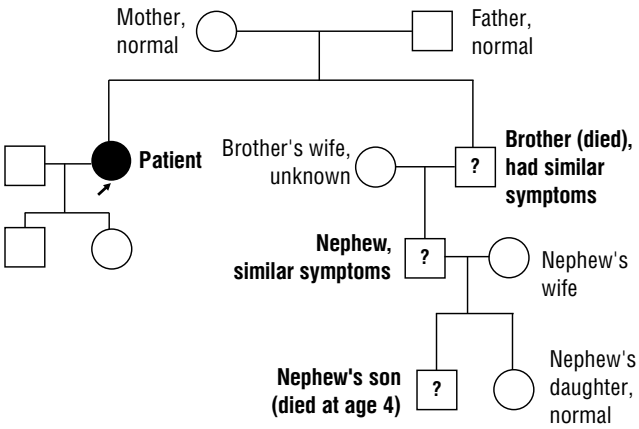

Fig. 1. Family history of patient A.

the left cerebellar hemisphere. She received neuroprotective and vascular therapy in the hospital. She was discharged for outpatient treatment in stable condition.

In April 2024, the patient was admitted to the Neurology Department No. 1 of the I.I. Mechnikov North-Western State Medical University with the status post hemorrhagic stroke with intracerebral hematoma in the left cerebellar hemisphere on 15 March 2024 with severe dynamic ataxia; early recovery period. During hospitalization, she received antiplatelet, lipid-lowering, hypotensive, neuroprotective, exercise and physical therapy, and biofeedback training. During the course of treatment, positive changes were observed with significant improvement in dynamic ataxia. Based on the identification of a hereditary myopathy and gait abnormalities not typical of a previous acute cerebrovascular event, it was recommended to perform additional examinations to rule out late-onset PD, such as alpha-1,4-glucosidase activity by dry spot assay and molecular genetic testing. It should be noted that there were no significantly elevated levels of CPK and other cytolytic enzymes according to the medical records provided.

On 5 November 2024 she was readmitted to Neurology Department No. 1 of the I.I. Mechnikov North-Western State Medical University. At admission, her condition was relatively satisfactory. She was alert and cooperative. The performance status was characterized by lower thoracic and lumbar S-shaped scoliosis. No abnormal changes in the cardiovascular, respiratory, digestive, or genitourinary systems were observed. Respiratory rate was 16 per minute; SpO_2 was 94%.

Sense of smell was not affected. Visual fields by the confrontational test were unrestricted. Eye-slits were D=S. Full range of eye movements was observed with no diplopia or pain. Pupils were OD=OS, light reflexes (direct and indirect) were brisk. The convergence and accommodation reflexes were reduced bilaterally. A horizontal fine end-point nystagmus was observed in the extreme positions. Facial sensitivity was not affected. The face was asymmetrical due to the reduction in the right nasolabial fold. The palpebral reflex was D=S and moderately brisk. The cochlear and vestibular systems were not affected. No dysphagia, dysarthria, dysphonia was observed. The tongue was in the midline. The soft palate was symmetrically tensed, and the uvula was in the midline. No primitive oral reflexes were revealed. The myopathic gait was observed with leaning on surrounding objects. The patient used Gowers' maneuvers to rise from the chair. Table 1 shows the results of strength testing of the major skeletal muscle groups using a Medical Research Council Scale for Muscle Strength (MRC-5). Moderate paresis of the shoulder and pelvic girdle muscles was noticed.

The range of active movements was restricted when lifting the arms above the horizontal position due to muscle weakness. The range of passive movements was normal. The muscle tone was diffusely reduced. Hypotrophy of the shoulder and pelvic girdle muscles was noticed, with winged scapulae (Fig. 2). No fasciculations or fibrillations were observed. Deep reflexes were as follows: equal and moderately brisk in the arms and uniformly reduced in the legs. As for pathological reflexes, the upper and lower Rossolimo reflexes were detected on the left side.

The finger-to-nose and finger-to-hammer tests were satisfactory bilaterally, while the heel-to-shin test showed bilateral ataxia, more severe on the left. The Romberg test showed unsteady gait without clear lateralization. Babinski asynergy test could not be reliably performed due to weakness of the back muscles. No dyshypermetry or dysdiadochokinesia was observed. The Stuart–Holmes test was negative. The patient presented with impaired surface sensitivity manifested as a mosaic pattern of the left-hand hypoesthesia. Deep sensitivity was intact. No signs of nerve root tension were detected.

No aphasic, apraxic, or agnosic disorders were observed. The Mini Mental State Examination (MMSE) score was 30. The Montreal Cognitive Assessment (MoCA) score was 28. The Frontal Assessment Battery (FAB) score was 18.

No meningeal signs were observed. Pelvic organ functions were intact.

The Hospital Anxiety and Depression Scale (HADS) showed an anxiety score of 2 and a depression score of 11. The patient was consulted by a psychotherapist. The depression subscale score identified was associated with a chronic, slowly progressive disease.

The patient's forced vital capacity (FVC) was 37% of the normal value adjusted for sex, height, and age in the sitting position and 29% of the normal value in the supine position.

The 6-minute walk test result was 318 meters.

Follow-up laboratory testing for myolysis showed a slight increase in lactate dehydrogenase level (235 U/L; reference range: 79–221 U/L), while the CPK level was 93 U/L and remained within the reference range of 26–174 U/L.

The patient's brain natriuretic peptide level was 118.6 pg/mL, which was below the reference range (300–900 pg/mL for subjects aged 50–75 years).

Abdominal ultrasound did not show any liver abnormalities, but revealed a gallbladder polyp. A decrease in the range

Table 1. Results of muscle strength testing in patient A.

Movement	Right	Left
Forward neck flexion	4	4
Backward neck flexion	4	4
Lifting arms to the horizontal position	3	3
Lifting arms above the horizontal position	2	2
External shoulder rotation	3	3
Internal shoulder rotation	3	3
Elbow flexion	4	4
Elbow extension	4	4
Forearm supination	4	4
Forearm pronation	4	4
Hand flexion	4	4
Hand extension	4	4
Finger flexion	5	5
Finger extension	5	5
Finger abduction	5	5
Finger adduction	5	5
Hip flexion	3	3
Hip extension	3–4	3–4
Hip adduction	4	4
Hip abduction	4	4
External hip rotation	4	4
Internal hip rotation	4	4
Knee flexion	4	4
Knee extension	4	4
Foot dorsiflexion	4–5	4–5
Foot plantar flexion	5	5
Foot abduction	5	5
Foot adduction	5	5
Toe extension	5	5
Toe flexion	5	5

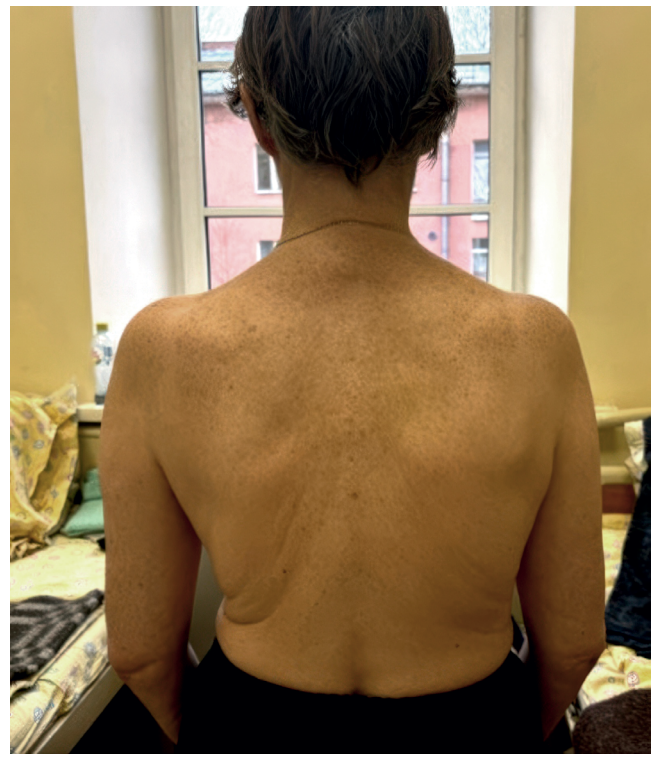

Fig. 2. Atrophy of the shoulder girdle muscles in Patient A, with signs of developing winged scapulae.

of movement to 7 mm for the right hemidiaphragm and to 10 mm for the left hemidiaphragm was noticed (reference range: 10–20 mm).

A diaphragmatic ultrasound was performed using 5–12 MHz sensors in B and M modes in the standing, semi-recumbent, and supine positions with an assessment of diaphragm thickness and excursion. At least 3 measurements were taken for each parameter, and the average value was calculated.

Tables 2 and 3 show the numerical parameters of the diaphragmatic ultrasound. All tested parameters were below reference ranges.

Triplex scanning of the brachiocephalic arteries showed dilation and curvature of the brachiocephalic trunk, subclavian arteries, S-shaped tortuosity of the common carotid arteries, tortuosity of the middle and distal segments of the internal carotid arteries, and VI segment of the vertebral arteries without evidence of significant stenosis. A prolonged heteroechoic atherosclerotic plaque with hyperechoic inclusions and a nodular outline was observed along the inferior wall of the right subclavian artery, stenosing the lumen by up to 44%. No evidence of subclavian (vertebral) steal syndrome was seen. Hypoplasia of the left vertebral artery could not be ruled out.

Previously recommended additional tests are presented. Dry spot assay of alpha-1,4-glucosidase activity showed a low level of 1.3 μmol/L/h (reference: > 2.32 μmol/L/h). Molecular

Table 2. Diaphragm thickness (mm) in patient A. according to ultrasound

Time of the examination	Position	Parameter			
		right		left	
		result	reference range	result	reference range
End-expiratory	Supine	1.1	1.7–2.2	1.1	1.7–2.2
	Semi-recumbent	1.2		1.1	
	Standing	1.4		1.4	
End-inspiration	Supine	1.4	1.9–2.7	1.4	2.0–2.8
	Semi-recumbent	1.4		1.5	
	Standing	1.5		1.6	
End of full inspiration	Supine	2.1	2.6–3.5	2.1	2.8–3.9
	Semi-recumbent	2.1		2.2	
	Standing	2.7		2.7	

Table 3. Range of diaphragm movements (mm) in patient A. according to ultrasound

Parameter	Position	Results	Reference range
Normal breathing	Standing	7.1	11.1–16.9
	Lying	6.5	
Deep breathing	Standing	27.6	39.3–63.6
	Lying	26.5	

genetic testing showed a pathogenic nucleotide variant chr17:80112604G>A in the heterozygous state and a likely pathogenic nucleotide variant chr17:80112993C>G in the heterozygous state in *GAA*.

The patient was referred to magnetic resonance imaging (MRI) of the soft tissues of the right and left thighs. Symmetrical fatty degeneration of the muscles of the posterior thigh compartment (semimembranosus, semitendinosus, biceps) and large adductor muscles of the thigh was observed with no signs of edema, involvement of fat tissue, fascial compartments, and vascular-nervous bundles (Fig. 3).

Severe symmetrical fatty degeneration of the posterior muscles of the thigh (black arrow) and moderate fatty degeneration of the adductor muscles of the thigh (white arrow) are visualized.

Based on clinical, laboratory, instrumental, and molecular genetic tests, the patient was diagnosed with late-onset progressive glycogenosis type II with myopathy of the proximal extremities and trunk, moderate hypotrophy, and moderate respiratory dysfunction.

The patient received previously prescribed antiplatelet, antihypertensive, and neuroprotective therapy. The subsequent management strategy included enzyme replacement therapy.

Discussion

Timely diagnosis of late-onset PD is challenging in most cases. When a patient first presents to a neurologist with a "typical" course of late-onset PD and data on complaints, history, and neurological status are obtained, myodystrophies, polymyositis, spinal amyotrophy, and myasthenic syndrome should be differentiated. However, patients adapt to the slowly progressive muscle weakness and atrophy, and clinical manifestations of damage to other organs may be the first complaint to seek medical attention from various specialists, such as pulmonologists, rheumatologists, orthopedic surgeons, gastroenterologists, etc. [2, 12, 14].

Late diagnosis, in turn, complicates patient management by delaying the initiation of pathogenetic therapy.

In our clinical case, patient A had been having progressive muscle weakness and atrophy for almost 30 years, but the reason for consulting a neurologist was a parenchymal hemorrhage in the left cerebellar hemisphere. Stroke is reported as one of the rare complications of PD. Stroke pathogenesis in this group of patients is caused by abnormal accumulation of lysosomal glycogen in the smooth muscle cells of brain arterioles and arteries, which affects the synthesis and structure of the extracellular matrix and reduces the elasticity and integrity of the vessel wall. In addition, the vertebrobasilar arteries are more vulnerable due to the lower expression of elastic fibers in their walls compared to the carotid arteries. These changes result in dilated arteriopathy, dolichoectasia of the basilar artery, and microaneurysms. This increases the risk of parenchymal hemorrhage into the brain matter (as in patient A), subarachnoid hemorrhage and microbleeds, as well as cerebral infarction and leukoencephalopathy. In addition, an increase in partial pressure of carbon dioxide as respiratory failure progresses may be a risk factor for vasodilation in patients with PD [15]. In our case, duplex scanning also showed multiple tortuosities of the brachiocephalic arteries, confirming the literature data. However, the parenchymal hemorrhage in the left cerebellar hemisphere was accompanied by a significant improvement in focal symptoms during the treatment.

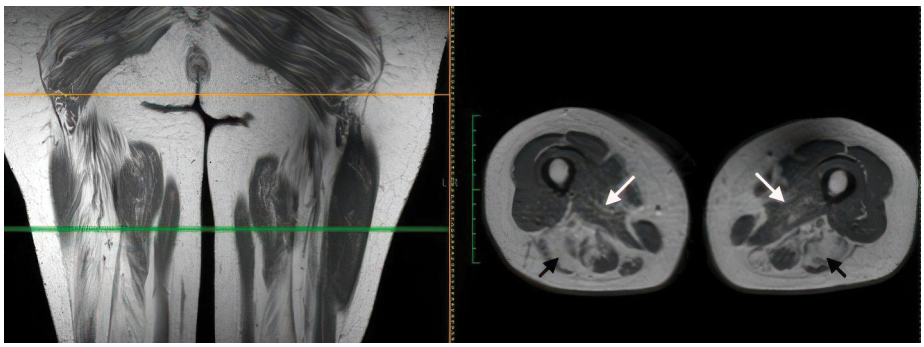

Fig. 3. Results of MRI of soft tissues of the thighs of patient A. (T1-weighted, frontal and axial slices).

During the hospitalization in the Neurology Department, which is the main base of the Department of neurology named after academician S.N. Davidenkov of the I.I. Mechnikov North-Western State Medical University, clinical manifestations of the patient's long-term neuromuscular disease were the focus of attention. The relevant medical history revealed the hereditary nature of the disease. Additional clinical and laboratory tests ruled out non-hereditary variants (inflammatory, toxic, endocrine, tumor-induced) of progressive muscle damage.

Analysis of the family history suggested a hereditary nature of the disease, but the mode of disease transmission was not typical of PD, as it appeared to be an autosomal dominant inheritance. However, autosomal recessive PD was not confirmed in deceased relatives (the patient's brother, the son of the nephew). Such a pseudodominant inheritance was only possible if the wives of the patient's brother and nephew were carriers of the mutation. Considering the low incidence of heterozygous carriers of *GAA* mutations, the probability of such a case is extremely low. Autosomal recessive inheritance is characterized by cases of disease in relatives of both sexes in different generations. Therefore, the family history was not typical of PD.

Moreover, the diagnosis in this case was challenging due to the lack of typical laboratory markers of myolysis in follow-up blood chemistry results, whereas in the majority of clinical cases described in the literature, hyper-creatine phosphokinase-emia was found [9, 16, 17]. Enzyme levels within the reference range may be associated with a long, slowly progressive disease (over 20 years) with severe chronic muscle damage [18].

In our case, the diagnosis was verified by measuring alpha-1,4-glucosidase activity in dried blood spots using tandem mass spectrometry, followed by molecular genetic testing. The patient had compound heterozygous mutations in the *GAA* alleles (pathogenic nucleotide variant chr17:80112604G>A and likely pathogenic nucleotide variant chr17:80112993C>G), which may explain the late onset of the disease with enzyme levels within the reference range. Currently, genotype-phenotype correlations in PD patients are of great interest because genetic and epigenetic mechanisms of the disease's clinical polymorphism are not fully understood.

We believe that assessment of respiratory muscle function is an important element of the evaluation of patients with neuromuscular disorders. FVC in the vertical and horizontal positions is the simplest and most commonly used clinical test to detect diaphragmatic paresis. In our case, FVC decreased to 37% of reference in the vertical position and to 29% in the horizontal position. The literature provides some data on the correlation between FVC and respiratory morbidity and mortality [11, 12, 15]. In our case, we used diaphragmatic ultrasound, which showed a decrease in diaphragm thickness and excursion below the reference ranges. In the available Russian literature, despite the simple and informative nature of ultrasound, we found no cases of diaphragmatic ultrasound in PD patients. Global literature describes only a few cases of diaphragmatic ultrasound [19].

The thigh muscle MRI of our patient showed a significantly more severe fatty degeneration of the posterior thigh muscles compared to the medial ones, which can be considered as an individual feature.

Current clinical guidelines [20] recommend watchful waiting for patients with PD, including monitoring of FVC, regular 6-minute walk tests, liver ultrasound, electrocardiography, echocardiography, follow-up measurement of myoglobin fraction of CPK, and brain natriuretic peptide.

The treatment strategy for PD patients involves a multidisciplinary approach based on the clinical manifestations of the disease. Recommended non-medication options include a high-protein, low-carbohydrate diet supplemented with L-alanine, psychotherapeutic support, and psychological adjustment. Lifelong enzyme replacement therapy is the primary pathogenetic treatment option used to slow disease progression, stabilize lung function, and prolong patients' lives before they require ventilatory support and a wheelchair.

Conclusions

Therefore, clinical diagnosis of late-onset PD may be significantly challenged. If myopathic syndrome is suspected in patients seen by different specialists for different diagnoses, additional testing, including FVC, is recommended. We believe that diaphragmatic ultrasound has great potential as a diagnostic tool for patients with PD and other neuromuscular disorders due to its availability, informative value, and non-invasiveness.

All patients with unspecified limb girdle myopathy, especially if associated with respiratory muscle weakness, should have a blood test for alpha-1,4-glucosidase activity. It should

be considered that family history is not always available and that elevated CPK and other markers of myolysis are not necessarily indicative.

The accumulation of lysosomal glycogen in the smooth muscle cells of brain arterioles and arteries, with associated structural alterations in the intercellular substance, is a risk factor for dilated arteriopathy, which increases the risk of stroke and leukoencephalopathy. Neurologists' awareness of cerebrovascular PD manifestations is the only way to suspect and diagnose this disease.

PD is a curable disease with pathogenetic treatment available, so early diagnosis and timely initiation of therapy are crucial. Pathogenetic treatment of PD appears to be the most optimal treatment strategy to prevent cerebrovascular complications, but the current scientific literature does not provide relevant data, so further research is needed.

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Thromboembolic Cerebral Aneurysms

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Abstract

Intracranial hemorrhage is the most common outcome of cerebral aneurysms, and, therefore, clinical guidelines for the management of patients with cerebral aneurysms are primarily based on assessing the risk of their rupture. Brain ischemia due to the cerebral aneurysms occur significantly less frequently (i.e. in 3–5% of cases), and, in most cases, it is caused by distal embolism with thrombotic masses from large and giant thrombosed aneurysms. A conclusion that ischemic stroke is associated with embolism of thrombi from the aneurysm sac can be made only after ruling out other risk factors, primarily cardioembolism and stenosis of intracranial and extracranial arteries. The management of patients with thromboembolism from aneurysms who developed ischemia is challenging because these patients require antithrombotic agents, which can result in recanalization and rupture of the aneurysm. In addition, the optimal timing for surgery for the aneurysm in the event of acute ischemia has not been determined, given the high risk of recurrent embolism and aneurysm rupture. We present an overview of recent studies on this issue and our experience in managing 4 patients with thromboembolic stroke caused by cerebral aneurysms.

Keywords: *cerebral aneurysm; ischemic stroke; embolism; thrombosis*

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Тромбоэмболический тип течения церебральных аневризм

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Аннотация

Геморрагический тип течения церебральных аневризм встречается наиболее часто, в связи с чем клинические рекомендации по ведению пациентов с аневризмами головного мозга в первую очередь основываются на оценке риска их разрыва. Ишемический тип течения церебральных аневризм встречается значимо реже (в 3–5% случаев) и чаще всего связан с дистальной эмболией тромботических масс из больших и гигантских тромбированных аневризм. Сделать заключение о том, что ишемический инсульт ассоциирован с эмболией тромбов из мешка аневризмы, можно только при исключении других факторов риска, в первую очередь кардиоэмболии и стенозов интра- и экстракраниальных артерий. Имеются сложности в определении тактики ведения пациентов с тромбоэмболией из аневризм и развитием ишемии, т. к. требуется назначение антитромботических препаратов, которые могут способствовать реканализации аневризмы и её разрыву. Кроме того, в настоящее время не определены оптимальные сроки для выполнения хирургического вмешательства на аневризме при развитии острой ишемии, учитывая высокий риск повторных эпизодов эмболии, а также риск разрыва аневризмы. В статье представлены краткий обзор современных исследований по данной проблеме и собственный опыт ведения 4 пациентов с тромбоэмболическим вариантом течения церебральных аневризм.

Ключевые слова: *церебральная аневризма; ишемический инсульт; эмболия; тромбоз*

Источник финансирования. Авторы заявляют об отсутствии внешних источников финансирования при проведении исследования.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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Introduction

Every year, 10–14 aneurysmal subarachnoid hemorrhages occur per 100 thousand population in Russia [1, 2]. Cerebral aneurysms (CAs) are found in 2–5% of the population, with most of them being small and asymptomatic [3]. The incidence of ischemic stroke (IS) is much higher (i.e. up to 2.5–3.0 cases per 1000 population per year) [2]. Based on these data on the prevalence aneurysm in the population and the annual incidence of IS, we can assume that some strokes and transient ischemic attacks occur in patients with asymptomatic CAs.

A history of aneurysm is a contraindication for systemic thrombolysis in patients with IS. However, angiography is not required to perform systemic thrombolysis, and most patients do not know if they have asymptomatic aneurysms. There have been isolated reports describing ruptures of asymptomatic aneurysms during thrombolytic therapy in patients with IS [4, 5]. However, these could be not asymptomatic aneurysms but those ruptured and partially thrombosed in the past, so the administration of a fibrinolytic agent could promote lysis of the thrombus and re-rupture of the aneurysm. A large cohort study did not detect any cases of rupture of asymptomatic aneurysms during thrombolytic therapy [6].

Therefore, in most cases CAs are a concomitant condition in IS patients. However, in 3–5% of cases, thrombosed aneurysms (TAs) can themselves cause IS, most often due to the embolism with thrombotic masses from the sac into the distal branches [7–9]. The rate is even higher in large and giant aneurysms. According to V.V. Krylov et al., in a group of patients with giant aneurysms of the middle cerebral artery, 6.9% of patients had embolism resulting in cerebral ischemia [10].

According to several studies, embolism of thrombi from the aneurysm sac was associated with an increased risk of rupture in the near future [9, 11–13].

Aim. The study aimed to review cases of thromboembolic CAs.

Materials and methods

We reviewed medical records of 240 patients with CAs who were treated at the regional vascular center of the Krasnoyarsk Regional Clinical Hospital in 2022–2023. Of these, 136 patients had surgery in the acute period of aneurysm rupture, 56 had a history of rupture, and 48 had unruptured aneurysms. In 4 (1.6%) patients, CAs manifested as IS.

A causal relationship between an aneurysm and stroke can be established based on the following:
- a large partially or completely TA;
- ischemia in the branches distal to the TA;
- no other risk factors for stroke, mainly cardioembolism and hemodynamically significant stenoses of extracranial and intracranial arteries.

We reviewed history, clinical, and neurovisualization data of patients with IS associated with TA.

Results

Our patients with thromboembolic cerebral aneurysms included 2 men and 2 women. Two patients were aged 22 and 24 years, and the other two were older (57 and 62 years old).

All patients had aneurysms larger than 10 mm (i.e. 13, 15, 15, and 20 mm). TAs were located in the middle cerebral artery (MCA) or internal carotid artery (ICA) in 3 and 1 cases, respectively. In 3 cases, the aneurysms had a small functioning part; in 1 case, the aneurysm was completely thrombosed.

One patient developed lacunar stroke in the internal capsule, and another had a large ischemic lesion in subcortical structures. One patient developed ischemia in the territory of the frontal branch of MCA M2, and another patient had multiple foci of subcortical and cortical ischemia.

In 3 patients with acute ischemia, native multislice computed tomography (MSCT) showed a hyperdense signal from a part of the TA, which could suggest acute formation of a thrombus, which partially migrated to the distal branches and caused cerebral infarction.

All patients were prescribed antiplatelet agents to treat acute IS. There were no recurrent ischemia episodes. Three patients with partially TAs underwent elective surgery (osteoplastic craniotomy, microsurgical clipping of the aneurysm) 3 months after IS or later. The patient with completely TA and MCA thrombosis did not have any surgery.

We present clinical cases of the patients with thromboembolic CAs.

Clinical case 1

Patient B., a 57-year-old woman, was admitted to the primary vascular department with severe right-sided hemiparesis (up to score 3 in the arm and leg). She had severe neurological deficit with an NIHSS score of 14. MSCT showed an ischemic area in the putamen, internal capsule, and head of the caudate nucleus on the left (Fig. 1, *A*). We can see a hyperdense area in the Sylvian fissure, which should be differentiated from hemorrhage and TA (Fig. 1, *B*). MSCT angiography showed a functioning part of the TA of MCA M1 (Fig. 1, *C*). After 3 months, the patient was hospitalized for planned surgery for MCA aneurysm. Follow-up native MSCT showed isodense signal from the aneurysm; follow-up MSCT angiography showed a functioning part of the aneurysm of up to 3.5 mm (Fig. 1, *D*, *E*).

In the acute period of IS, the thrombosed part of the aneurysm had an hyperdense signal, suggesting a fresh thrombus (Fig. 1, *B*), and after 3 months the signal from the thrombus became isodense (Fig.1, *D*). Based on the areas of ischemia in the territory of the lenticulostriate branches and a fresh thrombus in the aneurysm, we can assume that the ischemia was caused by thrombosis of the striatal branches of the MCA by an embolus from the aneurysm sac. The patient did not have any other IS risk factors. Microsurgical clipping of the aneurysm was performed. A small functioning part of the aneurysm and a large thrombosed part (13 mm) were identified during the surgery. The sac was opened, the blood clots were removed, and a permanent clip was placed on the neck formed. The patient was discharged with preoperative functional status of modified Rankin score 3.

Clinical case 2

Patient K., a 62-year-old man, was admitted to the primary vascular department with right-sided hemiparesis (score 2 in the arm, score 3 in the leg) and motor aphasia; his NIHSS score was 16. MSCT showed an ischemic area in the left frontal lobe extending to subcortical structures. Native MSCT suspected MCA TA of up to 2 cm (Fig. 2, *A*, *B*). Due to un-

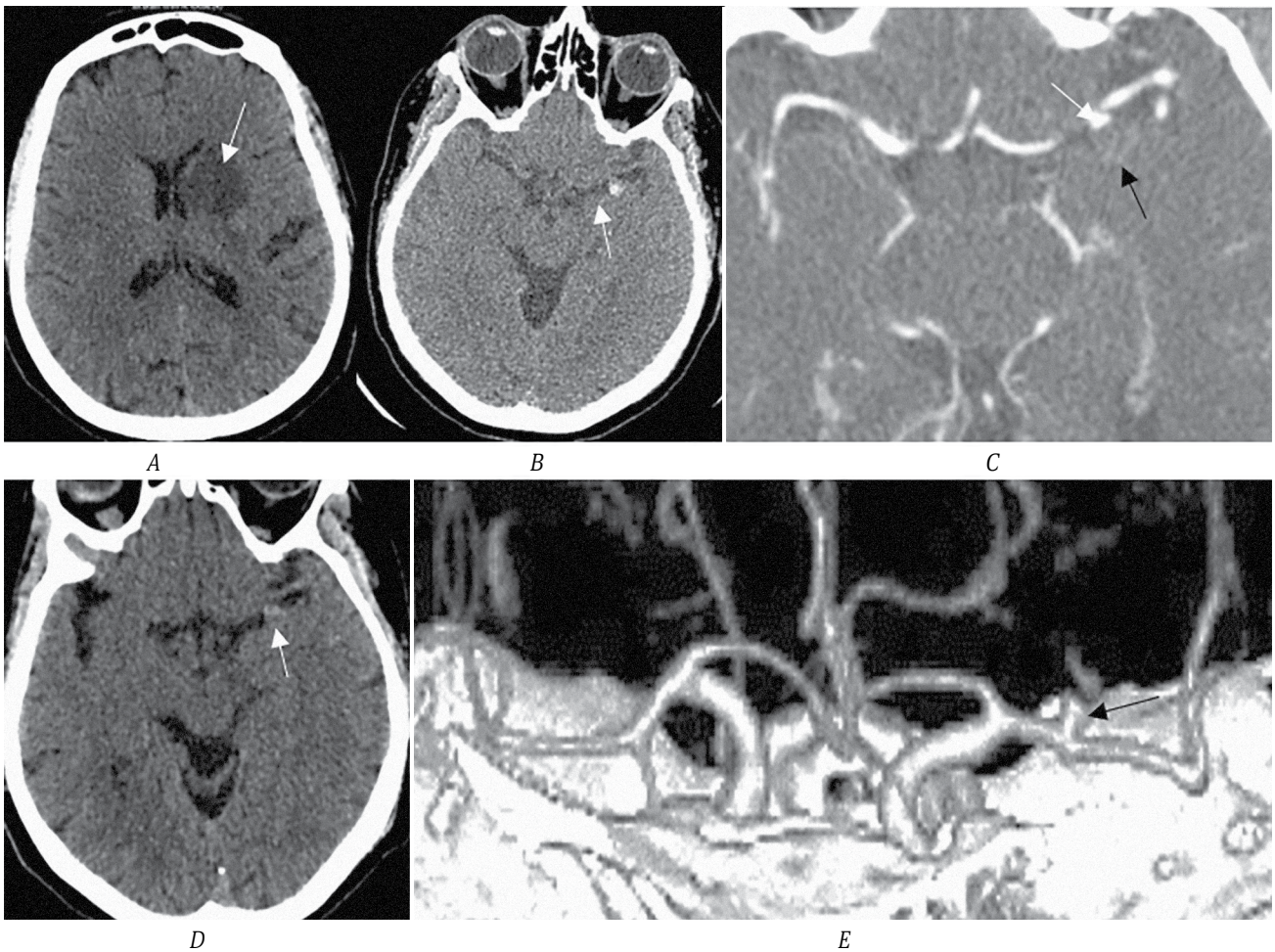

Fig. 1. Brain MSCT of patient B.
A, acute ischemia in the head of the caudate nucleus, internal capsule, and putamen (arrow); *B*, thrombosed part of the aneurysm (arrow); *C*, functioning part of the aneurysm (white arrow); the black arrow indicates the contours of the thrombosed part; *D*, MSCT 3 months after the stroke, the arrow indicates the thrombosed part of the aneurysm; *E*, 3D reconstruction of the functioning part of the aneurysm (arrow).

known reasons, MSCT angiography was not performed in the primary vascular department. Three months later, the patient was referred to the regional vascular center. MSCT showed a cystic-atrophic area in the area of the previous IS in the left frontal lobe. MSCT angiography showed a small functioning part of the M1 segment aneurysm and patent distal branches of the MCA (Fig. 2, *C*, *D*).

Considering a large thrombosed aneurysm sac in MCA M1 and ischemia in the territory of the frontal M2 branch of the MCA, thromboembolism from the aneurysm sac was considered. Three months later, angiography showed that all MCA branches were patent, which could be related to the recanalization of the thrombosed branch. An additional examination did not show any further risk factors for IS except for essential hypertension. Considering a high risk of recurrent thromboembolic complications, microsurgical clipping of the aneurysm was performed. After the surgery, his cognitive functions declined, and he and was discharged with a modified Rankin score of 4.

Clinical case 3

Patient I., a 22-year-old woman, was delivered by ambulance with complaints of weakness and difficulty to control her movements in the right limbs. The patient presented fully conscious, with right-sided hemiparesis scoring up to 3.5 in the arm and 4 in the leg. MSCT showed a round-shaped lesion of hyperdense density in the projection of the Sylvian fissure with a TA suspected. The lesion had areas of different densities, including a high-density area, which was a possible sign of acute thrombosis (Fig. 3, *A*). MSCT angiography showed no blood flow in the MCA on the left and no functioning part of the aneurysm. Considering the mild neurological deficit (NIHSS score 5), acute thrombosis of the MCA aneurysm with the artery itself was unlikely. Brain MRI was performed. A large MCA aneurysm with thrombosis along the whole MCA was identified. An ischemic lesion was seen in the internal capsule, which corresponded to the neurological deficit. Therefore, thrombosis of the MCA aneurysm and MCA itself was likely to be chronic, and

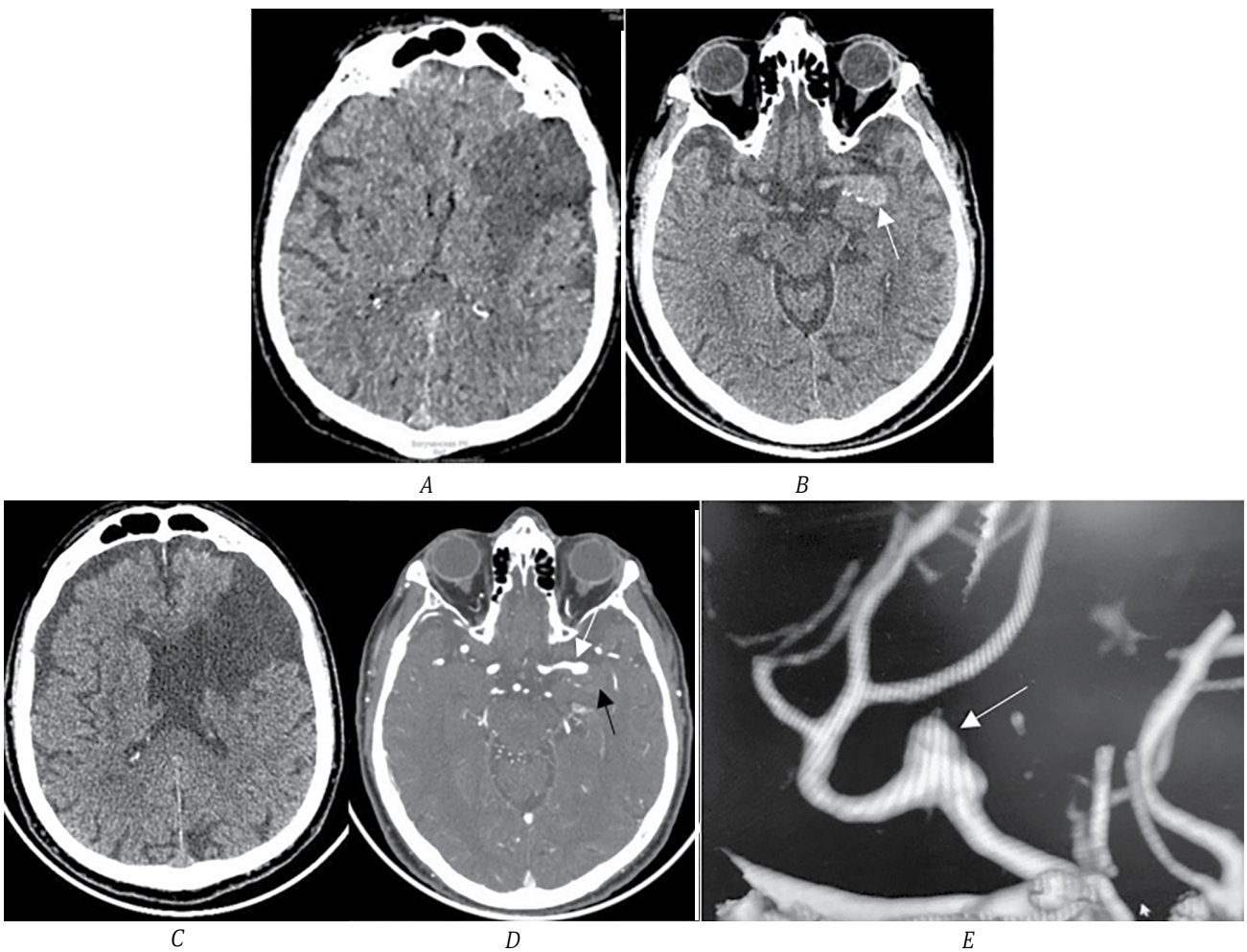

Fig. 2. Brain MSCT of patient K.
A, native MSCT, an ischemic area is seen in the left frontal lobe; *B*, the arrow indicates MCA TA with calcification of up to 2 cm; *C*, MSCT 3 months after IS, an area of cystic-atrophic changes in the left frontal lobe is seen; *D*, MSCT angiography, the white arrow indicates the functioning part of the aneurysm, the black arrow indicates the contour of the thrombosed part; *E*, 3D reconstruction of MSCT angiography. The arrow indicates the functioning part of the aneurysm.

the current clinical picture was caused by ischemia in the subcortical structures supplied with blood by the lenticulostriate arteries. Ischemia could be associated either with thromboembolism or enlargement of the aneurysm (a fresh thrombus) leading to an arterial occlusion.

This young patient did not have any other stroke risk factors. Cerebral angiography and MSCT perfusion were performed to determine treatment strategy. Angiography showed good collateral blood flow. MSCT perfusion did not show any significant differences in capillary blood flow in both hemispheres. Non-surgical treatment was chosen with follow-up angiography. The patient was followed up 6 months after; she had no recurrent ischemia episodes and had a modified Rankin score of 0. There were no MSCT angiography findings suggesting recanalization of the aneurysm and MCA during the administration of the antiplatelet therapy.

Clinical case 4

Patient A., a 24-year-old man, was admitted to the emergency room with acute right-sided hemiplegia. Brain MSCT showed an area of increased density in the chiasmatic-sellar region on the left, which had to be identified as a hemorrhagic focus or TA of the ICA (Fig. 4, *A*). MSCT angiography showed a small functioning part of the aneurysm in the left ICA. The ICA and MCA were patent along their entire length.

Brain MRI was performed. A 15-mm TA of the left ICA was seen. In DWI mode, areas of acute ischemia (restricted diffusion) were seen in the head of the caudate nucleus on the right, putamen, insular cortex, and lateral and medial regions of the right frontal lobe. Given this multifocal nature of ischemia and the presence of a TA of the ICA, embolism from the sac was likely to lead to the formation of multiple ischemic

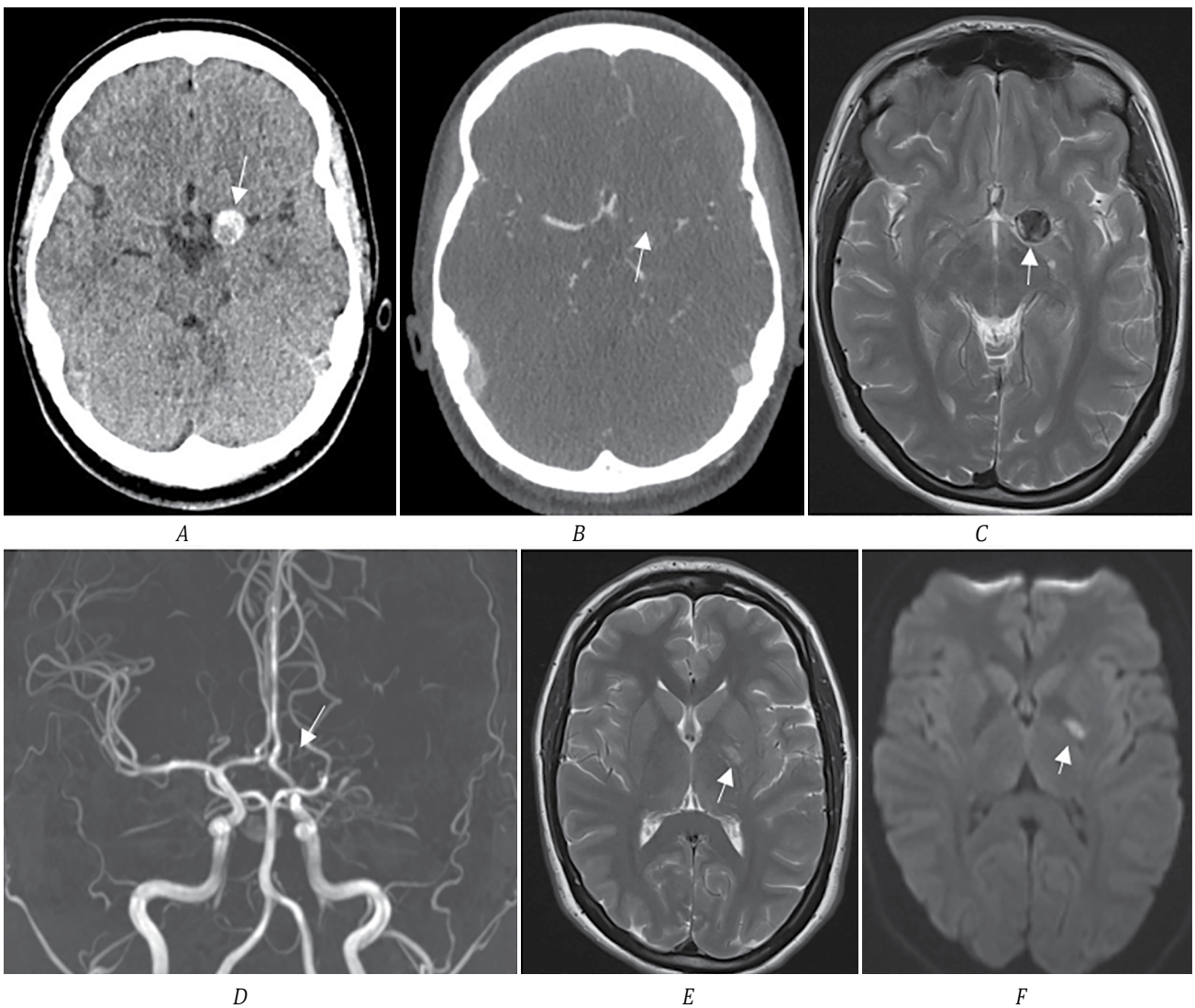

Fig. 3. Brain MSCT of patient I.
A, TA is indicated with the arrow; *B*, MSCT angiography, the white arrow indicates no blood flow in the left MCA; *C*, T2-WI MRI, the arrow indicates MCA TA; *D*, MR angiography, no blood flow in the left MCA (shown with the arrow); *E*, *F*, T2-WI and DWI, an ischemic lesion in the internal capsule is shown with the arrow.

areas. No other risk factors for IS were found in this young patient. The patient's neurological status was assessed an NIHSS score of 11. The patient had elective microsurgical clipping of the ICA aneurysm. He was discharged with a modified Rankin score of 2.

Discussion

Neurologists and neurosurgeons are aware of embolic CAs; however, there have been only isolated clinical cases described in scarce scientific publications. L. Calviere et al. [14] described one of the largest case series of embolic CA. They followed up 15 patients with IS and transient ischemic attack associated with thromboembolism from the CA. The mean age of patients was 49.7 years. The mean aneurysm diameter was 7.5 mm. During antiplatelet therapy, no patient had recurrent episodes of ischemia. However, the aneurysms ruptured in 2 patients.

In the near future, we can expect increased CA detectability in patients with IS due to the development and increasing use of the thrombectomy technique, which requires angiography. When identifying CAs in patients with IS, doctors should not only assess the risk of its rupture or determine contraindications to thrombolytic therapy but also assume whether the aneurysm, especially a large one, could contribute to the development of cerebral ischemia.

IS in patients with unruptured CAs may be explained by several mechanisms. Firstly, spontaneous thrombosis of the aneurysm can be associated with inflammatory changes in its wall [13]. A. Fomenko et al. presented a clinical case of a 56-year-old man with spontaneous thrombosis of the MCA aneurysm spreading into the lumen of the artery, which led to ischemia throughout the MCA territory [15]. In some cases, with giant aneurysms, adjacent branches can be mechanically compressed, resulting

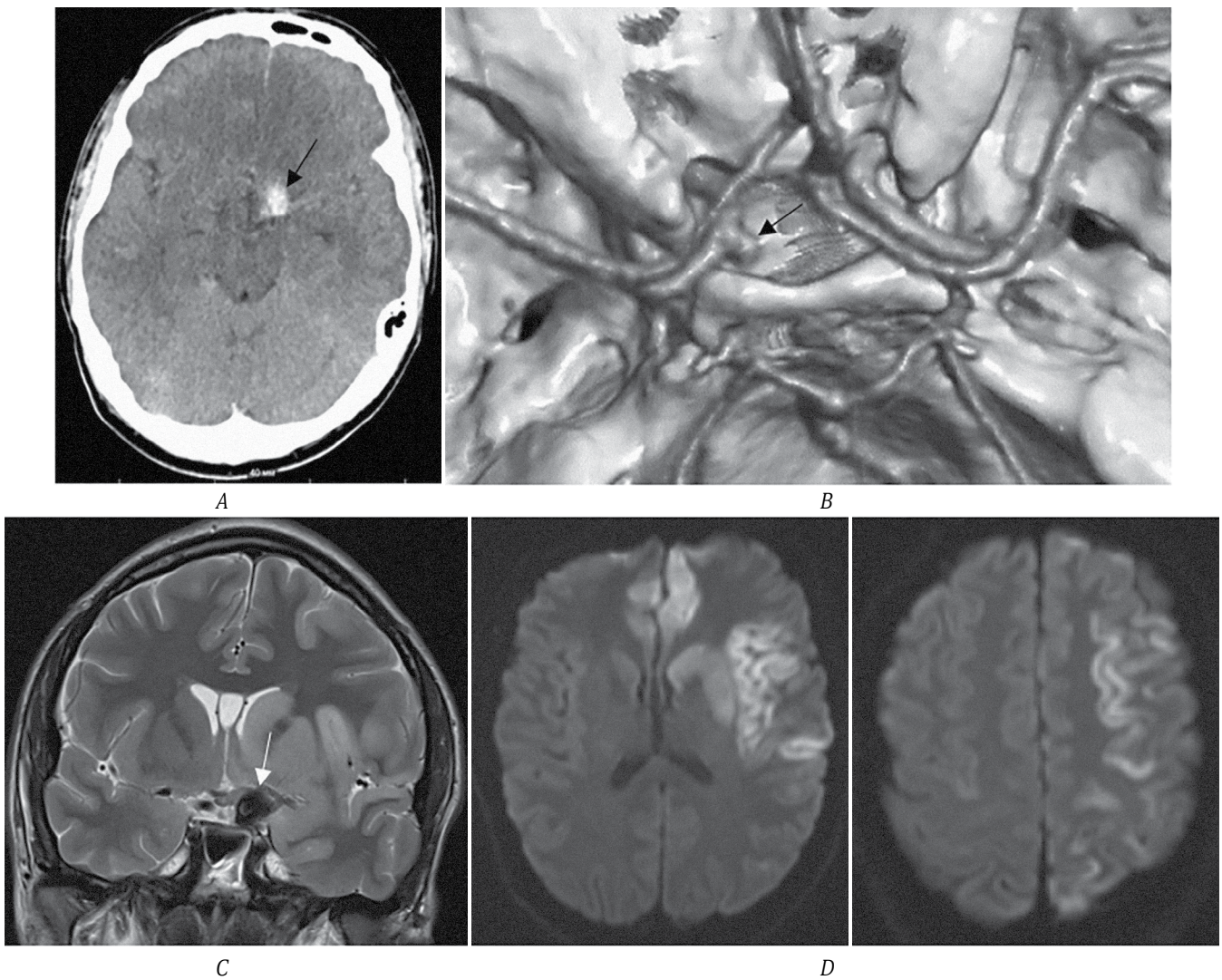

Fig. 4. Brain MSCT of patient A.
A, the arrow indicates the TA of the left ICA; *B*, MSCT angiography, 3D reconstruction, the arrow indicates the functioning part of the aneurysm of the left ICA; *C*, brain T1-WI MRI, the arrow indicates the TA of the left ICA; *D*, MRI in DWI mode, areas of acute ischemia in the subcortical structures of the left brain hemisphere.

in ischemia. However, thromboembolism most often occurs from a completely or partially thrombosed aneurysm sac into the distal cerebral branches [16].

It is not always possible to prove embolism of thrombotic masses from the aneurysm sac. To do so, it is necessary to verify the aneurysm with signs of thrombosis and exclude all other risk factors for IS, primarily cardioembolism and stenoses of extracerebral and intracerebral arteries. Paradoxical embolism through a patent foramen ovale should be ruled out in young patients.

Therefore, TA-associated stroke is more likely to be assumed in patients without other risk factors and with an infarction in the area of the blood supply of the artery carrying the aneurysm. However, as shown in our cases, this may also occur in patients of older age groups.

Thromboembolism can occur both from large/giant aneurysms and aneurysms of up to 1 cm in size [17–19]. All aneurysms in our case series were more than 1 cm in size.

However, what is more important is not the size of the aneurysm itself but whether it contains blood clots. Partial thrombosis of the aneurysm sac can be detected by MSCT angiography or cerebral angiography only based on indirect signs, such as uneven contour or unusual flow of contrast around the thrombosed part. Therefore, it is recommended to perform brain MRI, which can identify the thrombosed part of the aneurysm.

Completely TAs are especially challenging because they are not contrasted during angiography but can also be a source of thromboembolism. In these cases, completely TAs can be detected only by MRI, especially in T2-WI and SWI.

Considering that embolism from the CA may increase the risk of rupture in the near future [9, 11, 12], it is not known if such patients can be safely prescribed antithrombotic therapy, as required by the standards of care for IS. N. Kuroda et al. described a case of TA rupture on the fourth day of IS treatment with antithrombotic therapy [11].

There are no guidelines on the optimal timing of open or endovascular surgical management of the CA after stroke, including that to prevent recurrent episodes of thromboembolism from the aneurysm sac [20, 21].

Conclusion

Cases of cerebral thromboembolism due to CAs are rare and occur mainly in patients with large or giant TAs. In some cases, TA can be suspected based on native MSCT, especially if the patient has a fresh hyperdense thrombus. However, sometimes the aneurysm remains undetected, and recurrent embolism can develop from the sac followed by recurrent strokes and transient ischemic attacks. There are no current guidelines for the management of such patients, such as whether they can be administered with antithrombotic agents and what time is optimal for surgery, so further multicenter studies are needed.

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