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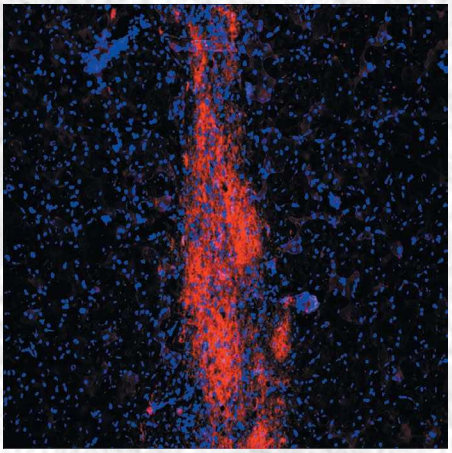

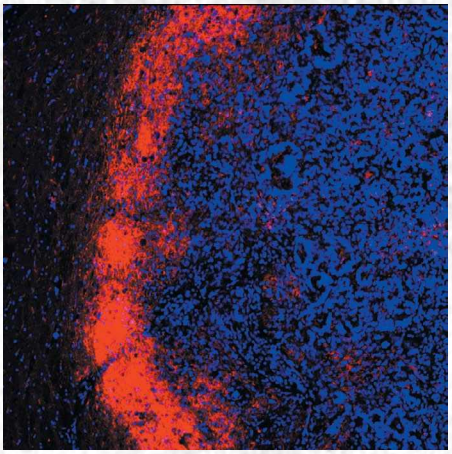

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E-mail: adv2@eco-vector.com

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On the front cover: part of the Figure 6 from the article of A.V. Stavrovskaya et al. (p. 50).

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Адрес: Россия, 125367, Москва, Волоколамское шоссе, д. 80.
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Адрес: 191181, Россия, Санкт-Петербург, Аптекарский переулок, д. 3, литера А, помещение 1Н.
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WEB: https://eco-vector.com

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E-mail: adv2@eco-vector.com

Отдел подписки
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E-mail: podpiska@eco-vector.com

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Адрес: Россия, 125367 Москва, Волоколамское шоссе, д. 80.
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РЕЦЕНЗИРУЕМЫЙ НАУЧНЫЙ МЕДИЦИНСКИЙ ЖУРНАЛ

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Accelerometry in Diagnosis of Functional Tremor

Konstantin M. Evdokimov, Ekaterina O. Ivanova, Amayak G. Brutyan, Ekaterina Yu. Fedotova, Sergey N. Illarioshkin

Research Center of Neurology, Moscow, Russia

Abstract

Introduction. *Functional tremor (FT) is the most common phenotype of functional movement disorders. Electrophysiological assessment is included in the diagnostic criteria for tremor; however, there is currently no consensus criteria for the differential diagnosis of FT.*

The ***objective*** *of this study was to evaluate the utility of tremor frequency characteristics derived from accelerometry for the differential diagnosis between FT and organic tremor (OT).*

Materials and methods. *Nineteen patients with FT, 20 patients with essential tremor, and 20 patients with Parkinson's disease were enrolled in the study and underwent electrophysiological examination with a two-channel accelerometer and subsequent data processing.*

Results. *The study results revealed the differences in the frequency peak widths in patients with FT and OT, predominantly while performing a cognitive load task. This criterion showed a high sensitivity (100%) and a high specificity (97.5%) for the diagnosis of FT in the study population.*

Conclusion. *Tremor characteristics recorded during accelerometry combined with cognitive load task can serve as an additional testing aid for differential diagnosis between functional and organic tremor.*

Keywords: *functional movement disorders; functional tremor; diagnosis, accelerometry*

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. 10-3/22, November 23, 2022).

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For correspondence: 80 Volokolamskoye shosse, Moscow, 125367, Russia. Research Center of Neurology.
E-mail: evdokimov@neurology.ru. Evdokimov K.M.

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Акселерометрический анализ в диагностике функционального тремора

Евдокимов К.М., Иванова Е.О., Брутян А.Г., Федотова Е.Ю., Иллариошкин С.Н.

Научный центр неврологии, Москва, Россия

Аннотация

Введение. *Функциональный тремор (ФТ) – наиболее часто встречающийся фенотип функционального двигательного расстройства. Электрофизиологическая оценка тремора входит в объём диагностики, однако нет единого стандарта дифференциальной диагностики ФТ.*

Целью *данного исследования являлась оценка возможности использования частотных характеристик тремора по данным акселерометрии для дифференциальной диагностики ФТ и органического тремора (ОТ).*

Материалы и методы. *В исследовании участвовали 19 пациентов с ФТ, 20 пациентов с эссенциальным тремором и 20 пациентов с болезнью Паркинсона, которым проводили электрофизиологическое исследование, включающее двухканальную акселерометрию с последующей обработкой полученных данных.*

Результаты. *В ходе исследования были выявлены различия в ширине частотного пика тремора по данным акселерометрии у пациентов с ФТ и ОТ, преимущественно на фоне когнитивной нагрузки. Данный показатель в исследуемой выборке продемонстрировал высокую чувствительность (100%) и специфичность (97,5%) для диагностики ФТ.*

Заключение. *Анализ характеристик тремора по данным акселерометрии с дополнительной задачей в виде когнитивной нагрузки может использоваться в качестве дополнительного теста для дифференциальной диагностики ФТ и ОТ.*

Ключевые слова: *функциональные двигательные расстройства; функциональный тремор; диагностика; акселерометрия*

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Адрес для корреспонденции: 125367, Москва, Волоколамское шоссе, д. 80. Научный центр неврологии. E-mail: evdokimov@neurology.ru. Евдокимов К.М.

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Introduction

Tremor is an involuntary, rhythmic, rapid back-and-forth (oscillatory) movement of a body part [1, 2]. This hyperkinesis is the most common movement disorder in clinical practice and can be observed in many diseases with various underlying pathophysiology [3].

In 1998, the International Parkinson and Movement Disorder Society (MDS) presented the first consensus criteria for classifying tremor disorders based on various types of tremor syndromes [4]. In 2018, this classification was revised, and, along with a description of tremor syndromes, two evaluation axes were added: Axis 1 – clinical characteristics and Axis 2 – etiology [2]. Axis 1 includes historical features (age at onset, family history, and temporal evolution), tremor characteristics, tremor-associated signs, and laboratory tests including electrophysiological study. For electrophysiological assessment of tremor, the authors of the classification suggested surface electromyography (SEMG): to document the presence of tremor, measure tremor frequency, evaluate pattern and rhythmicity of EMG-activity (e.g., to differentiate tremor from myoclonus). They also suggest a Fourier analysis of accelerometric and EMG recordings with and without weight loading to identify mechanical-reflex and central neurogenic tremors, and frequency and coherence analysis of EMG-activity from multiple limbs to diagnose primary orthostatic tremor [2]. In the literature, numerous reports are on other methods suitable for tremor recording and assessment: gyroscope, tremor video-recording with subsequent data processing, and various kinematic and tactile techniques [1].

Functional (former psychogenic) tremor is characterized by distractibility, changes in frequency during contralateral rhythmic movements (entrainment), antagonistic muscle co-activation, an increase in the oscillation amplitude during weight loading, and tremor regression during contralateral ballistic movements [2, 5]. A meta-analysis of the individual data obtained from 4,905 patients with functional movement disorders (FMD) revealed that FT was the most prevalent hyperkinesis, affecting 21.6% of the patients, which was also diagnosed within the mixed FMD phenotypes in 23% of the patients. Isolated functional tremor developed most frequently in females (71.2%) aged 40–42 years [6].

To date, there are no consensus criteria for FMD diagnosis. In clinical practice, the Fahn–Williams criteria are widely used [7]. Further, A. Gupta et al. proposed to extend these criteria with electrophysiological tests for FMD assessment, predominantly to differentiate tremor from myoclonus [8]. To identify functional tremor, several parameters are to be assessed: EMG recording frequency, acceleroяяяmeter oscillations (including the analysis of the frequency peak width), duration and pattern of EMG-recordings, variability, distractibility, tremor regression during ballistic movements, entrainment by rhythmic movements, an increase in the amplitude and frequency during weight loading, antagonistic muscle co-activation, and bilateral coherence analysis of EMG-recordings from muscles involved in tremor [5, 9–11]. In 2016, the international workgroup presented Tremor Test Battery (TTB) as the basis of validated laboratory-supported criteria for the diagnosis of FT [12]. The ETB consists of 10 parameters, each can be scored with 1 point (Table 1). A cut-off score of 3 points is indicative of FT. However, it should be mentioned that the ETB protocol requires special training, and ETB data recording and processing are time-consuming, which is rather challenging in clinical practice. Therefore, the search for more convenient diagnostic tools should be continued. To date, the members of the MDS Functional Movement Disorders Study Group and Clinical Neurophysiology Study Group have not agreed upon a consensus protocol for tremor assessment.

The **objective** of this study was to evaluate the utility of tremor frequency characteristics derived from accelerometry for the differential diagnosis between FT and organic tremor (OT).

Materials and methods

The study included 19 patients with FT (14 females aged 38 [26; 46] years) and 40 patients with OT assigned to 2 groups: 20 patients with essential tremor (ET; 13 females aged 71 [55; 75] years) and 20 patients with parkinsonian tremor (PD; 9 females aged 57.5 [49.5; 62.5] years). The type of tremor was identified according to MDS consensus criteria for classification of tremors [2]. The FT diagnosis was based on clinically positive diagnostic criteria: distractibility, entrainment by contralateral rhythmical movements, antagonistic muscle co-activation, and transition of tremor to another body part with external restraint of the affected hand. Exclusion criteria were a combination of various tremor types (for example, FT in PD patients, a combination of ET and parkinsonian tremor, etc.). Electrophysiological assessment of tremor was performed with a two-channel accelerometer (the accelerometer was attached to the back of the middle phalanx

Table 1. Tremor Test Battery (translated and adapted from [12])

Parameter	Assessment technique
Tremor amplitude with weight loading (1 point)	An increase in total power of the spectra derived from a 30-second epoch of accelerometer oscillations recorded from more affected hand before and after 500-g loading
Response to ballistic movements (1 point)	Tremor pause or > 50% reduction in tremor frequency or amplitude in at least 7 of 10 contralateral ballistic movement tests
EMG coherence in contralateral limbs (1 point)	The point was assigned in case of significant EMG-coherence between frequency spectra from right and left wrist extensors by comparing the frequency where coherence was detected with the frequency of tremor
Tonic co-activation (1 point)	The tonic co-activation phase was defined as tonic discharge of antagonist muscles (wrist flexors and wrist extensors) approximately 300 ms before the onset of tremor bursts
Tapping task performance by contralateral tapping (max. 3 points)	Tapping performance at 1, 3, and 5 Hz was considered correct if it fell within the range of 0.5–1.5 Hz, 2.5–3.5 Hz, and 4.5–5.5 Hz, respectively
Changes in tremor characteristics for more affected hand during contralateral tapping (max. 3 points)	Tremor in the ipsilateral hand during contralateral tapping was assessed for entrainment, tremor suppression, or a frequency shift, which was defined as pathological if the frequency peak shifted with 19.0, 26.9, and 25.7% during tapping at 1, 3, and 5 Hz, respectively

of the index or middle finger). The tremor was recorded with a Viking EDX Electrodiagnostic System (Natus Neurology Incorporated, USA) and assessed at rest, with arms extended (postural tremor, PT), with or without cognitive load (CL): a serial subtraction task (patients were asked to consecutively subtract each time 13 out of 100). The tremor was recorded for 30 seconds in each condition.

The recorded signals were exported and processed using an open-source tool for tremor analysis – Tremoroton (Fig. 1) [13]. Tremor frequency characteristics derived from accelerometry (the dominant frequency [the upper-frequency peak point], a peak width, upper and lower limits, a mean amplitude of oscillations) were assessed using the fast Fourier transformation test. The shift of minimum and maximum frequencies was defined as the modulus of the frequency difference with or without CL. A frequency peak/band splitting is defined as a tremor peak width > 0.5 Hz.

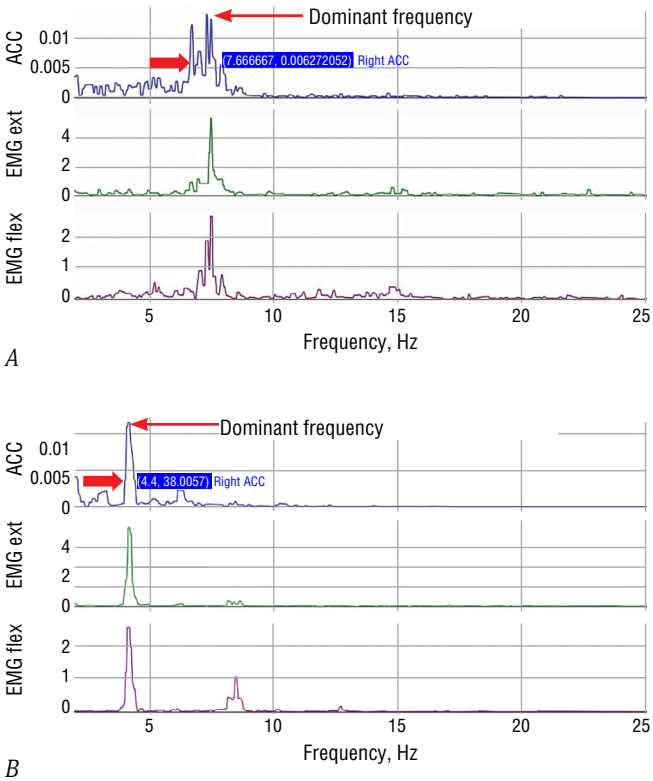

Fig. 1. Tremor frequency peak width was measured with Tremoroton software.
Upper and lower limits of the frequency peak of accelerometer oscillations were determined manually at 40–50% from the height of the frequency peak. The peak width is defined as a difference between upper and lower limits. The red thin arrow indicates the point where the dominant frequency of the peak is measured. The red bold arrow and blue bar indicate the points where the peak width was measured.
A – the frequency spectrum in a patient with FT; *B* – in a patient with PD; ACC – accelerometer oscillations; EMG ext – EMG-recordings from wrist extensors; EMG flex – EMG-recordings from wrist flexors.

The data were processed using Microsoft Excel and IBM SPSS Statistics v. 27 software. The Kruskal–Wallis nonparametric test was used to assess inter-group differences followed by ad hoc pairwise inter-group comparison with the Bonferroni correction. The Wilcoxon test was used to assess intra-group differences. The level of significance was set at 0.05. The ROC analysis was used to evaluate the sensitivity and specificity of the peak width differences.

Results

The dominant frequency of accelerometer oscillations with and without CL was similar in patients with FT and OT without any statistically significant differences (Table 2). Statistically significant differences in the width of the dominant frequency peak were observed in patients with FT compared with ET and PD patients, both without CL (Figure 2, *A*) and with CL (Figure 2, *B*). At the same time, a gradual increase in the peak width in FT patients with CL was noted (p_W = 0.002). The PT peak width in ET patients without CL was slightly greater than that in PD patients; however, with CL added, the frequency peak width decreased in ET patients (p_W = 0.002) and remained stable in PD patients (p_W = 0.538). Inter-group comparison of the differences between the PT peak width with or without CL yielded similar results (Figure 2, *C*).

The analysis of changes in upper and lower limits of the frequency peak (minimum and maximum frequency, respectively) revealed differences in the shift in minimum frequency peak in FT patients compared with that in ET and PD patients (p = 0.04), but these differences did not reach the level of statistical significance after the pairwise comparison (Figure 2, *D*). A change in the frequency peak width in FT patients was mainly associated with an upward shift of the upper limit of the frequency peak, which was further confirmed by the pairwise comparison with ET and PD patients (Figure 2, *E*).

An additional parameter that differed in the FT patients compared with the ET and PD patients was the ratio of the mean amplitude of accelerometer oscillations with CL to the same parameter measured without CL (Figure 2, *E*). In the OT group, the oscillation amplitude increased: 1.43 [1.23; 2]-fold in ET patients (p_W = 0.003), 1.63 [1.25; 3.38]-fold in PD patients (p_W = 0.008), while in FT patients the oscillation amplitude slightly decreased with the amplitude ratio of 0.7 [0.47; 1.4] (p_W = 0.031).

Among the studied accelerometric parameters, the frequency peak width with CL was of most interest. The utility of this method for the differential diagnosis of hyperkinetic movements, such as tremor, was evaluated with the ROC analysis. With the PT frequency peak width without CL of ⩾ 0.55 Hz, the sensitivity and specificity of this FT identification method were 94.7 and 85%, respectively. The frequency peak width with CL of ⩾ 0.6 Hz indicates FT with the sensitivity of 100% and specificity of 97.5%. In the studied sample, the diagnostic

Table 2. Accelerometric characteristics of tremor frequency

Parameter	FT	ET	PD	*p*
Dominant PT frequency without CL	6 [3,2; 7,8]	5,3 [4,8; 5,7]	5,25 [4,75; 6,20]	0,800
Dominant PT frequency with CL	4,9 [3,8; 8,4]	5,3 [4,65; 5,95]	5,4 [4,80; 5,95]	0,968
PT peak width without CL	1,4 [0,9; 1,9]	0,4 [0,3; 0,5]	0,3 [0,2; 0,4]	< 0,001
PT peak width with CL	1,9 [1,4; 3,0]	0,25 [0,2; 0,3]	0,3 [0,2; 0,4]	< 0,001
Difference in PT peak width without CL and with CL	0,6 [0; 1,2]	–0,1 [-0,3; 0]	–0,1 [–0,15; 0,10]	0,003
Shift of minimum frequency, with CL	0,4 [0,2; 2,8]	0,2 [0,10; 0,35]	0,2 [0,10; 0,40]	0,040
Shift of maximum frequency, with CL	1,3 [0,5; 3,0]	0,1 [0,10; 0,35]	0,25 [0,15; 0,4]	< 0,001

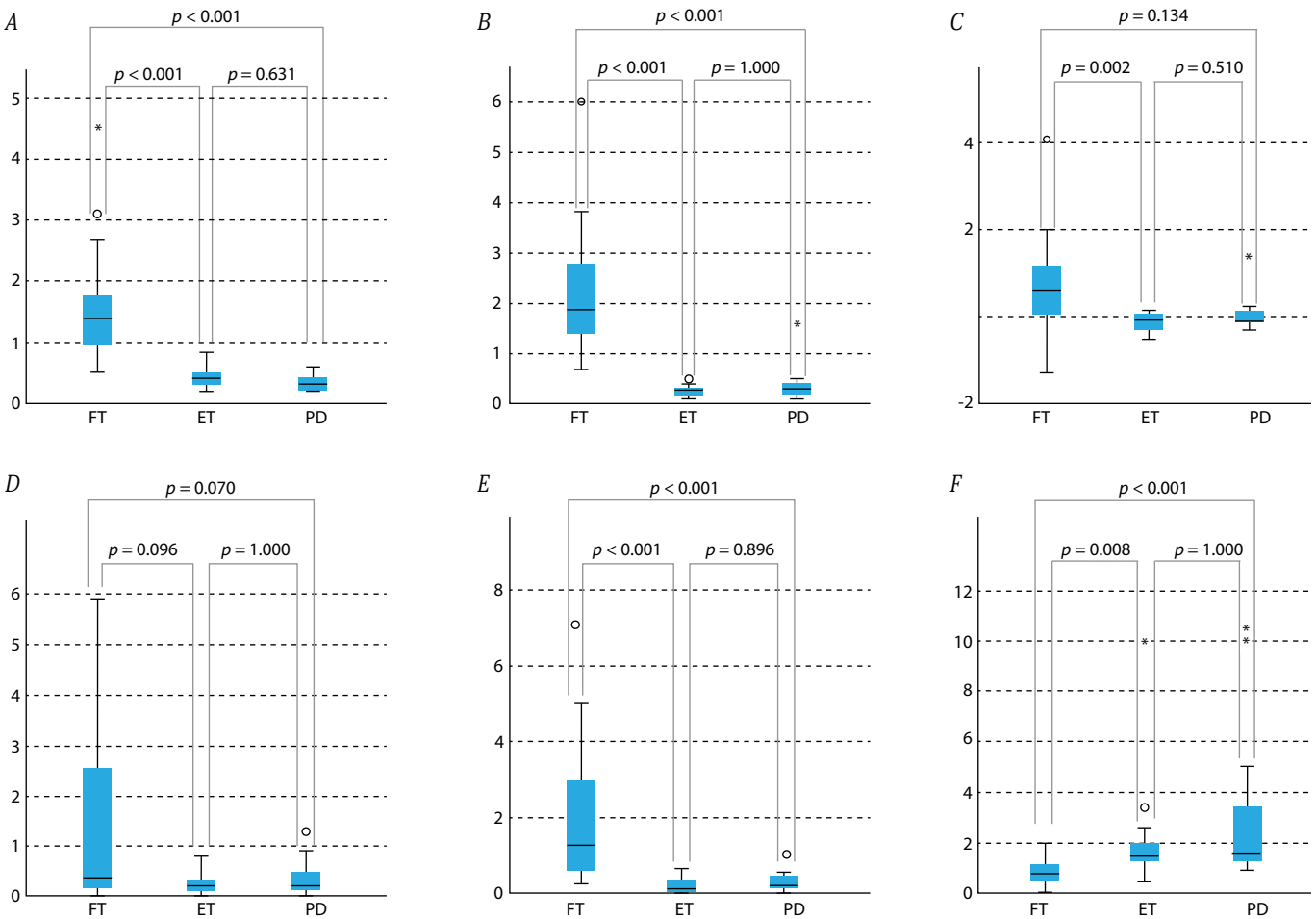

Fig. 2. Results of a posteriori pairwise comparison between FT, ET, and PD patients.
A – PT frequency peak width without CL; CL – cognitive load; PT – postural tremor; *B* – PT frequency peak width with CL; *C* – difference in PT frequency peak width without CL; *D* – shift of minimum frequency peak (lower limit); *E* – shift of maximum frequency peak (upper limit); *F* – ratio of the mean amplitude of PT oscillations with CL to the mean amplitude of PT oscillations without CL.

accuracy of the method without CL and with CL was 98.3% and 99%, respectively.

Discussion

Electrophysiological assessment of tremor is becoming more widely used. The results of our study confirm that the dominant tremor frequency alone cannot be used for differential diagnosis between various types of tremors (except for orthostatic tremor with the frequency of 13–18 Hz, which significantly exceeds the frequencies of 4–8 Hz typical for other types of OT) [4]. Therefore, the studies aimed to find additional FT markers and new methods of analyzing tremor, such as ETB, were conducted. Data recording and process-

ing in many of the techniques are quite time-consuming. For example, a complete ETB protocol followed by data analysis takes about 30–40 min, while accelerometric assessment of the frequency peak width and visual evaluation of the spectrogram of two recordings (PT with and without CL) takes about 5 min. Today, many types of wearable accelerometers are available for long-term ambulatory tremor analysis. It is worth mentioning that a consensus protocol for tremorography is not yet developed. However, this issue is being actively discussed by the members of the MDS Clinical Neurophysiology Study Group. Another obstacle to the implementation of the proposed techniques is the software used in laboratories, which is provided by a particular manufacturer of electrodiagnostic equipment or developed in-house for its own purposes. Tremoroton, an open-source tool for analyzing the txt files exported from the device, may facilitate the implementation of tremorography in clinics for widespread use by clinical neurophysiologists.

The accelerometer data obtained in our study showed that the frequency peak width in FT patients went above 0.6 Hz, while in OT patients it remained below 0.5 Hz. The frequency peak width of $\geqslant$ 0.6 Hz with CL may be used as a primary electrophysiological criterion for tremor assessment, which was confirmed by the ROC-analysis results. The frequency peak width has been used as a criterion for differential diagnosis between FT and OT in the Mayo Clinic, for example. A routine EMG screening for tremor is performed based on this criterion and according to its results, patients are selected for surgical treatment of tremor by deep brain stimulation or destruction by MRI-guided focused ultrasound. Z. Chou et al. reported that additional electrophysiological screening allowed them to identify FT in 12 (14%) of 87 patients, clinically pre-selected for surgery, thus avoiding inappropriate surgery and reserving the treatment opportunity for other patients [14]. Our study has shown that instead of labor-intensive surface EMG, easier-to-implement accelerometry can be used without any loss of accuracy.

The use of CL increases sensitivity and specificity of this method. The CL task is most commonly used for the clinical assessment of tremor and was not used in the electrophysiological test battery for the diagnosis of FT. However, the data obtained in our study confirm its significance in electrophysiological testing. The results of our study demonstrated the changes in the frequency peak width of accelerometric oscillations associated with CL introduction, which allows for rapid identification of tremor type. Additionally, electrophysiological data confirmed the distractibility phenomenon in FT patients during performing CL task. It appears as an extended frequency peak and a decreased mean oscillation amplitude, while in OT patients, on the contrary, the frequency peak narrows, and tremor amplitude increases when a patient's attention is diverted from tremor control to a cognitive task.

Nowadays, there are various wearable sensors proposed to assess tremor [15], predominantly to monitor the clinical features of Parkinson's disease [16]. G. Kramer et al. studied the objective daily duration of tremor recorded with a wrist-worn accelerometer compared with subjective symptom burden in FT and OT patients [17]. These easy-to-use and relatively inexpensive accelerometers may be a good basis for a fast, simple, and cost-efficient method of differential diagnosis between FT and OT, which would allow wider use of electrophysiological diagnostic tools in the outpatient clinical practice.

The proposed technique tested in an appropriate validation study with a sufficient number of patients could be used for the differential diagnosis between FT and OT. The versatility of export files and rapid data processing are additional factors that facilitate its widespread use.

A timely and accurate diagnosis of FT is of great importance, as the management of this condition differs significantly from that of OT and is based more on rehabilitation rather than on a pharmacological treatment.

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Information about the authors

Konstantin M. Evdokimov – postgraduate student, neurologist, 5th Neurology department, Institute of Clinical and Preventive Neurology, Research Center of Neurology, Moscow, Russia, https://orcid.org/0000-0001-6217-4151
Ekaterina O. Ivanova – Cand. Sci. (Med.), researcher, neurologist, 5th Neurology department, Institute of Clinical and Preventive Neurology, Research Center of Neurology, Moscow, Russia, https://orcid.org/0000-0003-3337-1759
Amayak G. Brutyan – Cand. Sci. (Med.), Head, Laboratory of clinical neurophysiology, Institute of Clinical and Preventive Neurology, Research Center of Neurology, Moscow, Russia, https://orcid.org/0000-0002-6381-2925
Ekaterina Yu. Fedotova – D. Sci. (Med.), leading researcher, Head, 5th Neurology department, Institute of Clinical and Preventive Neurology, Research Center of Neurology, Moscow, Russia, https://orcid.org/0000-0001-8070-7644
Sergey N. Illarioshkin – D. Sci. (Med.), prof., RAS Full Member, Director, Brain Institute, Deputy director, Research Center of Neurology, Moscow, Russia, https://orcid.org/0000-0002-2704-6282

Authors' contribution. *Evdokimov K.M.* – creating the research concept, conducting the research, analyzing the data; *Ivanova E.O.* – conducting the research, methodology development; *Brutyan A.G.* – data curation, software development; *Fedotova E.Yu.* – creation of the research concept, methodology development, supervising the research work; *Illarioshkin S.N.* – supervising the research work.

Информация об авторах

Евдокимов Константин Михайлович – аспирант, врач-невролог 5-го неврологического отделения с молекулярно-генетической лабораторией Института клинической и профилактической неврологии Научного центра неврологии, Москва, Россия, https://orcid.org/0000-0001-6217-4151
Иванова Екатерина Олеговна – канд. мед. наук, н. с., врач-невролог 5-го неврологического отделения с молекулярно-генетической лабораторией Института клинической и профилактической неврологии Научного центра неврологии, Москва, Россия, https://orcid.org/0000-0003-3337-1759
Брутян Амаяк Грачевич – канд. мед. наук, в. н. с., рук. лаб. клинической нейрофизиологии Института клинической и профилактической неврологии Научного центра неврологии, Москва, Россия, https://orcid.org/0000-0002-6381-2925
Федотова Екатерина Юрьевна – д-р мед. наук, в. н. с., зав. 5-м неврологическим отделением Института клинической и профилактической неврологии Научного центра неврологии, Москва, Россия, https://orcid.org/0000-0001-8070-7644
Иллариошкин Сергей Николаевич – д-р мед. наук, проф., акад. РАН, зам. директора по научной работе, директор Института мозга Научного центра неврологии, Москва, Россия, https://orcid.org/0000-0002-2704-6282

Вклад авторов: *Евдокимов К.М.* – создание концепции исследования, проведение исследования, анализ данных; *Иванова Е.О.* – проведение исследования, разработка методологии; *Брутян А.Г.* – курирование данных, программное обеспечение; *Федотова Е.Ю.* – создание концепции исследования, разработка методологии, руководство научно-исследовательской работой; *Иллариошкин С.Н.* – руководство научно-исследовательской работой.

Characteristics of Patients with Hereditary Transthyretin Amyloid Polyneuropathy and Chronic Idiopathic Axonal Polyneuropathy in Russia: PRIMER Study Results

Natalia A. Suponeva[1], Olga E. Zinovyeva[2], Fatima R. Stuchevskaya[3, 4, 5], Tatiana G. Sakovets[6, 7], Daria A. Grishina[1], Maria S. Kazieva[1], Elvira I. Safiulina[2], Anton P. Solovyov[8], Eugenia A. Zorina[8]

[1]Research Center of Neurology, Moscow, Russia;
[2]I.M. Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russia;
[3]Medical Center "Reavita Med SPb", Saint Petersburg, Russia;
[4]City Multidisciplinary Hospital No. 2, Saint Petersburg, Russia;
[5]First Pavlov State Medical University of St. Petersburg, Saint Petersburg, Russia;
[6]Republican Clinical Hospital, Kazan, Russia
[7]Kazan State Medical University, Kazan, Russia;
[8]AstraZeneca Pharmaceuticals LLC, Moscow, Russia

Abstract

Introduction. *Hereditary transthyretin amyloidosis with polyneuropathy (hATTR-PN) is a severe progressive hereditary disease. Even with the availability of genetic testing for transthyretin (TTR) gene variants, timely hATTR-PN diagnosis remains challenging due to a great variability in its clinical presentation. Patients with hATTR-PN are often misdiagnosed with chronic idiopathic axonal polyneuropathy (CIAP).*

The ***objective of our study*** *is to describe the baseline electrophysiological, clinical, and demographic characteristics of hATTR-PN and CIAP patients and to establish patients' pre-selection criteria for genetic testing.*

Materials and methods. *Retrospective analysis was performed in 42 hATTR-PN patients and 58 CIAP patients (according to diagnosis defined in medical records from 1 January 2017 to 1 March 2024). Demographic, clinical, and electrophysiological data were collected at diagnosis. To identify factors influencing the likelihood of the hATTR-PN presence, a logistic regression model including clinically relevant variables was developed.*

Results. *The mean age of hATTR-PN and CIAP patients was 57.7 and 60.9 years, respectively. As compared with CIAP patients, those with hATTR-PN more frequently exhibited gait disturbances (64.3% vs 37.9%), autonomic (47.6% vs 12.1%), cardiac (35.7% vs 10.3%) and gastrointestinal symptoms (64.3% vs 12.1%), unintentional weight loss (45.2% vs 12.1%), and heart failure with preserved ejection fraction (26.2% vs 6.9%). Peripheral nerve conduction scores were also lower in the hATTR-PN group. In predicting hATTR-PN, the logistic regression model had a sensitivity of 91% and a specificity of 97%.*

Conclusion. *Demographic, clinical, and electrophysiological characteristics of patients with hATTR-PN and CIAP were described. Based on the screening data, it is feasible to predict hATTR-PN in CIAP patients with relatively high accuracy, sensitivity, and specificity.*

Keywords: *transthyretin amyloidosis; polyneuropathy; transthyretin; screening tool*

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For correspondence: 80 Volokolamskoye shosse, Moscow, 125367, Russia. Research Center of Neurology.
E-mail: suponeva@neurology.ru. Suponeva N.A.

For citation: Suponeva N.A., Zinovyeva O.E., Stuchevskaya F.R., Sakovets T.G., Grishina D.A., Kazieva M.S., Safiulina E.I., Solovyov A.P., Zorina E.A. Characteristics of patients with hereditary transthyretin amyloid polyneuropathy and chronic idiopathic axonal polyneuropathy in Russia: PRIMER study results. *Annals of Clinical and Experimental Neurology*. 2024;18(4):12–26.

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Характеристики пациентов с наследственной формой транстиретиновой амилоидной полинейропатии и хронической идиопатической аксональной полинейропатией в российской популяции: результаты исследования «ПРАЙМЕР»

Н.А. Супонева[1], О.Е. Зиновьева[2], Ф.Р. Стучевская[3, 4, 5], Т.Г. Саковец[6, 7], Д.А. Гришина[1], М.С. Казиева[1], Э.И. Сафиулина[2], А.П. Соловьев[8], Е.А. Зорина[8]

[1]Научный центр неврологии, Москва, Россия;
[2]Первый Московский государственный медицинский университет имени И.М. Сеченова (Сеченовский университет), Москва, Россия;
[3]Медицинский центр «Реавита Мед СПб», Санкт-Петербург, Россия;
[4]Городская многопрофильная больница № 2, Санкт-Петербург, Россия;
[5]Первый Санкт-Петербургский государственный медицинский университет имени академика И.П. Павлова, Санкт-Петербург, Россия;
[6]Республиканская клиническая больница, Казань, Россия;
[7]Казанский государственный медицинский университет, Казань, Россия;
[8]«АстраЗенека Фармасьютикалз», Москва, Россия

Аннотация

Введение. *Наследственная транстиретиновая амилоидная полинейропатия (hATTR-PN) – прогрессирующее тяжёлое наследственное заболевание. Несмотря на доступность генетического тестирования для выявления вариантов гена транстиретина (TTR), своевременная диагностика затруднена вследствие разнообразия клинических проявлений. Частым ошибочным диагнозом является хроническая идиопатическая аксональная полинейропатия (ХИАП).*

Цель исследования *– описание исходных электрофизиологических, клинических и демографических характеристик пациентов с hATTR-PN и ХИАП и подбор критериев для отбора пациентов, которые подлежат генетическому тестированию.*

Материалы и методы. *Ретроспективный анализ проведён у 42 пациентов с hATTR-PN и 58 пациентов с ХИАП (диагноз установлен в медицинской документации с 01.01.2017 по 01.03.2024). Демографические и клинические характеристики, результаты электрофизиологического исследования были собраны на момент постановки диагноза. Клинически релевантные параметры включили в модель логистической регрессии для выявления факторов, влияющих на вероятность наличия hATTR-PN.*

Результаты. *Средний возраст составил 57,7 (hATTR-PN) и 60,9 (ХИАП) года. В группе hATTR-PN по сравнению с ХИАП чаще встречались нарушения походки (64,3 и 37,9%), вегетативные симптомы (47,6 и 12,1%), проявления со стороны сердца (35,7 и 10,3%), желудочно-кишечного тракта (64,3 и 12,1%), непреднамеренная потеря веса (45,2 и 12,1%), сердечная недостаточность с сохранённой фракцией выброса (26,2 и 6,9%), были хуже показатели проводящей функции периферических нервов. Модель логистической регрессии показала чувствительность 91% и специфичность 97% в отношении предсказания наличия hATTR-PN.*

Заключение. *Описаны демографические, клинические и электрофизиологические характеристики пациентов с hATTR-PN и ХИАП. На основании скрининговых данных возможно с хорошей точностью, чувствительностью и специфичностью предсказать наличие hATTR-PN у пациентов с ХИАП.*

Ключевые слова: *транстиретиновый амилоидоз; полинейропатия; транстиретин; скрининговый инструмент*

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Адрес для корреспонденции: 125367, Россия, Москва, Волоколамское шоссе, д. 80. Научный центр неврологии.
E-mail: suponeva@neurology.ru. Супонева Н.А.

Для цитирования: Супонева Н.А., Зиновьева О.Е., Стучевская Ф.Р., Саковец Т.Г., Гришина Д.А., Казиева М.С., Сафиулина Э.И., Соловьев А.П., Зорина Е.А. Характеристики пациентов с наследственной формой транстиретиновой амилоидной полинейропатии и хронической идиопатической аксональной полинейропатией в российской популяции: результаты исследования «ПРАЙМЕР». *Анналы клинической и экспериментальной неврологии*. 2024;18(4):12–26.

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Introduction

Hereditary transthyretin amyloidosis is a severe progressive multisystem disease caused by mutations in the gene encoding transthyretin (*TTR*) [1]. The *TTR* gene composed of four exons is located on chromosome 18. Over 160 *TTR* gene variants have been identified so far [2]. The majority of hATTR-PN cases (formerly referred to as Familial Amyloid Polyneuropathy) are caused by a point mutation leading to methionine-for-valine substitution at position 30 of the mature protein (Val30Met, or p.Val50Met) [3]. The mutated tetrameric TTR protein is unstable and dissociates into misfolded monomers that accumulate mainly in the heart and the peripheral nervous system, causing cardiomyopathy and progressive axonal polyneuropathy, respectively [4].

hATTR-PN is an adult-onset disease with variable penetrance and an autosomal-dominant mode of transmission [5, 6]. Accumulation of TTR amyloid fibrils in the peripheral nervous system results in rapidly progressing sensorimotor and autonomic polyneuropathy leading to patient's disability. Patients die within an average of 10 years from the onset of symptoms [7].

The prevalence of hATTR-PN per 1 million population ranges from 0.9 to 204 and 0.3 to 56 in endemic and non-endemic countries, respectively [8]. Portugal, Japan, Sweden, and Brazil are recognized as endemic countries; however, the global incidence of hATTR-PN continues to increase and cases are mainly sporadic. It is expected that the accuracy of diagnosis will improve with the expanded use of genetic testing, particularly in non-endemic regions, thereby increasing the detection of new hATTR-PN cases [1, 4, 6]. No data on the hATTR-PN prevalence in Russia are currently available. Based on available data extrapolation [9], we can assume that the estimated prevalence for Russia would be 0.32 (per 1 million population). This estimate is tentative and is based on the lowest prevalence rates in other countries.

Timely hATTR-PN diagnosis is challenging mainly due to a great variability in symptoms, with signs of damage not only to peripheral nerves, but also to many internal organs and systems. Clinical presentation of hATTR-PN often mimics that of other, more prevalent, diseases [10]. Hence, the early diagnosis of this rare disease poses a significant challenge for a neurologist practicing in non-endemic areas. In these areas, hATTR-PN is suspected in only 26–38% of initial evaluations [5]. A delay in diagnosis can be as long as 3–4 years, which directly affects the functional and vital prognosis for patients.

The symptom complex of chronic symmetric sensorimotor or peripheral neuropathy associated with hATTR-PN is non-specific. Neurological disturbances similar to these may accompany a variety of conditions, each with the potential for misdiagnosis [11]. Initial hATTR-PN misdiagnoses commonly include chronic inflammatory demyelinating polyradiculoneuropathy (CIDP), lumbar spinal stenosis, diabetic polyneuropathy, carpal tunnel syndrome (CTS), paraneoplastic polyneuropathy, paraproteinemic polyneuropathy, and, more rarely, inherited polyneuropathy, and amyotrophic lateral sclerosis [10]. To date, patients with hATTR-PN are often misdiagnosed with chronic idiopathic axonal polyneuropathy (CIAP), which is a peripheral nerve disease of uncertain etiology. In Russia, such patients are usually diagnosed with polyneuropathy of unspecified or

mixed etiology. CIAP is diagnosed in 20–30% of patients with polyneuropathy. The disease is slowly progressive and most patients remain ambulatory with mild to moderate disability, but the quality of life is affected in all patients [12].

In patients without a family history of amyloidosis who present with progressive idiopathic axonal polyneuropathy or atypical CIDP, current guidelines suggest that the diagnosis of hATTR-PN should be considered first. Red flag symptoms and manifestations are autonomic dysfunction, early gait disturbances, gastrointestinal manifestations, CTS or a history of surgically corrected bilateral CTS, concomitant cardiac abnormalities, or unexplained weight loss [5]. In patients with such red flag symptoms, genetic testing should be performed to establish mutation status of the *TTR* gene.

We conducted a present multicenter observational study to define the baseline electrophysiological, clinical, and demographic characteristics of the patients diagnosed with hATTR-PN and CIAP in Russia. The secondary objective of this study was to develop a screening tool to preselect patients with axonal polyneuropathy for the *TTR* gene sequencing to detect its variants.

Materials and Methods

Study Design and Population

A multicenter, non-interventional, observational, retrospective study with secondary data collection was conducted at four institutions specialized in neurology and located in Russia:
- Research Center of Neurology (Moscow);
- M. Sechenov First Moscow State Medical University (Sechenov University, Moscow);
- Medical Center "Reavita Med SPb" (Saint Petersburg);
- Republican Clinical Hospital of the Ministry of Health of the Republic of Tatarstan (Kazan).

Given its non-interventional design, this study did not interfere with routine clinical practice or procedures and examinations of the patients. All the examinations were conducted in accordance with the standard clinical practice protocols of the study sites and their findings were retrospectively obtained from medical records.

The study included adult patients with a confirmed diagnosis of hATTR-PN or CIAP, as per primary medical records, who met the following inclusion criteria:
- hATTR-PN or CIAP diagnosis or its equivalents: polyneuropathy of unspecified etiology, polyneuropathy of mixed etiology (patients with axonal polyneuropathy carrying a pathogenic *TTR* gene mutation were classified as having hATTR-PN);
- hATTR-PN or CIAP was diagnosed between 1 January 2017 and 1 March 2024;
- at least 1 month between the hATTR-PN or CIAP diagnosis and study inclusion date;
- age of ⩾ 18 years at hATTR-PN or CIAP diagnosis.

Non-inclusion criterion was participation in any clinical trial of an investigational product from the date of hATTR-PN or CIAP diagnosis until the end of the retrospective follow-up period.

Due to retrospective design of the study, no written informed consent was required. All data were collected retrospectively and anonymously from the medical records available in the study sites.

Data Collection

This study was conducted using secondary data. Authorized and duly trained study site staff transferred all protocol-required data from medical records available at the study sites to the electronic Case Report Form (eCRF) developed for each patient included into the study. All the patients were identified by a unique code in their eCRF, which included no data allowing the identification of the patient's identity.

The retrospective data collection started on 23 January 2023 and ended on 27 June 2024. The database was closed on 18 July 2024.

A patient was enrolled in the study once the investigator deemed a patient eligible and decided to input the patient's data into the eCRF. Patients were enrolled in the study consecutively, beginning with the earliest diagnosis of hATTR-PN or CIAP and continuing to the later date, within the pre-specified period from 1 January 2017 to 1 March 2024. Retrospective follow-up began on the date of diagnosis of hATTR-PN or CIAP and continued until the patient was enrolled in the study, died or was lost to follow-up, whichever occurred first. Thus, if patients died or were lost for retrospective follow-up at the study site, their data were also included in the study.

The data obtained were based on three consecutive patient's visits during the retrospective data collection period, which were carried out as a part of routine clinical practice and registered in the medical records. We collected all available data from these visits during the retrospective follow-up period, even if the data were not fully available at the time of any visit.

Once a patient was recognized eligible and thus included in the study, we collected their baseline demographic, electrophysiological, and clinical characteristics and other baseline data (medical history, comorbidities, etc.) registered at hATTR-PN or CIAP diagnosis (this time point was designated as Visit 1). The changes in the selected endpoints were evaluated at the two subsequent follow-up visits (Visit 2 and Visit 3) relative to the baseline assessment at Visit 1.

Statistical analysis

Considering that hATTR-PN is a rare condition (with a prevalence of about 0.32 cases per 1 million in Russia), the sample size was established based on the available number of patients diagnosed with hATTR-PN. The planned sample size included approximately 50 patients with hATTR-PN and a similar number of patients with CIAP, for a total of 100 patients.

Statistical data processing was performed using R-Studio v. 2023.06.1 software and the R programming language v. 4.2.2. The results are presented using descriptive statistics for all patients included in the analysis (full analysis set) and for each group (mean and standard deviation, absolute frequencies, and percentages).

In intergroup analysis of demographic, clinical, and electrophysiological characteristics, Fisher's exact test or Pearson's χ^2 test were used to compare qualitative variables, and Student's test or Wilcoxon–Mann–Whitney test were used for quantitative variables (depending on the distribution patterns).

A logistic regression model was used to detect the factors impacting the likelihood of hATTR-PN diagnosis. This model included clinically relevant variables. Based on this model, a screening tool for patients with axonal polyneuropathy was developed, which allows to preselect them for the *TTR* gene sequencing.

Results

Clinical and demographic characteristics of patients

The study included 42 hATTR-PN patients as per medical records and 58 patients with CIAP diagnosed according to medical records (or its equivalents – polyneuropathy of unspecified etiology, polyneuropathy of mixed etiology) in 4 clinical centers in Russia. All 100 patients were included in the analysis set. Two patients were deceased at the time of inclusion (both from hATTR-PN group, with the cause of death unknown).

Baseline demographic and clinical characteristics of patients are presented in Table 1. The study sample was represented

Table 1. Baseline demographic and clinical characteristics of patients

Parameter	hATTR-PN (*n* = 42)	CIAP (*n* = 58)	*p*
Age, years	57.7 ± 12.8	60.9 ± 11.9	0.201
Male	24 (57.1%)	23 (39.7%)	0.127
Female	18 (42.9%)	35 (60.3%)	
Federal district (region of residence):			0.020
Central	17 (40.5%)	25 (43.1%)	
Northwestern	10 (23.8%)	19 (32.8%)	
Volga	10 (23.8%)	4 (6.9%)	
Southern	3 (7.1%)	2 (3.5%)	
North Caucasian	1 (2.4%)	1 (1.7%)	
Ural	1 (2.4%)	0 (0%)	
unknown	0 (0%)	7 (12.1%)	
Body mass index, kg/m^2	22.6 ± 5.0	27.4 ± 4.0	< 0.001
Underweight (Body mass index < 18.5 kg/m^2)	2 (4.8%)	0 (0%)	0.317
History of excessive alcohol use	4 (9.5%)	3 (5.2%)	0.669
Family history:			
premature cardiovascular death (age < 50) in close relatives	4 (9.5%)	0 (0%)	0.058
heart failure in close relatives	7 (16.7%)	2 (3.4%)	0.033
progressive polyneuropathy in close relatives	22 (52.4%)	7 (12.1%)	< 0.001

Continuation of the Table 1

Parameter	hATTR-PN (*n* = 42)	CIAP (*n* = 58)	*p*
Time from symptom onset to polyneuropathy diagnosis, years:			0.166
number of valid cases	40	58	
M ± SD	2.48 ± 3.33	2.03 ± 3.11	
median	1.5	1.0	
Time from polyneuropathy symptom onset to hATTR-PN or CIAP diagnosis, years:			0.088
number of valid cases	40	58	
M ± SD	3.10 ± 3.26	2.45 ± 3.21	
median	2.0	1.0	
Time from polyneuropathy diagnosis to hATTR-PN or CIAP diagnosis, years:			0.170
number of valid cases	42	54	
M ± SD	0.64 ± 1.32	0.39 ± 1.29	
median	0	0	
Chronic sensory or sensorimotor polyneuropathy*	–	55 (94.8%)	–
Chronic progressive polyneuropathy*	–	3 (5.2%)	–
Initially suggested polyneuropathy etiology, as per physician opinion**:			
number of valid cases	40	45	
diabetes mellitus	0 (0%)	2 (4.4%)	0.497
alcohol-related	0 (0%)	0 (0%)	–
toxicity-related	0 (0%)	2 (4.4%)	0.497
other hereditary factors	26 (65.0%)	4 (8.9%)	< 0.001
vitamin deficiency	0 (0%)	4 (8.9%)	0.120
immunity-related	2 (5.0%)	6 (13.3%)	0.275
hematology-related	1 (2.5%)	0 (0%)	0.465
infection-related	0 (0%)	0 (0%)	–
idiopathic	9 (22.5%)	19 (42.2%)	0.104
other causes***	4 (10.0%)	11 (24.4%)	0.153
TTR-gene sequencing:			< 0.001
performed, gene mutation (gene variant) detected	38 (90.5%)	–	
performed, no gene mutation (gene variant) detected	–	9 (15.5%)	
no data available in patient's medical record	4 (9.5%)	49 (84.5%)	
TTR-gene variants detected**** (*n* = 38):			
NM_000371.4(TTR):c.148G>A (p.Val50Met)	20 (52.6%)	–	–
NM_000371.4(TTR):c.379A>G (p.Ile127Val)	6 (15.8%)	–	

End of the Table 1

Parameter	hATTR-PN (*n* = 42)	CIAP (*n* = 58)	*p*
NM_000371.4(TTR):c.220G>C (p.Glu74Gln)	4 (10.5%)	–	
NM_000371.4(TTR):c.368G>A (p.Arg123His)	1 (2.6%)	–	
NM_000371.4(TTR):c.200G>C (p.Gly67Ala)	1 (2.6%)	–	
NM_000371.4(TTR):c.323A>G (p.His108Arg)	1 (2.6%)	–	
NM_000371.4(TTR):c.233T>A (p.Leu78His)	1 (2.6%)	–	
NM_000371.4(TTR):c.157T>A (p.Phe53Ile)	1 (2.6%)	–	
NM_000371.4(TTR):c.179C>A (p.Thr60Asn)	1 (2.6%)	–	
NM_000371.4(TTR):c.272T>C (p.Val91Ala)	1 (2.6%)	–	
gene variant is not specified in patient's medical record	1 (2.6%)	–	
Heart failure with preserved ejection fraction	11 (26.2%)	4 (6.9%)	0.016
Hypertension with predominant cardiac involvement	5 (11.9%)	19 (32.8%)	0.018
Systolic blood pressure, mm Hg:			0.006
number of valid cases	25	34	
M ± SD	114.7 ± 17.7	127.4 ± 15.5	
Diastolic blood pressure, mm HG:			0.016
number of valid cases	25	34	
M ± SD	72.4 ± 10.7	80.2 ± 10.8	
Heart rate, bpm:			0.911
number of valid cases	28	34	
M ± SD	72.8 ± 9.7	73.1 ± 9.3	

Note. *The parameter was assessed only in patients with CIAP. **Missing data were not included in the analysis due to unequal distribution of patients whose data was missing; a patient could have more than 1 variant etiology indicated. ***Other etiology encompassed depression with anorexia, radiation therapy, hypothyroidism, hereditary conditions, chemotherapy, deficit-, dysmetabolic-, and inflammatory-related conditions. ****Gene variant names according to HGVS (Human Genome Variation Society) nomenclature.

by patients from six federal districts. Almost half of them (42/100) resided in the Central Federal District. The mean age of the patients at diagnosis (Visit 1) was 57.7 ± 12.8 years in the hATTR-PN group and 60.9 ± 11.9 years in the CIAP group ($p = 0.201$). The hATTR-PN group was predominantly male (57.1%) and the CIAP group was predominantly female (60.3%; $p = 0.127$). There were no statistically significant intergroup differences for age and sex, whereas the groups differed in body mass index (BMI): in the hATTR-PN group BMI was lower (22.6 ± 5.0 kg/m^2 vs 27.4 ± 4.0 kg/m^2 in the CIAP group; $p < 0.001$). There were also two (4.8%) patients in the hATTR-PN group with BMI < 18.5 kg/m^2 (0% in the CIAP group).

According to medical records, the most frequent (> 50%) clinical manifestations of polyneuropathy at hATTR-PN or CIAP diagnosis (Visit 1) in the hATTR-PN group were sensory (88.1% of patients), motor (85.7%), gastrointestinal (64.3%), and autonomic symptoms (47.6%). In the CIAP group, the most frequent (> 50%) clinical manifestations were sensory (82.8%) and motor (67.2%) symptoms. Some polyneuropathy manifestations were reported significantly more often in the hATTR-PN group compared with the CIAP group. These included gait disturbances such as walking imbalance, foot weakness, unsteadiness, and coordination disorders (64.3 vs 37.9%; p = 0.016), gastrointestinal (64.3 vs 12.1%; $p < 0.001$) and autonomic symptoms (47.6 vs 12.1%; $p < 0.001$), unintentional weight loss (45.2 vs 12.1%; $p < 0.001$), and heart failure (23.8 vs 1.7%; $p = 0.001$; see Table 2).

In the hATTR-PN group compared with the CIAP group, there were significantly more patients with HFpEF as per their medical record (11 [26.2%] vs 4 [6.9%], $p = 0.016$). Ejection fraction considered preserved at ⩾ 50% (Table 1).

Table 2. Clinical manifestations of polyneuropathy at hATTR-PN or CIAP diagnosis

Clinical manifestations	hATTR-PN (*n* = 42)	CIAP (*n* = 58)	*p*
Sensory symptoms:	37 (88.1%)	48 (82.8%)	0.575
paresthesia	22 (52.4%)	26 (44.8%)	0.587
hypoalgesia/analgesia	11 (26.2%)	13 (22.4%)	0.842
neuropathic pain	21 (50.0%)	25 (43.1%)	0.631
Balance disorder	25 (59.5%)	22 (37.9%)	0.053
Motor symptoms:	36 (85.7%)	39 (67.2%)	0.061
muscular weakness	28 (66.7%)	30 (51.7%)	0.197
gait disturbances (walking imbalance, foot weakness)	27 (64.3%)	22 (37.9%)	0.016
Gastrointestinal symptoms:	27 (64.3%)	7 (12.1%)	< 0.001
diarrhea	11 (26.2%)	1 (1.7%)	< 0.001
constipation	6 (14.3%)	0 (0%)	0.004
switching between diarrhea and constipation	5 (11.9%)	0 (0%)	0.011
persistent nausea and vomiting	3 (7.1%)	0 (0%)	0.071
early satiety	0 (0%)	0 (0%)	–
Autonomic symptoms:	20 (47.6%)	7 (12.1%)	< 0.001
orthostatic hypotension	17 (40.5%)	3 (5.2%)	< 0.001
sweating disorders	9 (21.4%)	1 (1.7%)	0.002
dysuria	8 (19.1%)	4 (6.9%)	0.116
sexual dysfunction	4 (9.5%)	0 (0%)	0.029
Unintentional weight loss	19 (45.2%)	7 (12.1%)	< 0.001
Cardiac disorders:	15 (35.7%)	6 (10.3%)	0.005
heart failure	10 (23.8%)	1 (1.7%)	0.001
arrhythmias	5 (11.9%)	4 (6.9%)	0.486
heart block	3 (7.1%)	3 (5.2%)	0.694
Central nervous system disorders:	9 (21.4%)	10 (17.2%)	0.788
ataxia	5 (11.9%)	6 (10.3%)	1.000
seizures	2 (4.8%)	3 (5.2%)	1.000
progressive dementia	0 (0%)	1 (1.7%)	1.000
headache	0 (0%)	0 (0%)	–
Eye disorders:	7 (16.7%)	5 (8.6%)	0.350
abnormal changes in fundus blood vessels	4 (9.5%)	1 (1.7%)	0.158
vitreous opacities	3 (7.1%)	3 (5.2%)	0.694
glaucoma	1 (2.4%)	0 (0%)	0.420
pupil abnormalities	0 (0%)	0 (0%)	–
dry eyes	1 (2.4%)	1 (1.7%)	1.000
Carpal tunnel syndrome	8 (19.0%)	4 (6.9%)	0.116
Renal disorders:	4 (9.5%)	3(5.2%)	0.449
renal failure	4 (9.5)	2 (3.5%)	0.235
proteinuria	1 (2.4%)	0 (0%)	0.420
Lumbar spinal stenosis	2 (4.8%)	1 (1.7%)	0.571
Biceps tendon rupture	1 (2.4%)	2 (3.5%)	1.000

CTS was diagnosed in 8 (19.0%) hATTR-PN patients and 4 (6.9%) CIAP patients. Two patients in each group had a history of surgically corrected CTS.

Other Comorbidities

In the hATTR-PN group, the most common (> 10%) comorbidities were chronic gastritis – in 8 (19.1%) patients; hypertension with predominant cardiac involvement – in 5 (11.9%), and chronic heart failure – in 5 (11.9%) patients. In the CIAP group, the most common (> 10%) comorbidities were hypertension with predominant cardiac involvement – in 19 (32.8%) patients, chronic gastritis – in 12 (20.7%), osteochondrosis – in 6 (10.3%), and varicose veins of lower limbs – in 7 (12.1%) patients. Statistically significant differences were detected for hypertension with predominant cardiac involvement (p = 0.018) and varicose veins of lower limbs (p = 0.020).

Treatment

Thirty-three (78.6%) patients in the hATTR-PN group and 47 (81.0%) patients in the CIAP group received medicines to treat their primary disease (p = 0.804). Namely, tafamidis was prescribed to 18 (42.9%) hATTR-PN patients. Fourteen (33.3%) hATTR-PN patients and 29 (50%) CIAP patients received medicines to treat their concomitant disease (p = 0.107).

Polyneuropathy dysfunction scores

The following polyneuropathy disability score (PND) is used to evaluate the impact of polyneuropathy on locomotion [13]:
- PND 0 – no impairment;
- PND I – sensory disturbances, preserved walking capability;
- PND III – impaired walking capability but ability to walk without a stick or crutches;
- PND IIIA – walking only with the help of one stick or crutch;
- PND IIIB – walking with the help of two sticks or crutches;
- PND IV – patient confined to a wheelchair or bedridden.

In the hATTR-PN group, 16 (38.1%) patients had PND I, 9 (21.4%) – PND II, 6 (14.3%) – PND IIIA, 4 (9.5%) – PND IIIB, and 4 (9.5%) – PND IV; 3 (7.1%) patients had no PND score data in their medical records. In the CIAP group, 31 (53.5%) patients had PND I, 12 (20.7%) – PND II, 6 (10.3%) – PND IIIA, 3 (5.2%) – PND IIIB, and 2 (3.5%) – PND IV; 4 (6.9%) patients had no PND score data in their medical records. In either group, there were no patients with PND 0. No statistically significant intergroup differences in PND scores were detected (p = 0.577).

Modified Rankin Scale

The modified Rankin Scale, mRS is a universal tool to measure the degree of disability [14].

A single mRS grade should be assigned based on the following criteria:
0 – no symptoms;
1 – no significant disability despite symptoms: able to carry out all usual duties and activities;
2 – slight disability: unable to carry out all previous activities but able to look after own affairs without assistance;
3 – moderate disability: requiring some help, but able to walk without assistance;
4 – moderately severe disability: unable to walk without assistance, and unable to attend to own bodily needs without assistance;
5 – severe disability: bedridden, incontinent, and requiring constant nursing care and attention;
6 – dead.

MRS scores were available in medical records of 42 hATTR-PN patients and 56 CIAP patients. In the hATTR-PN group, the mean mRS score was significantly higher than that in the CIAP group (2.50 ± 1.35 vs 1.82 ± 0.92; p = 0.014). mRS scores ranged from 1 to 5 in the hATTR-PN group and from 1 to 4 in the CIAP group, with a median of 2.5 and 2.0, respectively. Thus, hATTR-PN patients were characterized by more severe functional impairment.

INCAT disability score

The INCAT (Inflammatory Neuropathy Cause and Treatment) disability score is widely used for assessment of activity limitation in CIDP patients. A Russian version of the INCAT scale is developed [15]. The 5-point INCAT score is meant for separate assessment of upper and lower limb function, with 0 representing no disability and 5 representing no limb function, and a 10-point INCAT total score as the sum of points for upper and lower limbs.

In the hATTR-PN group, lower limb INCAT scores were 1.38 ± 1.41 vs 1.19 ± 1.21 in the CIAP group (difference statistically insignificant). The number of patients with data available for analysis was 39 in the hATTR-PN group and 53 in the CIAP group. Differences were identified for upper limb INCAT scores: 1.36 ± 1.16 vs 0.54 ± 0.80, respectively (p = 0.001; number of patients with data available for analysis: 39 in the hATTR-PN group and 48 in the CIAP group) and for INCAT total scores: 2.74 ± 2.36 vs 1.57 ± 1.60, respectively (p = 0.021; number of patients with data available for analysis: 39 in the hATTR-PN group and 47 in the CIAP group). Thus, activity limitations, including those associated with upper limbs, were more pronounced in hATTR-PN patients than in CIAP patients.

Electrophysiological findings

Results of nerve conduction studies (NCS) performed at diagnosis presented in Table 3. Patients with hATTR-PN generally had worse peripheral nerve conduction function compared

Table 3. Results of nerve conduction study

Nerve	Parameter	hATTR-PN			CIAP			
		n	mean	SD	*n*	mean	SD	*p*
Median nerve	mV	15	3.70	3.28	20	8.96	12.37	0.012
	ms	15	6.39	2.68	20	4.98	2.61	0.129
	m/s	15	48.64	8.50	19	55.02	8.80	0.040
	μV	6	9.11	12.76	13	16.12	9.61	0.267
	SNCV at wrist level, m/s	6	41.27	14.40	13	55.75	12.63	0.064
Peroneal nerve	mV	8	2.95	2.61	16	3.12	2.63	0.883
	ms	8	5.41	1.64	13	5.76	4.93	0.818
	m/s	8	44.06	9.82	14	43.06	10.58	0.825
Superficial peroneal nerve	μV	2	5.80	3.96	4	3.08	2.39	0.500
	m/s	2	41.80	8.20	4	47.45	8.44	0.509
Sural nerve	μV	2	15.50	7.78	6	4.17	2.60	0.278
	m/s	2	43.40	3.39	6	47.88	9.44	0.365
Tibial nerve	mV	8	4.64	5.58	15	3.40	3.08	0.574
	ms	9	6.49	3.39	14	6.84	6.42	0.867
	m/s	9	42.80	7.77	13	40.25	7.07	0.443
Ulnar nerve	mV	13	4.97	3.42	17	6.84	1.90	0.094
	ms	13	4.55	2.54	17	4.47	3.08	0.217
	m/s	13	44.12	8.03	15	52.95	7.39	0.006
	μV	9	9.97	9.33	12	12.07	8.45	0.601
	m/s	9	44.44	12.72	12	50.89	9.74	0.225

Note. DML — distal motor latency. The number of patients with non-zero values of these parameters is indicated.

with CIAP patients. The greatest intergroup differences were observed for the median, sural, ulnar and superficial peroneal nerves. The following parameters were statistically significantly lower in the hATTR-PN group compared with those in the CIAP group: the compound muscle action potential (CMAP) of the median nerve: 3.70 ± 3.28 mV vs 8.96 ± 12.37 mV (p = 0.012); the motor nerve conduction velocity (MNCV) of the median nerve: 48.64 ± 8.50 m/s vs 55.02 ± 8.80 m/s (p = 0.040) and MNCV of the ulnar nerve: 44.12 ± 8.03 m/s vs 52.95 ± 7.39 m/s (p = 0.006), respectively.

Additionally, there were intergroup differences in the number of patients in whom it was not possible to record a response during the nerve conduction study . Statistically significant intergroup differences were found for the sensory nerve action potential (SAP) of the superficial peroneal nerve and sural nerve: in 8 hATTR-PN patients (19.1%) vs 1 (1.7%) CIAP patient; p = 0.004 for both nerves) and for sensory nerve conduction velocity (SNCV) of the superficial peroneal nerve and sural nerve: in 7 hATTR-PN patients (16.7%) vs 1 (1.7%) CIAP patient; p = 0.009 for both nerves).

Changes in clinical and electrophysiological characteristics over time

The exploratory objective of the study was to assess the changes in clinical and electrophysiological characteristics of the patients from the date of hATTR-PN or CIAP diagnosis to Visits 2 and 3 of the retrospective follow-up. The assessment was challenging because of the significant number of patients with missing data. Noteworthy, during the retrospective dynamic follow-up period, a decrease in PND scores from baseline to Visit 2 was detected in 2 (4.8%) hATTR-PN patients compared to none in the CIAP group. By Visit 3, the number

Table 4. Prognostic value of the hATTR-PN diagnosis predictors in a logistic regression model

Factor	Significance score
Other hereditary factors (of polyneuropathy etiology)	3.05
Body mass index	2.65
History of hypertension with predominant cardiac involvement	1.89
Cardiac manifestations	1.25
Median nerve, CMAP	1.14
Heart failure in close relatives	1.14
Median nerve, MNCV	0.93
Diastolic blood pressure, mm Hg	0.87
Heart failure with preserved ejection fraction	0.78
Gastrointestinal symptoms	0.73
Ulnar nerve, MNCV	0.63
INCAT total score	0.56
Upper limb INCAT score	0.56
Autonomic symptoms	0.41
Progressive polyneuropathy in close relatives	0.38
mRS score	0.31
Systolic blood pressure, mm Hg	0.27

of patients with decreased PND score was 3 (7.1%) in the hATTR-PN group and 1 (1.7%) in the CIAP group. These data may indicate a more rapid progression of neurological impairment in hATTR-PN patients.

Pre-selection of patients eligible for genetic testing for hATTR

Variables influencing disease prediction were included in a logistic regression model for assessment of the likelihood of hATTR-PN or CIAP diagnosis. To identify the factors that most contribute to the prediction of the diagnosis, the variables were scored according to their significance in the model (Table 4). Variables that were considered clinically insignificant (certain comorbidities and aspects of neurological examination, etc.) were excluded from the model. This model demonstrated a predictive accuracy of 94%, a sensitivity of 91%, and a specificity of 97% for the likelihood of hATTR-PN diagnosis. The AUC (area under the ROC curve displaying the trade-off between sensitivity and specificity) was 0.96. Based on these findings, we developed a screening tool that considers factors indicating the likelihood of hATTR-PN in a patient.

Discussion

Hereditary transthyretin amyloidosis with polyneuropathy is a rare disease. Timely diagnosis of hATTR-PN is challenging due to a great variability of clinical manifestations that can be mistaken for those of other neurological diseases.

In this non-interventional, observational, retrospective study with secondary data collection, we described the baseline (at diagnosis) electrophysiological, clinical, and demographic characteristics of hATTR-PN and CIAP patients in Russia. Additionally, the obtained data allowed us to develop a screening tool predicting the likelihood of hATTR-PN diagnosis. A hATTR-PN or CIAP diagnosis was documented in primary medical records.

No statistically significant differences for age and sex were observed between hATTR-PN and CIAP groups. Mean age at diagnosis was approximately 60 years in both groups. Results of routine genetic testing were available in medical records of 90% of hATTR-PN patients (38/42). Val30Met/Val50Met (p.Val50Met) mutation was detected in 53% cases, which corresponds to previously published data [1, 7].

The study revealed fundamental differences between hATTR-PN and CIAP patients, which are typical for the Russian population. Hereditary factors are known to be one of the red flags for suspected transthyretin amyloidosis. In this study, the proportion of patients with polyneuropathy of hereditary origin, as assessed by the physician, was significantly greater in the hATTR-PN group compared with the CIAP group (65% vs 8.9%). In the hATTR-PN group compared with the CIAP group, there was also a higher incidence of heart failure in close relatives (16.7% vs 3.4%) and progressive polyneuropathy in close relatives (52.4% vs 12.1%).

Patients with hATTR-PN more often exhibited gait disturbances (64.3% vs 37.9%), autonomic (47.6% vs 12.1%), cardiac (35.7% vs 10.3%), and gastrointestinal (64.3% vs 12.1%)

symptoms, unintentional weight loss (45.2% vs 12.1%), and heart failure with preserved ejection fraction (26.2% vs 6.9%) compared with CIAP patients. Intergroup differences in the history of CTS (this syndrome is often associated with early- or late-onset hATTR-PN due to local deposits of amyloid in the palmar carpal ligament) did not reach the level of statistical significance. However, in the hATTR-PN group, CTS incidence was higher than that in the CIAP group (19.0% vs 6.9%). These findings are also confirmed by results of a nerve conduction velocity (NCV) test for the median nerves, which showed a significant decrease in the M-wave amplitude at distal stimulation, and slowing of NCV in the forearms in hATTR-PN patients compared to CIAP patients (Table 3).

Patients with hATTR-PN had lower mean systolic and diastolic blood pressure values (approximately 10 mm Hg lower than in the CIAP group), suggesting that arterial hypotension may be considered an autonomic symptom of hATTR-PN. Further, hATTR-PN patients generally exhibited more severely impaired peripheral nerve conduction compared to CIAP patients.

In the study with similar design (n = 90) conducted in Italy by S. Tozza et al., hATTR-PN patients, compared with CIAP patients, more often presented with motor symptoms (86 vs 54%) and a CTS history (57% vs 24%) as polyneuropathy manifestations. Intergroup differences for gait disturbances did not reach statistical significance [16]. In another study conducted by J.K. Warendorf et al., hATTR-PN patients, compared with CIAP patients, more often had bilateral CTS (80.0% vs 23.9%), cardiac involvement (60.0% vs 2.2%), family history suggestive of hATTR (86.7% vs 12.0%), and autonomic symptoms (86.7% vs 51.1%) [17].

We identified the factors contributing to the likelihood of hATTR-PN diagnosis using a logistic regression model with predictive sensitivity of 91% and specificity of 97%. Based on the model, we developed a screening tool to pre-select patients with axonal polyneuropathy eligible for *TTR* gene sequencing. Modeling results demonstrate high levels of predictive accuracy, sensitivity, and specificity for this screening tool in assessing the likelihood of an hATTR-PN diagnosis, enabling the pre-selection of patients with axonal polyneuropathy for genetic testing.

The study presented in this article confirmed the variability of clinical manifestations of polyneuropathy in hATTR-PN patients [18], which makes the differential diagnosis of this disease quite challenging. At the same time, early diagnosis and timely treatment help slow down the progression of neurological and other signs of the disease, confirming the relevance of the comparative data obtained and the screening tool developed. Nowadays, all necessary methods for screening of hATTR patients are available in Russia — first of all, genetic testing. Therefore, timely referral of patients to specialized institutions is of key importance for early diagnosis, which is most effective in improving the disease course.

Strengths and limitations of the study. This study was conducted in the study sites focusing on the management of hATTR-PN patients, which allowed a comprehensive retrospective assessment of their clinical and electrophysiological characteristics. The study included a selected group (cohort) of hATTR-PN or CIAP patients according to the inclusion/non-inclusion criteria. The sample size was limited by the available number of patients diagnosed with hATTR-PN. The patients enrolled in the study were diagnosed with hATTR-PN or CIAP at a pre-specified time interval. This time limitation was essential to evaluate the patients' characteristics over the past several years (since 2017), as standard clinical practices and the required data may have significantly changed over time. In the non-interventional study design, all procedures that yielded results collected from primary medical records are to be the part of standard clinical practice. Hence, there were missing data in the statistical analyses due to their absence in the medical records, particularly for follow-up visits after diagnosis. At the same time, baseline data (at diagnosis) were almost complete. In an observational study, it is impossible to standardize procedures and management of patients, which naturally leads to heterogeneity in the data obtained from the study sites. However, given that the study sites underwent thorough selection for the purposes of this study, this limitation can be considered insignificant. To assess functional impairment in hATTR-PN and CIAP patients, we used INCAT scores originally developed for CIDP, which is another form of polyneuropathy. The Russian version of INCAT scale is developed and validated only for CIDP, not for hATTR-PN or CIAP. Taking into account that CIDP is the first to rule out in the hATTR-PN differential diagnosis, which reflects the similarity of their clinical symptom complexes, this choice of evaluation scale was considered appropriate. Moreover, it was important to evaluate functional impairment in both the lower and upper limbs, as the median nerve is more commonly affected in hATTR-PN patients, which was confirmed by intergroup differences: severity and disability were more pronounced in hATTR-PN patients than in CIAP patients.

Additionally, to minimize data heterogeneity, a standardized data collection form (eCRF) was created and introduced across all the study sites. Detailed instructions for data collection and assessment were also provided to all investigators.

Conclusion

In this study, we described demographic, clinical, and electrophysiological characteristics collected at diagnosis for hATTR-PN and CIAP patients in Russia. Patients with hATTR-PN more often exhibited autonomic, cardiac, and gastrointestinal symptoms, gait disturbances, unintentional weight loss, heart failure with preserved ejection fraction, and declined peripheral

nerve conduction. Based on the results of clinical and electrophysiological tests (screening data), we demonstrated high predictive accuracy, sensitivity and specificity of the screening tool for the likelihood of an hATTR-PN diagnosis in patients with axonal polyneuropathy. Based on the screening tool scores, the patients can be referred for genetic testing.

Reference medical institutions for transthyretin amyloid polyneuropathy:

1. Federal medical centers:
   1) Research Center of Neurology, Moscow;
   2) A.Ya. Kozhevnikov Clinic for Nervous Diseases, E.M. Tareev Clinic of Rheumatology, Nephrology and Occupational Diseases in I.M. Sechenov First Moscow State Medical University (Sechenov University), Moscow;
   3) The Loginov Moscow Clinical Scientific Center, Moscow;
   4) N.I. Pirogov National Medical and Surgical Center, Moscow;
   5) First Pavlov State Medical University of St. Petersburg, Saint Petersburg;
   6) S.M. Kirov Medical Military Academy, Saint Petersburg;
   7) I.I. Mechnikov North-Western State Medical University, Saint Petersburg;
   8) Almazov National Medical Research Centre, Saint Petersburg.

2. Regional centers:
   1) Northern State Medical University, Arkhangelsk;
   2) Alexander-Mariinsky Regional Clinical Hospital, Astrakhan;
   3) Profimed Ltd – Siberian Medical Center, Barnaul;
   4) Primorye Regional Clinical Hospital No. 1, Vladivostok;
   5) Volgograd Regional Clinical Hospital No. 1, Volgograd;
   6) Medical Center “Healthy Child”, Voronezh;
   7) Voronezh Regional Clinical Hospital No. 1, Voronezh;
   8) Grozny Clinical Hospital No. 4, Grozny;
   9) Sverdlovsk Regional Clinical Hospital No. 1, Ekaterinburg;
   10) Medical Association “New Hospital”, Ekaterinburg;
   11) First Regional Clinical Hospital, Izhevsk;
   12) Irkutsk Regional Clinical Hospital, winner of the “Mark of the Honor” award, Irkutsk;
   13) M.N. Sadykov City Clinical Hospital No.7, Kazan;
   14) Medical Center for Vascular diseases “Impuls-Angio”, Kazan;
   15) Republican Clinical Hospital, Kazan;
   16) Kaliningrad Regional Clinical Hospital, Kaliningrad;
   17) Kaluga Regional Clinical Hospital, Kaluga;
   18) S.V. Belyaev Kuzbass Regional Clinical Hospital, Kemerovo;
   19) Research Center of Cardiology and Neurology, Kirov;
   20) Prof. S.V. Ochapovsky Regional Clinical Hospital No. 1, Krasnodar;
   21) Regional Clinical Hospital No. 2, Krasnodar;
   22) Regional Clinical Hospital, Krasnoyarsk;
   23) Lipetsk Regional Clinical Hospital, Lipetsk;
   24) A.V. Vishnevsky Republican Clinical Hospital, Makhachkala;
   25) The Loginov Moscow Clinical Scientific Center, Moscow;
   26) M.F. Vladimirsky Moscow Regional Research Clinical Institute, Moscow;
   27) N.A. Semashko Regional Clinical Hospital, Nizhny Novgorod;
   28) State Novosibirsk Regional Clinical Hospital, Novosibirsk;
   29) EZRAMED Clinic Ltd, Omsk;
   30) Regional Clinical Hospital No. 2, Orenburg;
   31) Penza Institute for Advanced Medical Education – branch of the Russian Medical Academy of Continuing Professional Education, Penza;
   32) Perm Regional Clinical Hospital, Perm;
   33) Medical Center “Beauty and Health Philosophy”, Perm;
   34) Rostov State Medical University, Rostov-on-Don;
   35) Regional Consultative and Diagnostic Centre, Rostov-on-Don;
   36) Rostov Regional Clinical Hospital, Rostov-on-Don;
   37) V.D. Seredavin Regional Clinical Hospital, Samara;
   38) City Multidisciplinary Hospital No. 2, Saint Petersburg;
   39) V.I. Razumovsky Saratov State Medical University, Saratov;
   40) Stavropol Regional Clinical Hospital, Stavropol;
   41) Tula Regional Clinical Hospital, Tula;
   42) Regional Treatment and Rehabilitation Centre, Tyumen;
   43) Regional Clinical Hospital No. 1, Tyumen;
   44) Ulyanovsk Regional Clinical Hospital, Ulyanovsk;
   45) Republican Center of Medical Genetics, Ufa;
   46) G.G. Kuvatov Republican Clinical Hospital, Ufa;
   47) Psychology and Childhood Development Centre “Psylogia”, Khabarovsk;
   48) Regional Clinical Hospital, Khanty-Mansiysk;
   49) Chelyabinsk Regional Clinical Hospital, Chelyabinsk;
   50) City Clinical Hospital No. 1, Chelyabinsk;
   51) Clinical Hospital No. 2, Yaroslavl.

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Information about the authors

Natalya A. Suponeva – Dr. Sci. (Med.), Corresponding Member of RAS, Director of the Institute of Neurorehabilitation and Restorative Technologies, Research Center of Neurology, Moscow, Russia,
https://orcid.org/0000-0003-3956-6362
Olga E. Zinovieva – Dr. Sci. (Med.), Professor, Department of nervous system diseases and neurosurgery, N.V. Sklifosovsky Institute of Clinical Medicine, I.M. Sechenov First Moscow State Medical University, Moscow, Russia, https://orcid.org/0000-0001-5937-9463
Fatima R. Stuchevskaya– Cand. Sci. (Med.), Associate Professor, Neurology department, Pavlov First Saint Petersburg State Medical University, St. Petersburg, Russia; neurologist, Medical Center "Reavita Med SPb", Saint-Petersburg, Russia; Head, Neurological department No. 3, City Multidisciplinary Hospital No. 2, St. Petersburg, Russia,
https://orcid.org/0000-0003-3181-4229
Tatyana G. Sakovets – Cand. Sci. (Med.), Associate Professor, Neurology and rehabilitation department, Kazan State Medical University, Kazan, Russia; neurologist, Republican Clinical Hospital, Kazan, Russia,
https://orcid.org/0000-0002-0713-9836
Darya A. Grishina – Cand. Sci. (Med.), Head, Center for Peripheral Nervous System Diseases, Institute of Clinical and Preventive Neurology, Research Center of Neurology, Moscow, Russia,
https://orcid.org/0000-0002-7924-3405
Maria S. Kazieva – neurologist, Research Center of Neurology, Moscow, Russia, https://orcid.org/0009-0007-5683-0934
*Elvira I. Safiulin*a – Cand. Sci. (Med.), neurologist, I.M. Sechenov First Moscow State Medical University, Moscow, Russia,
https://orcid.org/0000-0002-1233-7626
Anton P. Soloviev – medical advisor, AstraZeneca Pharmaceuticals LLC, Moscow, Russia, https://orcid.org/0009-0001-3407-7220
Evgenia A. Zorina – Head, Therapeutic direction, AstraZeneca Pharmaceuticals LLC, Moscow, Russia,
https://orcid.org/0009-0004-9283-5714

Authors' contribution. *Suponeva N.A.* – conceptualization, investigation, data curation, writing – original draft preparation, writing – review and editing; *Zinovieva O.A., Stuchevskaya F.R., Sakovets T.G., Kazieva M.S., Safiulina E.I.* – investigation; *Grishina D.A.* – investigation, data curation, writing – original draft preparation; *Solovyov A.P.* – conceptualization, methodology; *Zorina E.A.* – conceptualization.

Информация об авторах

Супонева Наталья Александровна – д-р мед. наук, член-корреспондент РАН, директор Института нейрореабилитации и восстановительных технологий Научного центра неврологии, Москва, Россия,
https://orcid.org/0000-0003-3956-6362
Зиновьева Ольга Евгеньевна – д-р мед. наук, профессор кафедры нервных болезней и нейрохирургии Института клинической медицины им. Н.В. Склифосовского Первого Московского государственного медицинского университета им. И.М. Сеченова, Москва, Россия,
https://orcid.org/0000-0001-5937-9463
Стучевская Фатима Рамазановна – канд. мед. наук, доцент каф. неврологии Первого Санкт-Петербургского государственного медицинского университета им. акад. И.П. Павлова, Санкт-Петербург, Россия; врач-невролог Медицинского центра «Реавита Мед СПб», Санкт-Петербург, Россия; врач-невролог, зав. неврологическим отделением № 3 Городской многопрофильной больницы № 2, Санкт-Петербург, Россия,
https://orcid.org/0000-0003-3181-4229
Саковец Татьяна Геннадьевна – канд. мед. наук, врач-невролог Республиканской клинической больницы, Казань, Россия; доцент каф. неврологии и реабилитации Казанского государственного медицинского университета, Казань, Россия, https://orcid.org/0000-0002-0713-9836
Гришина Дарья Александровна – канд. мед. наук, рук. Центра заболеваний периферической нервной системы Института клинической и профилактической неврологии Научного центра неврологии, Москва, Россия, https://orcid.org/0000-0002-7924-3405
Казиева Мария Сергеевна – врач-невролог Научного центра неврологии, Москва, Россия, https://orcid.org/0009-0007-5683-0934
Сафиулина Эльвира Ирековна – канд. мед. наук, врач-невролог Первого Московского государственного медицинского университета им. И.М. Сеченова, Москва, Россия, https://orcid.org/0000-0002-1233-7626
Соловьев Антон Павлович – медицинский советник ООО «АстраЗенека Фармасьютикалз», Москва, Россия, https://orcid.org/0009-0001-3407-7220
Зорина Евгения Александровна – руководитель терапевтического направления ООО «АстраЗенека Фармасьютикалз», Москва, Россия, https://orcid.org/0009-0004-9283-5714

Вклад авторов: *Супонева Н.А.* – создание концепции исследования, проведение исследования, курирование данных, подготовка черновика и редактирование рукописи; *Зиновьева О.А., Стучевская Ф.Р., Саковец Т.Г., Казиева М.С., Сафиулина Э.И.* – проведение исследования; *Гришина Д.А.* – проведение исследования, курирование данных, подготовка черновика и редактирование рукописи; *Соловьев А.П.* – создание концепции исследования, разработка методологии; *Зорина Е.А.* – создание концепции исследования.

Serum Cholinesterase Activity in Elderly Female Patients with Different Screening Cognitive Status and Frailty Assessment Scores

Anton N. Kodincev[1], Larisa I. Volkova[2]

[1]Institute of High Temperature Electrochemistry, Ekaterinburg, Russia;
[2]Ural State Medical University, Ekaterinburg, Russia

Abstract

Introduction. *Frailty and mild cognitive impairment (MCI) are common geriatric syndromes. Peripheral serum cholinesterase (pChE) is a laboratory indicator that may reflect dysfunction of cholinergic processes in the central nervous system. Published data demonstrate the potential utility of pChE as a marker for a range of neurodegenerative disorders.*

Aim. *This study aimed to identify and investigate the relationship between serum pChE levels in patients and various screening scores of cognitive status, frailty, and metabolic parameters.*

Materials and methods. *The study included 50 women aged over 60 years. Screening clinical examinations were conducted, including Montreal Cognitive Assessment (MoCA), Mini-Mental State Examination (MMSE), Frontal Assessment Battery (FAB), Age Is Not a Hindrance questionnaire, and Charlson Comorbidity Index. A blood chemistry analysis was performed, including a kinetic colorimetric assay of serum pChE.*

Results. *The Age Is Not a Hindrance score and pChE activity exhibited a moderate inverse correlation with a Spearman coefficient (r_S) of –0.31; 95% confidence interval (CI) –0.5 to –0.03; $p < 0.05$. The MoCA scores and pChE levels also showed a moderate inverse correlation with r_S of –0.32; 95% CI: –0.55 to –0.05, $p < 0.05$. A high risk of MCI is defined by a pChE activity threshold point of 9978 U/L, with a sensitivity of 47% and a specificity of 97%. The association between pChE activity and the prevalence of cognitive impairment remained significant even when different socio-demographic and metabolic parameters were included in the regression model, odds ratio (OR) 1.0005; 95% CI: 1.0001–1.009; $p = 0.01$).*

Conclusion. *Women over 60 years of age in an outpatient setting exhibited an inverse correlation between the Age Is Not a Hindrance questionnaire score and the pChE activity. A pChE activity of 9978 U/L or higher was associated with an elevated risk of concomitant mild cognitive impairment. However, it is important to consider the high probability of false negatives in this context. This association persisted across a variety of clinical and metabolic factors.*

Keywords: *frailty; cognitive impairment; cholinesterase; biomarker; diagnosis*

Ethics approval. The study was conducted with the informed consent of patients. The study protocol was approved by the local Ethical Committee of the Ural State Medical University (protocol No 9, December 18, 2020).

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

For correspondence: 20 Akademicheskaya str., Ekaterinburg, 620066, Russia. Institute of High Temperature Electrochemistry of the Ural Branch of the Russian Academy of Sciences. E-mail: antonkodintsev@mail.ru. Kodincev A.N.

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Активность сывороточной холинэстеразы у пожилых пациенток с различными скрининговыми показателями оценки когнитивного статуса и старческой астении

А.Н. Кодинцев[1], Л.И. Волкова[2]

[1]Институт высокотемпературной электрохимии, Екатеринбург, Россия;
[2]Уральский государственный медицинский университет, Екатеринбург, Россия

Аннотация

Введение. *Старческая астения (СА) и умеренные когнитивные нарушения (УКН) являются распространёнными гериатрическими синдромами. Периферическая холинэстераза (ПХЭ) сыворотки крови является потенциальным лабораторным показателем, отражающим дисфункцию холинергических процессов в центральной нервной системе. Опубликованы данные, свидетельствующие о возможности использования ПХЭ в качестве маркера различных нейродегенеративных заболеваний.*

Цель *– выявление и изучение взаимосвязи активности ПХЭ сыворотки крови у пациенток с различными скрининговыми показателями когнитивного статуса, СА и метаболических параметров.*

Материалы и методы. *В исследование были включены 50 женщин старше 60 лет. Проведено скрининговое клиническое обследование: Монреальская когнитивная шкала, Краткая шкала оценки когнитивного статуса, батарея тестов на лобную дисфункцию, опросник «Возраст не помеха», индекс коморбидности Чарлсона. Выполнено биохимическое обследование, включавшее определение ПХЭ сыворотки крови кинетическим колориметрическим методом.*

Результаты. *Показатели опросника «Возраст не помеха» и активность ПХЭ обладают обратной умеренной корреляцией, коэффициент Спирмена (r_S) = –0,31, 95% доверительный интервал (ДИ): –0,54–(–0,03); p < 0,05. Показатели шкалы МоСА и активность ПХЭ также обладали умеренной обратной корреляцией: r_S = –0,32; 95% ДИ –0,55–(–0,05); p < 0,05. Пороговая точка активности ПХЭ 9978 ЕД/л позволяет с чувствительностью 47% и специфичностью 97% определить высокий риск умеренных когнитивных нарушений. Ассоциация между показателями ПХЭ и распространённостью когнитивных нарушений сохранялась при введении в регрессионную модель социально-демографических и метаболических параметров: отношение шансов 1,0005; 95% ДИ 1,0001–1,009; p = 0,01.*

Заключение. *У женщин старше 60 лет, наблюдающихся амбулаторно, выявлена обратная корреляция показателей опросника «Возраст не помеха» и активности ПХЭ. Уровень активности ПХЭ 9978 ЕД/л и выше ассоциировался с высоким риском сопутствующих умеренных когнитивных нарушений, при этом важно учитывать большую вероятность ложноотрицательных результатов. Данная ассоциация сохранялась в условиях воздействия различных клинических и метаболических факторов.*

Ключевые слова: *старческая астения; когнитивные нарушения; холинэстераза; биомаркер; диагностика*

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Адрес для корреспонденции: 620066, Свердловская область, Екатеринбург, ул. Академическая, д. 20. Институт высокотемпературной электрохимии УрО РАН. E-mail: antonkodintsev@mail.ru. Кодинцев А.Н.

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Introduction

The increase in life expectancy is responsible for the growing proportion of elderly and senile individuals in Russia, which requires the development of personalized medical care for patients over 60 years of age. Aging is associated with the onset of geriatric symptoms, a multifactorial age-related clinical condition that increases the risk of adverse outcomes and functional impairment [1]. In this regard, physicians of various specialties are increasingly encountering clinical manifestations of frailty and cognitive impairment in their practice. Pre-frailty and frailty are more common in females than in males [2]. Data suggesting an association between frailty and cognitive impairment have been published. In particular, frailty is associated with an increased risk of cognitive impairment of various origin and vice versa [3, 4]. Furthermore, cognitive frailty (CF) is currently being recognized as a distinct nosological entity, combining features of frailty and cognitive impairment. The prevalence of CF is 6 to 16% in the population over the age of 60 years [5].

In this context, there is an increasing need for timely diagnosis and personalized management strategy for patients at high risk of developing frailty in combination with cognitive impairment. A relevant diagnostic focus is to search for potential biomarkers that can be used as a patient stratification system, since frailty and cognitive impairment are currently diagnosed primarily by clinical examination.

Laboratory and instrumental diagnosis of cognitive impairment in Alzheimer's disease (A/T/N (β-amyloid/tau protein/neurodegeneration) system) is expensive and inaccessible and involves the use of labor-intensive methods (positron-emission tomography, lumbar puncture, biopsy) [6]. This approach is not commonly employed in clinical practice, thus the pursuit of more accessible and comparable biomarkers in terms of accuracy continues [7].

Specifically, the level of peripheral serum cholinesterase (pChE) is an available indicator whose level changes may serve as a predictor of the development and progression of neurodegenerative processes associated with cognitive impairment [8]. PChE is an α-glycoprotein synthesized by the liver and existing in two main forms: acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). In blood, the ratio of BChE to AChE is 412.5 : 1, but AChE is the more active enzyme [9]. In clinical practice, the assessment of BChE and pChE levels is used to diagnose organophosphate poisoning [10].

Recently, more and more data have emerged indicating a decrease in pChE activity in frailty, but a number of scientific papers show different changes in the levels of AChE and BChE in Alzheimer's disease and other neurodegenerative processes, making it difficult to formulate unified diagnostic algorithms [11–13]. Despite the clear correlation of pChE activity with frailty and cognitive function scores, the validity and clinical significance of this parameter have not been studied, including in the presence of concomitant metabolic disorders. There is a lack of data on threshold enzyme levels that can be used as predictive values. Therefore, investigating the diagnostic role of pChE may be a promising area of personalized medicine. This parameter may be potentially employed not only as a laboratory marker of cognitive impairment and frailty, but also as a laboratory indicator of the efficacy of drug therapy for these conditions.

The **aim** of the study was to identify and investigate the relationship between serum pChE activity in elderly patients and various screening scores of cognitive status, frailty, and metabolic parameters.

Materials and Methods

A total of 50 women over 60 years of age on the record of the outpatient clinic of the Institute of High Temperature Electrochemistry, Ural Branch of the Russian Academy of Sciences (Ekaterinburg) were randomly selected to participate in a single-time cross-sectional study. A comprehensive clinical examination was conducted to form the main referral sample, with inclusion and non-inclusion criteria applied. The Study Protocol and the Informed Consent Form (ICF) were approved by the Local Ethics Committee of the Ural State Medical University (meeting minutes No. 9 dated 18 December, 2020).

Study design: single-time cross-sectional study.

Inclusion criteria:
- female;
- age > 60 years;
- signed ICF.
- Non-inclusion criteria:
- severe decompensated somatic, neurological, or psychiatric conditions;
- inability to perform neuropsychological testing due to the severity of somatic condition and mental disorders (dementia and/or depression according to neuropsychological testing: Geriatric Depression Scale-15 score 5 or higher, life history data);
- treatment with parasympathomimetics, muscarinic receptor antagonists, AChE inhibitors;
- chronic hepatitis, severe and decompensated liver disease.

A validated Age Is Not a Hindrance questionnaire was used to evaluate the frailty severity. The questionnaire scores were evaluated as follows: 0 – no signs of frailty; 1–2 – signs of pre-frailty; 3 and more – signs of frailty. For a more reliable assessment, scores of 0–2 were considered as low risk of frailty, while scores of 3 or more were considered as high risk of frailty [14]. The Age Is Not a Hindrance questionnaire scores demonstrate greater sensitivity for detecting an elevated risk of frailty compared to the thresholds recommended

in the Clinical Guidelines on frailty [15, 16]. O.N. Tkacheva et al. showed that the sensitivity was 87% for threshold score ⩾ 3 and 46.7% for the threshold score ⩾ 5 in relation to the frailty index. Compared to the frailty phenotype model, the sensitivity was 93% for the threshold score ⩾ 3 and 46.4% for the threshold score ⩾ 5. Thus, the threshold score point ⩾ 3 on the Age Is Not a Hindrance questionnaire is more valuable as a screening tool for frailty [16].

Three validated scales were used to assess cognitive status: Mini-Mental State Examination (MMSE), Montreal Cognitive Assessment (MoCA), and Frontal Assessment Battery (FAB). Mild cognitive impairment was diagnosed at MoCA score < 26 and MMSE score > 24. The FAB results were interpreted in conjunction with the MMSE and MoCA scores.

The Charlson Comorbidity Index was used to assess the long-term prognosis of patients. Comorbidity (hypertension, type 2 diabetes mellitus (T2DM), postmenopausal osteoporosis, nonalcoholic fatty liver disease) data were established from medical history (outpatient record data).

Measuring peripheral cholinesterase activity

Venous blood was drawn in the treatment room to measure pChE levels. The pChE level was estimated by means of a kinetic colorimetric assay (Cobas 6000, Roche Diagnostics) at NPF HELIX LLC. This assay is based on the method published by E. Schmidt et al. [17].

Statistical analysis

Statistica v. 10 (StatSoft Inc.), MedCalc, OpenEpi (http://www.openepi.com) were used for data processing. The selection of the criterion and test for statistical analysis was based on the evaluation of the normality of the distribution of each parameter conducted using Kolmogorov–Smirnov and Shapiro–Wilk tests. In the event that the data exhibited a normal distribution, the mean and standard deviation values were used for description, with the analysis conducted using parametric methods. In other instances, the median, lower and upper quartiles were employed for descriptive purposes, and nonparametric tests were utilized for analysis. The qualitative data were compared using the χ^2 criterion and Fisher's exact test (when the χ^2 criterion was not applicable) due to the independence of the samples. A ROC analysis with area under curve (AUC) estimation, univariate and multivariate logistic regression, and multiple linear regression were employed in the study. The threshold for statistical significance is $p < 0.05$. In the event of negative results, the probability of a type II error was estimated, and the study power was calculated.

Results

A total of 50 women aged over 60 years (mean age 70.2 ± 4.2 years) were included in the study. Patients were categorized into 4 groups according to their frailty and cognitive function scores (Table 1). The majority of patients (37–74%)

Table 1. Patient demographic and clinical profile

Group	*n*	Mean age, years	Body mass index	Hypertension, *n*	Type 2 diabetes mellitus, *n*	Higher education, *n*	Postmenopausal osteoporosis, *n*
Low frailty risk, no MCI	24	70.8 ± 3.3	27.7 ± 4.8	19	4	15	10
Low frailty risk, MCI	13	69.5 ± 4.2	27.3 ± 3.6	10	3	7	6
High frailty risk, no MCI	8	72.5 ± 3.5	26.5 ± 4.5	7	0	3	1
High frailty risk, MCI	5	69.6 ± 5.9	31.5 ± 6.0	4	1	2	1
Total	50			40	8	27	18

Table 2. Median serum cholinesterase levels in patient groups

Group	*n*	Serum cholinesterase, U/l	
		median	min–max
Low frailty risk, no MCI	24	8195	6962–9467
Low frailty risk, MCI	13	9603*	9061–10 952
High frailty risk, no MCI	8	8137	7772–9339
High frailty risk, MCI	5	8685*	7206–8714

Note. *$p < 0.05$ as compared to groups without MCI.

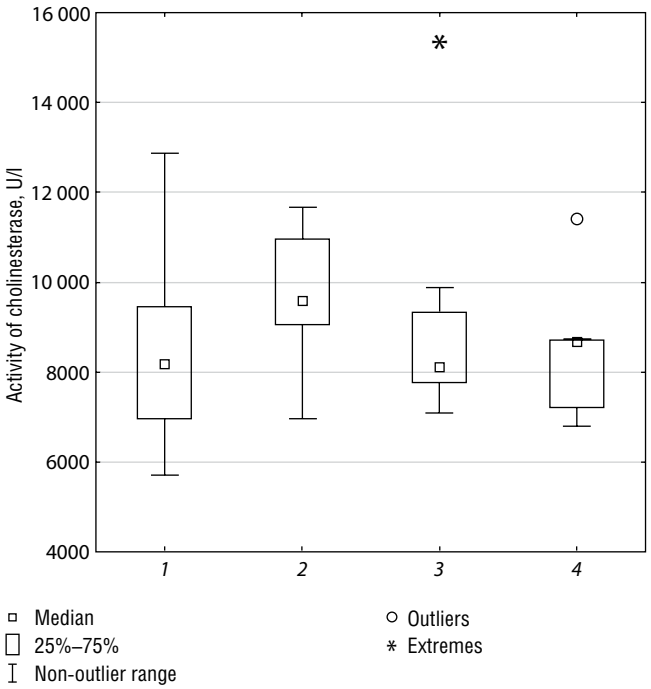

Fig. 1. Median blood cholinesterase levels in patient groups.
1 – low frailty risk, no MCI; *2* – low frailty risk, MCI; *3* – high frailty risk, no MCI; *4* – high frailty risk, MCI.

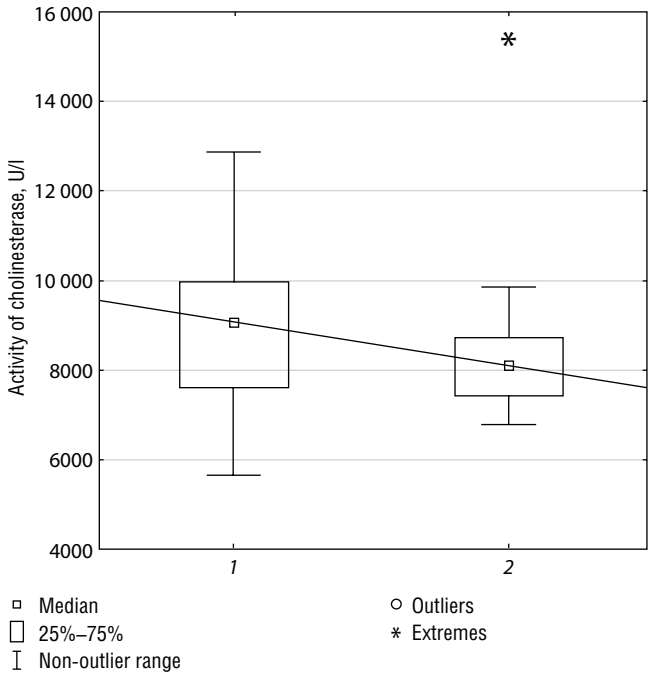

Fig. 2. Median cholinesterase levels in low (*1*) and high (*2*) frailty risk groups.

exhibited low frailty risk, with a higher prevalence of those without MCI (n = 24).

The patients in the groups did not differ significantly by age and BMI (two-way ANOVA; $p > 0.05$), prevalence of hypertension, T2DM, postmenopausal osteoporosis, and presence of higher education (χ^2 criterion > 0.05). Therefore, the obtained samples were comparable in terms of the main socio-demographic and certain clinical characteristics.

A Kruskal–Wallis test, followed by subgroup analysis, revealed that, although there was no statistically significant difference in pChE activity (H = 5.6; p = 0.13), higher pChE levels were observed in the patient groups with MCI (irrespective of the frailty risk) (Table 2).

Therefore, it can be assumed that the level of pChE is more strongly correlated with cognitive impairment than with the high frailty risk (Figure 1). To clarify this relationship, it was necessary to examine pChE levels, frailty scores, and cognitive status.

Relationship of cholinesterase levels to frailty assessment

When the correlation was evaluated, an inverse relationship was found between the pChE level and the Age Is Not a Hindrance questionnaire scores, Spearman's rank correlation coefficient (r_S) = –0.31, 95% CI: –0.541–(–0.0334); $p < 0.05$. However, pChE activity was not significantly different between the low and high frailty risk groups (9095 U/L (7613–9978) and 8137 U/L (7457–8756), respectively), Mann–Whitney test significance level (pMW) > 0.05 (Figure 2). Thus, changes in pChE levels were not significantly associated with the high frailty risk, despite the inverse correlation of the Age Is Not a Hindrance questionnaire with pChE level.

The constructed logistic regression model did not yield a significant association between elevated pChE levels and the increased frailty risk, odds ratio (OR) = –1; 95% CI 0.99–1.0003. Moreover, the absence of a significant relationship (p = 0.09) precluded the construction of a viable linear regression model evidenced by the exceedingly low value of the coefficient of determination ($R^2 < 0.3$), not normally distributed residuals (Shapiro–Wilk test = 0.0075). To minimize the likelihood of false-negative results, the power of the study was calculated and found to be less than 80%, suggesting a high probability of a type II error. Therefore, although a correlation was identified between pChE activity and the Age Is Not a Hindrance questionnaire scores, further investigation is required in a larger sample in order to assess a reliable relationship between these parameters.

Relationship of cholinesterase levels to cognitive status assessment

The relationship between pChE level and MoCA score was assessed, revealing a moderate inverse correlation: r_S = –0.32 (95% CI –0.55–(–0.05)); $p < 0.05$. The Mann–Whitney test revealed a statistically significant difference in pChE levels between the two patient groups: 8173 U/L (7110–9256) without MCI and 9603 U/L (8267–11418) with MCI, pMW = 0.008.

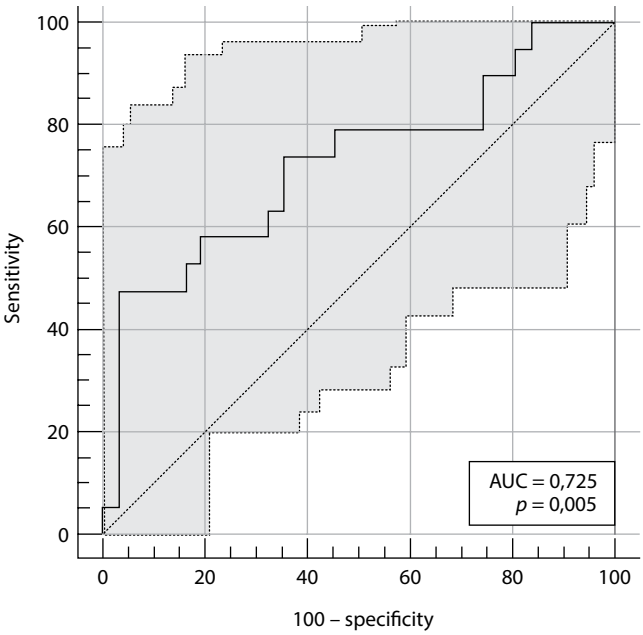

Fig. 3. ROC curve assessing the sensitivity and specificity of serum pChE level for the diagnosis of MCI.

Therefore, an inverse correlation was identified between MoCA scores and pChE levels. Higher enzyme levels were found to be associated with a lower score on this scale: OR = –1.0005; 95% CI 1.0001–1.009; p = 0.01, confirming the role of cholinergic deficiency in developing the cognitive impairment.

Although there is a correlation between pChE level and MoCA score, no significant association was found between changes in pChE level and MMSE score or FAB. In clinical practice, the MoCA scale is more sensitive than MMSE in diagnosing different variants of MCI in patients over 60 years of age [18]. We can therefore conclude an association of pChE level and MCI. A ROC analysis was performed for a more detailed evaluation (Figure 3).

According to the ROC-analysis, the sensitivity of the ChE threshold level of 9978 U/L (according to the Youden index) for detecting MCI was 47% (95% CI 24.4–71.1); specificity 97% (95% CI 83.3–99.9); AUC = 0.725; p = 0.005. The positive predictive value of the test was 55.6%; negative predictive value of the test –83%; diagnostic accuracy – 78%; likelihood ratio for the positive test – 14.68; for the negative test – 0.54; Cohen's kappa – 48.6%. The low sensitivity of pChE level does not allow the use of this enzyme level as a laboratory screening for MCI, but the high specificity allowed to suspect MCI in patients with pChE level > 9978 U/L due to a very low risk of false-positive results.

Despite the correlation between pChE levels, MoCA scores, and Age Is Not a Hindrance questionnaire scores, no correlation was found between the MoCA scores and the Age Is Not a Hindrance questionnaire scores.

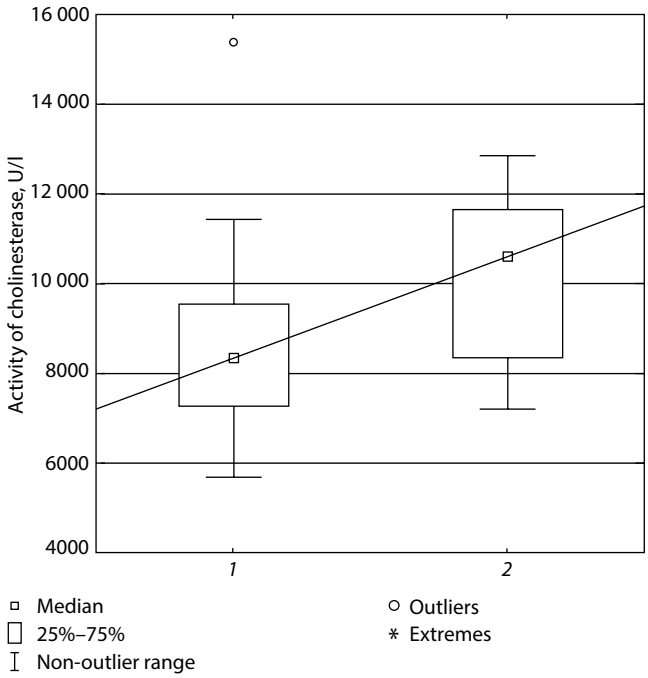

Fig. 4. Cholinesterase level in group of patients without T2DM (*1*) and with T2DM (*2*).

Relationship between cholinesterase level and metabolic parameters

Data on the effect of a number of metabolic factors on serum pChE level have recently been published and should be considered when assessing the relationship between this enzyme level and indicators of cognitive function. The main source of pChE is the liver, and evaluation of the effect of key metabolic parameters (including liver disease) on this enzyme level plays an important role in the development of a diagnostic model. Specifically, patients with T2DM had significantly higher pChE level compared to patients without T2DM: 10,614 (8337–11,646) and 8346 (7279–9535) U/L, respectively; $p_{MW} < 0.05$ (Fig. 4). No correlation was found between pChE level and Charlson Comorbidity Index scores (r_S = 0.21; $p > 0.05$). Also, the median pChE level was not significantly different between the group of patients with postmenopausal osteoporosis (8465 U/L) and the group without osteoporosis (8741 U/L), $p_{MW} > 0.05$.

pChE activity correlated with alanine aminotransferase (ALT: r_S = 0.43; $p < 0.05$) but not aspartate aminotransferase (AST) levels, confirming the relationship between liver functional status and pChE levels. Despite this association, pChE level in patients with nonalcoholic fatty liver disease (NAFLD), 9110 U/L (8173–9880), was not significantly different from that in patients without NAFLD, 8465 U/L (7242–9733); p_{MW} = 0.43. However, it should be noted that a small number of patients with confirmed NAFLD (n = 5) could not reliably exclude a false-negative result.

pChE activity did not correlate with total cholesterol (r_S = –0.21; $p > 0.05$), alkaline phosphatase (r_S = 0.11; $p > 0.05$), triglyceride

Table 3. Multiple linear regression analysis of socio-demographic and clinical factors. laboratory parameters. and peripheral cholinesterase level

Parameter	β-Coefficient	*p*
Age	0.56	0.764
Higher education	0.183	0.659
Body mass index	0.272	0.621
Hypertension	–0.213	0.702
Type 2 diabetes mellitus	0.095	0.897
Non-alcoholic fatty liver disease	0.489	0.589
Postmenopausal osteoporosis	–0.336	0.548
Charlson Comorbidity Index	0.333	0.485
Statins	0.547	0.593
Alanine aminotransferase	–0.651	0.411
Aspartate aminotransferase	0.673	0.395
Total cholesterol	–0.142	0.874
Triglycerides	0.073	0.865
Total protein	0.251	0.754

Table 4. Multiple logistic regression analysis to examine the correlation between social and clinical factors, laboratory parameters, and mild cognitive impairment

Parameter	Odds ratio	95% CI
Age	0.941	0.736–1.203
Higher education	0.674	0.099–4.559
Body mass index	0.987	0.741–1.312
Hypertension	13.038	0.968–187.399
Type 2 diabetes mellitus	3.102	0.097–99.57
Non-alcoholic fatty liver disease	2.006	0.073–55.388
Postmenopausal osteoporosis	0.423	0.06–2.981
Charlson Comorbidity Index	2.263	0.961–5.33
Statins	0.89	0.109–7.25
Alanine aminotransferase	0.827	0.613–1.115
Aspartate aminotransferase	1.091	0.922–1.291
Total cholesterol	1.136	0.618–2.089
Triglycerides	0.996	0.159–6.253
Total protein	0.929	0.718–1.204
Peripheral cholinesterase	1.0008	1.0001–1.0015

(r_S = –0.03; $p > 0.05$), or total protein (r_S = –0.11; $p > 0.05$) levels. pChE level did not significantly depend on the intake of lipid-lowering agents of the statin group either ($p_{MW} = 0.66$). A multiple linear regression model was constructed to assess the multicollinearity of the factors under study and pChE (Table 3). Under the influence of various parameters, the correlation between pChE and ALT or T2DM became statistically insignificant.

To test the hypothesis regarding the relevance of serum pChE level as a potential biomarker of cognitive impairment, a multiple logistic regression model was constructed taking into account the main metabolic parameters: T2DM, hypertension, NAFLD, BMI, ALT, AST, triglycerides, total cholesterol, total protein levels, and intake of statins. Even in the presence of a direct relationship with ALT (the primary liver function test), pChE level was significantly associated with MCI:

Table 5. Multiple linear regression analysis to examine the correlation between social and clinical factors, laboratory parameters, and MoCA scores

Parameter	β-Coefficient	*p*
Age	–0.082	0.871
Higher education	0.004	0.311
Body mass index	–0.389	0.676
Arterial hypertension	0.205	0.394
Type 2 diabetes mellitus	–0.038	0.458
Non-alcoholic fatty liver disease	–0.138	0.148
Postmenopausal osteoporosis	0.042	0.13
Charlson Comorbidity Index	–0.07	0.454
Statins	–0.06	0.175
Alanine aminotransferase	0.306	0.825
Aspartate aminotransferase	–0.263	0.724
Total cholesterol	0.031	0.753
Triglycerides	–0.175	0.718
Peripheral cholinesterase	–1.15	0.01

OR = –1.0008; 95% CI 1.0001–1.0015 (Table 4). Alkaline phosphatase level was not included in the model due to insufficient data. Furthermore, when a multiple linear regression model was constructed to assess the effect of socio-demographic and metabolic factors on the MoCA score, only pChE level was significantly associated with this cognitive assessment score (Table 5). The coefficient of determination (R^2) is 0.42, indicating a moderate degree of effect of the characteristic. The normal distribution of residuals (Shapiro–Wilk test > 0.05) and the acceptable model quality according to analysis of variance (F = 2.69; p = 0.34) confirm the effect of pChE level on MoCA scores.

Therefore, even when metabolic factors are considered, pChE level may serve as a potential laboratory marker of cognitive impairment, as evidenced by the regression analysis.

Discussion

There is currently an ongoing search for cost-effective and available biomarkers for laboratory diagnosis of frailty and cognitive impairment. Specifically, in patients with established frailty, the primary laboratory diagnostic focus is on the evaluation of hematologic (hemoglobin level) and endocrinologic (thyroid-stimulating hormone, T3, T4) parameters. The role of vitamin D and changes in the level of inflammatory markers such as C-reactive protein and interleukin-6 have also been studied [19]. A small study by R.E. Hubbard et al. performed on 30 hospitalized patients is published, indicating an inverse relationship between the severity of frailty and the level of AChE, BChE, and benzoylcholinesterase [11]. The authors mention that malnutrition in elderly age, particularly in patients with frailty, may be a potential mechanism for the decrease in esterase level. These findings are consistent with the obtained data on the inverse correlation between the Age Is Not a Hindrance questionnaire and serum pChE activity. The higher the risk of frailty, the lower the pChE activity. Nevertheless, the potential diagnostic utility of pChE in the diagnosis of frailty remains incompletely understood.

Frailty is significantly associated with the risk of development and progression of cognitive impairment, and the study of blood pChE levels in patients with various cognitive status scores is a key area of investigation [20]. It is established that cholinergic deficiency plays a pivotal role in the progression of cognitive impairment, including in Alzheimer's disease [21]. Cholinergic system dysfunction may be associated with increased serum cholinesterase activity and central nervous system, resulting in high levels of acetylcholine catabolism and impaired cholinergic transmission. R.C. Smith et al. found a 100% increase in plasma pseudocholinesterase (BChE) level in patients with Alzheimer's disease compared to controls [22]. The paper by M. Hosoi et al. points to the potential role of pChE (mainly BChE) as a biomarker for Alzheimer's disease. Activation of neuroinflammation and hyperexpression of BChE by astrocytes and microglia are accompanied by changes in the permeability of the blood-brain barrier, suggesting a relationship between increased BChE level in the central nervous system and serum pChE level [23].

High BChE level is detected in amyloid plaques and neurofibrillary tangles. Increased accumulation of β-amyloid in the hippocampus, thalamus and amygdala is associated with the modulating effect of BChE [13, 24]. Therefore, BChE level plays a pivotal role in the processes of amyloidogenesis and formation of neurofibrillary tangles, which permits the consideration of this enzyme not only in the context of laboratory diagnostics, but also as a potential therapeutic target. For example, when BChE level is high, rivastigmine is the preferred antidementia agent because, unlike donepezil, it also inhibits BChE [23].

Since the liver is involved in the synthesis of pChE, esterase (particularly BChE) level serves as a dynamic indicator of liver synthetic function and lipid metabolism. Reduced pChE levels may be observed in liver pathology [25]. In this study, metabolic parameters did not significantly affect the association between increased pChE level and the prevalence of MCI. Nevertheless, in the study sample, a markedly elevated pChE level was found in patients with T2DM substantiating earlier hypotheses regarding the hyperfunction of this enzyme in experimentally-induced diabetes mellitus [26]. Common mechanisms of increased pChE level in patients with T2DM and MCI include defects in insulin signaling, mitochondrial metabolism, SIRT-PGC-1α axis, Tau signaling, autonomic function, and neuroinflammatory pathways [27]. Furthermore, elevated levels of pChE are associated with the development of diabetic retinopathy [9]. Thus, high cholinesterase level (specifically BChE) may be a predictor of the development of T2DM and MCI (including of the Alzheimer type) [28]. The lack of a notable correlation between pChE, osteoporosis prevalence, and Charlson Comorbidity Index scores permits the consideration of this parameter as a specific marker of cognitive impairment. However, larger studies involving additional population groups are required.

Notwithstanding the findings of this study, R.C. Smith et al. [22], and M. Hosoi et al. [23], which indicate an increase in serum pChE level with the progression of neurodegenerative processes, there are published papers that demonstrate an inverse relationship. M.X. Dong et al. found a decrease in BChE level in patients with Parkinson's disease (PD) compared to controls [29]. The optimal cut-off point for BChE of 6864.08 U/L allows for distinguishing PD patients with a sensitivity of 61.8% and specificity of 72.1%. A reduction in BChE level to a value below 6550 U/L is significantly associated with a high probability of dementia, with a sensitivity of 70.6% and specificity of 76.3% [29]. Y.C. Chen et al. revealed a decrease in serum pChE, AChE, and BChE activities in patients with post-stroke vascular dementia [30]. Similar findings of lower plasma cholinesterase levels in patients with Alzheimer's disease and dyscirculatory encephalopathy were published by Russian authors [31, 32].

The disparate outcomes of studies examining pChE level in MCI, Alzheimer's disease, Parkinson's disease, and vascular dementia may be attributed to the heterogeneity of the samples and the varied methodological and research approaches employed. Furthermore, there is evidence of a markedly reduced BChE level in patients with dementia with Lewy bodies in comparison to patients with AD and controls. This does not preclude an association between reduced pChE level and synucleinopathies [29, 33]. A parabolic change in the pChE level cannot be excluded with a gradual level increase with the development of MCI and a subsequent level decrease as the dysfunction of the cholinergic system progresses with dementia manifestation. A potential role is played by the notable advancement of comorbid conditions, particularly alterations in metabolic status and synthetic liver function in patients with dementia and synucleinopathies (Parkinson's disease, dementia with Lewy bodies).

Limitations and advantages of the study. The single-time cross-sectional study did not yield sufficient evidence to establish a causal relationship between the studied parameters. Furthermore, the sample was comprised of female outpatients over the age of 60, which precludes the possibility of extrapolating the results to other populations. Cognitive impairment was diagnosed based on the common validated scales without additional in-depth neuropsychological examination or additional stratification.

To determine the frailty risk, the screening Age Is Not a Hindrance questionnaire was applied without additional comprehensive geriatric examination (phenotype model, frailty index) and the use of objective methods of assessment of various geriatric domains (dynamometry, Timed Up and Go test), which does not allow to reliably exclude the subjective nature of the assessment.

Patients with dementia were not included in the study. Total serum pChE levels were measured without verification of AChE and BChE. Further evaluation of pChE levels in the context of additional comorbidities and laboratory markers (including systemic inflammatory parameters) is advisable.

However, the homogeneous nature of the sample, the sufficiently strict selection criteria, the availability of standardized assessment tools, and the consistency of the identified trends with the data of other researchers allow us to anticipate more significant results when studying a larger sample that includes male patients.

Conclusion

A significant correlation was identified between pChE activity and changes in MoCA scores and the prevalence of MCI. The high degree of specificity of the test, coupled with the exceedingly low probability of a false-positive result, renders it feasible to suspect MCI at a pChE level of 9978 U/L or above. However, the low level of sensitivity implies a high risk of false-negative results. The data obtained from the multiple linear and logistic regression analyses corroborate the established relationship between pChE activity, MoCA scores, and MCI, even when accounting for the potential influence of metabolic parameters and comorbidities. Despite the correlation between the Age Is Not a Hindrance questionnaire scores and pChE activity, no significant differences in this enzyme levels were identified between patients at low and high risk of frailty. A study on a larger sample size is needed to reliably assess the association of pChE and frailty risk parameters and to further investigate pChE level changes in patients with cognitive impairment of different origin.

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Information about the authors

Anton N. Kodincev – general neurologist, ultrasound specialist, junior researcher, Institute of High Temperature Electrochemistry of the Ural Branch of the Russian Academy of Sciences, Ekaterinburg, Russia, https://orcid.org/0000-0002-3978-8902
Larisa I. Volkova – Dr. Sci. (Med.), Associate Professor, Chief freelance neurologist of the Ural Federal District, Head, Department of nervous diseases, neurosurgery and medical genetics, Ural State Medical University, Ekaterinburg, Russia,
https://orcid.org/0000-0002-2478-727X

Authors' contribution. *Kodincev A.N.* – creating a research concept, conducting research, analyzing data, preparing a draft manuscript and graphic material; *Volkova L.I.* – participating in research concept developing, checking of research methodology and material description, checking and text correction.

Информация об авторах

Кодинцев Антон Николаевич – врач-невролог, врач ультразвуковой диагностики поликлиники, м. н. с. Института высокотемпературной электрохимии Уральского отделения РАН, Екатеринбург, Россия, https://orcid.org/0000-0002-3978-8902
Волкова Лариса Ивановна – д-р мед. наук, доцент, главный внештатный специалист-невролог Уральского федерального округа, зав. каф. нервных болезней, нейрохирургии и медицинской генетики Уральского государственного медицинского университета, Екатеринбург, Россия, https://orcid.org/0000-0002-2478-727X

Вклад авторов: *Кодинцев А.Н.* – создание концепции исследования, проведение исследования, анализ данных, подготовка черновика рукописи, подготовка графического материала; *Волкова Л.И.* – участие в разработке концепции исследования, проверка методологии исследования и оформления материала, правка текста.

Mini Balance Evaluation Systems Test (Mini-BESTest): Cultural and Linguistic Adaptation in Russia

Dzhamilya G. Yusupova[1], Ekaterina V. Salina[2], Aleksandr B. Zaytsev[3], Aleksey A. Zimin[1], Aleksandra O. Moskvina[4], Oleg V. Kolokolov[2], Natalia V. Polekhina[1], Aleksandr S. Fedonnikov[2], Natalia A. Suponeva[1]

[1]Research Center of Neurology, Moscow, Russia;
[2]V.I. Razumovsky Saratov State Medical University, Saratov, Russia;
[3]I.M. Sechenov First Moscow State Medical University, Moscow, Russia;
[4]I.I. Mechnikov North-Western State Medical University, St. Petersburg, Russia

Abstract

Introduction. *In clinical practice, a comprehensive assessment of the systems responsible for balance is important both for correct diagnosis and the right choice of therapy. To provide accurate assessment of all the systems involved in balance control, in 2009, F.B. Horak et al. from the Oregon Health and Sciences University developed a universal Balance Evaluation Systems Test (BESTest) consisting of 36 tasks. Subsequently, the authors improved this method by selecting 14 tasks that evenly belonged to four of the six sections of the original BESTest, which collectively constituted the Mini-BESTest. The Mini-BESTest is a unique brief assessment tool that is actively used worldwide for the diagnostics and dynamic evaluation of balance in various nervous system disorders. However, the absence of a validated Russian version makes it challenging to use this test in Russia. The* ***objective*** *of the study is to develop an official Russian version (cultural and linguistic adaptation) of the Mini-BESTest to consider the target language and culture (1st stage of the linguistic validation study).*

Materials and methods. *The author of the test, F.B. Horak, granted her consent for the linguistic validation of Mini-BESTest in Russia. Forward and backward translations of the test and its materials, pilot testing (cognitive debriefing), and development of the Russian version were carried out with the participation of a linguistic philologist and neurologists specializing in working with patients with balance disorders in various neurological diseases.*

Results. *Based on the results of the expert committee meeting, a cultural and linguistic adaptation of the test was carried out and the final Russian version presented in this article was approved.*

Conclusion. *The first developed Russian version of Mini-BESTest is officially presented and recommended for use both in clinical and research practice in Russia and other Russian-speaking countries. The psychometric properties (reproducibility, inter-rater reliability, and sensitivity of the test) of the Russian version are currently being assessed.*

Keywords: *balance systems evaluation test; Mini-BESTest; linguistic validation; cultural and linguistic adaptation*

Ethics approval. The research protocol was approved by the Ethics Committee of the V.I. Razumovsky Saratov State Medical University (Protocol No. 6, January 16, 2024).

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For correspondence: 125367, Russia, Moscow, Volokolamskoye shosse, 80. Research Center of Neurology.
E-mail: dzhamilya-d@mail.ru. Yusupova D.G.

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Тест оценки равновесия (Mini Balance Evaluation Systems Test – Mini-BESTest): лингвокультурная адаптация в России

Д.Г. Юсупова[1], Е.А. Салина[2], А.Б. Зайцев[3], А.А. Зимин[1], А.О. Москвина[4], О.В. Колоколов[2], Н.В. Полехина[1], А.С. Федонников[2], Н.А. Супонева[1]

[1]Научный центр неврологии, Москва, Россия;
[2]Саратовский государственный медицинский университет имени В.И. Разумовского, Саратов, Россия;
[3]Первый Московский государственный медицинский университет имени И.М. Сеченова, Москва, Россия;
[4]Северо-Западный государственный медицинский университет имени И.И. Мечникова, Санкт-Петербург, Россия

Аннотация

Введение. *В клинической практике всесторонняя оценка функционирования систем, обеспечивающих равновесие, важна не только для диагностики, но и для выбора тактики терапии. С целью точного определения функции всех систем, участвующих в поддержании равновесия, в 2009 г. F.B. Horak и соавт. из Орегонского университета медицины и естественных наук разработали универсальный тест оценки равновесия (Balance Evaluation Systems Test – BESTest), который включает 36 заданий. В последующем авторы усовершенствовали данный метод, отобрав 14 тестовых заданий, равномерно принадлежащих 4 из 6 разделов BESTtest, которые получили в совокупности общее название Mini-BESTest. Этот уникальный краткий оценочный тест активно применяется во всём мире для диагностики и динамической оценки функции равновесия при различных заболеваниях нервной системы. Отсутствие валидированной русскоязычной версии данного теста затрудняет его применение в России.*

Цель *работы – разработка официальной русскоязычной версии (лингвокультурная адаптация) Mini-BESTest с учётом языковых и культурных особенностей (1-й этап валидационного исследования).*

Материалы и методы. *Получено согласие автора теста F.B. Horak на проведение валидации Mini-BESTest в России. Проведены прямой и обратный переводы теста и материалов к нему, пилотное тестирование, разработка русскоязычного варианта при участии филолога-лингвиста и неврологов, специализирующихся на работе с пациентами с нарушениями функции равновесия при различных неврологических заболеваниях.*

Результаты. *По результатам заседания экспертной комиссии была проведена лингвокультурная адаптация текста теста, утверждена финальная русскоязычная версия, которая представлена в данной статье.*

Заключение. *Русскоязычная версия Mini-BESTest впервые официально представлена и рекомендована к использованию как в клинической, так и в исследовательской практике в России и других русскоговорящих странах. Проводится оценка психометрических свойств (воспроизводимости, межэкспертной согласованности и чувствительности) русскоязычной версии теста.*

Ключевые слова: *тест оценки равновесия; Mini-BESTest; валидация; лингвокультурная адаптация*

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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.

Адрес для корреспонденции: Россия, 125367, Москва, Волоколамское шоссе, д. 80. Научный центр неврологии. E-mail: dzhamilya-d@mail.ru. Юсупова Д.Г.

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Introduction

Balance control is a fundamental complex function required for normal human activity. Coordinating vestibular, visual, oculomotor, and proprioceptive systems ensure control of muscle tone, several higher cortical functions, posture, complex motor activities, and gait on various levels of the nervous system, from receptors to a cortical representation [1]. Moreover, recent discoveries suggest that balance control is involved in cognitive processes, and that balance deficits may cause disorders associated with spatial memory, learning, and navigation [2, 3].

The primary symptoms of balance disorder include dizziness, unsteadiness and falls. 15–20% of the adult population annually experience sensation described as "dizziness" [4]; this same condition, occurring suddenly, accounts for 2.1–3.6% of emergency department visits [5]. Dizziness can occur in any patient, with middle and inner ear disorders being the most common causes in patients < 50 years, while in patients > 70 years a more comprehensive assessment of the factors causing unsteadiness and balance problems is required due to the high risk of falls and related complications [6–8]. The balance control is negatively affected by aging. Age-related changes in the body are multifaceted and encompass a decrease in deep and superficial sensation, vision disturbances (especially contrast detection and depth perception), vestibular and cochlear dysfunctions, a decrease in strength and tone across various muscle groups, and impaired regulatory mechanisms of the central nervous system (CNS), including slowing down of afferent processing and executive functioning. Severe impairment in any of the above systems may predispose the elderly patients to falls, with such risk increasing significantly with the number of functions affected [9]. Fall-related injuries are the sixth leading cause of mortality in the elderly people worldwide, with up to $10 billion annual costs for the treatment of these injuries [10, 11].

Balance may be disturbed by a variety of somatic and neurological disorders affecting both central and peripheral nervous systems. Risk factors associated with balance disorders include female gender, low level of education, age over 40 years, cardiovascular diseases (CVDs), and anxiety and depression [12, 13]. Finding the true cause of these symptoms is often challenging due to the multi-component nature of the balance system. Patients may be confused when describing their sensations, using terms such as "dizziness", "unsteadiness", "discomfort", "rocking sensation" etc. and therefore a multidisciplinary approach to the management of these patients is required. Incorrect topical diagnosis or late detection of the cause underlying poor functional balance often leads to the gravely limited motor function and significantly reduced quality of life in these patients.

In clinical practice, a comprehensive assessment of the balance system functioning is of significant importance, not only from a diagnostic standpoint, but also for the selection of an appropriate therapeutic intervention. Consequently, a multitude of specialized assessment tools, questionnaires, scales, and devices have been developed, which have now become integral components of international standards for the diagnosis of diseases. The current assessment techniques have significant drawbacks and are not universally applicable for the majority of disorders associated with vestibular dysfunction, dysbasia, and postural disorders. Most of them are published in English, which also complicates their use by Russian-speaking medical professionals, and a direct word-for-word translation of a scale or a test does not necessarily ensure consistent application within a single country. Therefore, linguistic validation and evaluation of the psychometric properties of the Russian versions of these assessment tools is a necessity.

Recently, new tools for comprehensive assessment of the balance systems have been developed with the potential for use in clinical practice. Mini-BESTest is one such tool that has already been recommended and adapted by numerous researchers of methods to detect balance deficits and postural disorders [14–17]. This test was developed by the Head of the Balance Disorders Laboratory at Oregon Health and Sciences University, Prof. Fay B. Horak et al. Their intention initially was to create a universal tool to assess the functioning of all the systems involved in maintaining balance and to identify the localization of the disorder causing balance impairment. Their work yielded in the BESTest (Balance Evaluation Systems Test). They conducted a study including 22 participants aged 50–88 years. The study sample included a control group with no signs of balance problems and participants with balance impairment caused by various factors (uni- and bilateral vestibulopathy, Parkinson's disease, peripheral neuropathy, hip arthroplasty). The study findings showed that patients with different diagnoses scored poorly on different sections of the BESTest. For example, patients with unilateral vestibular insufficiency had worse results in Section V (Sensory Orientation), while patients with Parkinson's disease had worse results in Section IV (Postural Responses) [18].

The authors reported that their methodology allowed clinicians to identify the specific mechanism underlying impaired balance, but they also admitted the need for further studies to improve the test. The Mini-BESTest has become such a modification. Initially, the BESTest consisted of 36 tasks, grouped into six sections evaluating different balance control systems and mechanisms. The authors selected 14 tasks belonging evenly to four of the six sections from the original BESTest using Rasch psychometric analysis. The new 14-item scale was referred to as the Mini-BESTest [19].

The Mini-BESTest study was conducted at a rehabilitation center where 115 patients (mean age 62.7 years) were recruited. The patients had various neurological diagnoses, including stroke-related hemiparesis, Parkinson's disease, neuromuscular disorders, hereditary ataxia, multiple sclerosis, nonspecific age-related balance system disorders, peripheral vestibular

disorders, traumatic brain injury, diffuse encephalopathy, cervical myelopathy, and CNS neoplasm. Inclusion criteria were the ability to walk with or without a cane and the absence of severe cognitive or communication impairments. The authors note that the novel Mini-BESTest offers a unique brief clinical rating scale for balance and can be used for assessment of severity for different neurological disorders. For example, in a study of 80 patients with Parkinson's disease, the sensitivity and specificity of the BESTest and Mini-BESTest were compared, and it was determined that both tests were suitable for balance assessment. However, with the Mini-BESTest, the difference in results between patients with and without history of falls was greater than with the BESTest (27% vs. 19%, respectively), suggesting that the Mini-BESTest had better sensitivity. The most significant benefit of the Mini-BESTest in clinical practice is that it takes half as much time compared to the BESTest.[20] The Mini-BESTest is widely used in various countries to assess the balance in a range of neurological disorders, both in neurological clinics and rehabilitation centers [21–25].

The **objective** of our study is to develop an official Russian version for cultural and linguistic adaptation of the Mini-BESTest to ensure its conceptual equivalence to the original source document (stage 1 of the linguistic validation) and to carry out cognitive debriefing of this translation.

Materials and methods

The developer of the original test, F.B. Horak, granted her written consent for adaptation of the Mini-BESTest. The first stage of linguistic adaptation was performed by specialists of the Center for Validation of International Scales and Questionnaires of the Research Center of Neurology. The cultural and linguistic adaptation was performed according to general requirements. Forward translation was done by two Russian-speaking medical translators and the backward translation – by native speakers with medical education. The Russian version was reviewed by an expert committee chaired by an expert translator who was not involved in the translation of the Mini-BESTest. The committee included medical translators and neurologists with more than 5 years of experience.

The cognitive debriefing was performed at the Research Center of Neurology and K.N. Tretyakov Department of Neurology in V.I. Razumovsky Saratov State Medical University at the stroke unit and at the neurology department. The study was approved by the local ethical committee of the V.I. Razumovsky Saratov State Medical University (protocol No. 6 of 16 January, 2024).

The inclusion criteria for the cognitive debriefing were the age of patients ⩾ 18 years and a patient's informed consent. The recruited patients had central vestibular disorders and were diagnosed with ischemic stroke, cerebral microangiopathy, Parkinson's disease, and multiple sclerosis. Important criteria included the ability to walk independently or with technical support (with a cane), without an assistant, and the absence of severe cognitive impairment according to the Mini Mental State Examination (MMSE).

Exclusion criteria were severe sensory impairment (major visual, hearing, and deep sensory impairment), decompensation of somatic diseases, class 3 obesity, and severe musculoskeletal disorders.

The cognitive debriefing included 18 patients (10 males and 8 females with neurological diseases: cerebral microangiopathy (n = 5), Parkinson's disease (n = 4), multiple sclerosis (n = 3), vertebrobasilar stroke (n = 3), and carotid artery-related stroke (n = 3). All patients were native Russian speakers, and the patients' diagnosis met the international criteria.

Results and discussion

The Mini-BESTest consists of 14 tasks developed to evaluate various mechanisms that are responsible for balance. The tasks are organized into four sections (domains) assessing various balance control systems. The first section includes three tasks for preliminary assessment of the balance. The second section consists of three items to assess postural responses. Three tasks of the third section assess the sensory orientation. The final section includes five tests that focus on stability in gait. The methodology of the Mini-BESTest requires additional equipment, including a vestibular cushion, a chair without armrests and wheels, a stopwatch, a platform with a slope, a box approximately 23 cm high, and adhesive tape for measuring and marking the distance on the floor. A patient has to follow instructions to perform each task and the patient's performance is scored from 0 to 2, with a maximum test score of 28. Based on the score, it is possible to determine whether the patient has a balance disorder and to identify the underlying mechanism.

The translation and development of the final Russian version of Mini-BESTest posed some challenges related to conceptual equivalence of the English and Russian versions. Since each task includes instructions, the Russian text should be clear for both physicians and patients. In the course of the work, several minor adjustments were made to the test instructions. The instructions for performing the tasks and interpretation of the obtained results in the Postural Responses section and the Sensory Orientation section were clarified. For instance, to make the instructions for the Compensatory Stepping Forward, Backward, and Lateral tasks more understandable, the phrasing "do whatever is necessary, including taking a step, to avoid a fall" was replaced by "take a step to avoid falling". In the instructions for the Stand on One Leg task, the phrasing "Lift your leg off of the ground behind you without touching or resting your raised leg upon your other standing leg " has been replaced by "You need to raise

one leg without touching the opposite leg". In the Sit to Stand task, the phrase "thrusting of the arms forward" was changed to "compensatory forward arm movement".

The cognitive debriefing was performed by neurologists independently. The interval between examinations by the two investigators was < 24 h. On average, the test took 20 min and the scoring took 5 min. Cognitive debriefing revealed no confusions in understanding the task instructions and further interpretation of the results. Based on the results of cognitive debriefing, the final Russian version was approved during the meeting of the expert committee.

Conclusion

The 1st stage of the linguistic validation was completed, cultural and linguistic adaptation of the Mini-BESTest was performed, and a Russian version was developed with account to the target language and culture. The Mini-BESTest is an accessible and user-friendly universal tool for comprehensive assessment the balance and neurological localization. The Russian version of the questionnaire is available for download at the official website of the Research Center of Neurology, as well as using a QR code. The psychometric properties of the Russian version are currently being evaluated.

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Information about the authors

Dzhamilya G. Yusupova – Cand. Sci. (Med.), researcher, Head, Validation group for international scales and questionnaires, Institute of Neurorehabilitation and Restorative Technologies, Research Center of Neurology, Moscow, Russia, https://orcid.org/0000-0002-5826-9112
Ekaterina A. Salina – Cand. Sci. (Med.), Associate Professor, Department of neurology named after K.N. Tretyakov, V.I. Razumovsky Saratov State Medical University, Saratov, Russia, https://orcid.org/0000-0002-8945-2120
Aleksandr V. Zaitsev – Cand. Sci. (Philology), Associate Professor, Institute of Linguistics and Intercultural Communication, I.M. Sechenov First Moscow State Medical University, Moscow, Russia,
https://orcid.org/0000-0003-3774-3070
Aleksey A. Zimin – Cand. Sci. (Med.), researcher, Group for validation of international scales and questionnaires, Institute of Neurorehabilitation and Restorative Technologies, Research Center of Neurology, Moscow, Russia, https://orcid.org/0000-0002-9226-2870
Aleksandra O. Moskvina – resident in neurology, Department of neurology named after S.N. Davydenkov, I.I. Mechnikov North-Western State Medical University, St. Petersburg, Russia, https://orcid.org/0000-0002-2318-9788
Oleg V. Kolokolov – Dr. Sci. (Med.), Associate Professor, Head, Department of Neurology named after K.N. Tretyakov, V.I. Razumovsky Saratov State Medical University, Saratov, Russia, https://orcid.org/0000-0001-6672-6873
Natalia V. Polekhina – neurologist, Research Center of Neurology, Moscow, Russia, https://orcid.org/0000-0002-4364-5947
Aleksandr S. Fedonnikov – Dr. Sci. (Med.), Associate Professor, Vice-rector for research, V.I. Razumovsky Saratov State Medical University, Saratov, Russia, https://orcid.org/0000-0003-0344-4419
Natalia A. Suponeva – Dr. Sci. (Med.), Prof., Corresponding Member of RAS, Director, Institute of Neurorehabilitation and Restorative Technologies, Research Center of Neurology, Moscow, Russia,
https://orcid.org/0000-0003-3956-6362

Authors' contribution. *Yusupova D.G.* – concept and design development, organization and coordination of the study, editing and final approval of the manuscript; *Salina E.V.* – organization and coordination of the study, data collection and analysis, writing and final approval of the manuscript; *Zaytsev A.B., Polekhina N.V.* – analysis of the data; *Zimin A.A.* – analysis of the data, editing the manuscript; *Moskvina A.O.* – analysis of the data, editing the manuscript; *Kolokolov O.V.* – coordination of the study, final approval of the manuscript; *Fedonnikov A.S.* – final approval of the manuscript; *Suponeva N.A.* – coordination of the study, final approval of the manuscript.

Информация об авторах

Юсупова Джамиля Гереевна – канд. мед. наук, н. с., руководитель Группы валидации международных шкал и опросников Института нейрореабилитации и восстановительных технологий Научного центра неврологии, Москва, Россия, https://orcid.org/0000-0002-5826-9112
Салина Екатерина Анатольевна – канд. мед. наук, доцент каф. неврологии им. К.Н. Третьякова Саратовского ГМУ им. В.И. Разумовского, Саратов, Россия, https://orcid.org/0000-0002-8945-2120
Зайцев Александр Владимирович – канд. филол. наук, доцент Института лингвистики и межкультурной коммуникации Первого Московского государственного медицинского университета им. И.М. Сеченова, Москва, Россия, https://orcid.org/0000-0003-3774-3070
Зимин Алексей Алексеевич – канд. мед. наук, н. с. Группы валидации международных шкал и опросников Института нейрореабилитации и восстановительных технологий Научного центра неврологии, Москва, Россия, https://orcid.org/0000-0002-9226-2870
Москвина Александра Олеговна – клинический ординатор каф. неврологии им. С.Н. Давыденкова СЗГМУ им. И.И. Мечникова, Санкт-Петербург, Россия, https://orcid.org/0000-0002-2318-9788
Колоколов Олег Владимирович – д-р мед. наук, доцент, зав. каф. неврологии им. К.Н. Третьякова Саратовского ГМУ им. В.И. Разумовского, Саратов, Россия, https://orcid.org/0000-0001-6672-6873
Полехина Наталья Вадимовна – врач-невролог Научного центра неврологии, Москва, Россия, https://orcid.org/0000-0002-4364-5947
Федонников Александр Сергеевич – д-р мед. наук, доцент, проректор по научной работе Саратовского ГМУ им. В.И. Разумовского, Саратов, Россия, https://orcid.org/0000-0003-0344-4419
Супонева Наталья Александровна – д-р мед. наук, проф., член-корр. РАН, директор Института нейрореабилитации и восстановительных технологий Научного центра неврологии, Москва, Россия,
https://orcid.org/0000-0003-3956-6362

Вклад авторов: *Юсупова Д.Г.* – разработка концепции и дизайна исследования, организация и координация исследования, редактирование и финальное утверждение рукописи; *Салина Е.А.* – организация и координация исследования, сбор и анализ полученных данных, написание текста рукописи, финальное утверждение рукописи; *Зайцев А.Б.* – анализ полученных данных; *Зимин А.А., Москвина А.О.* – анализ полученных данных, редактирование текста рукописи; *Колоколов О.В.* – координация исследования, финальное утверждение рукописи; *Полехина Н.В.* – анализ полученных данных; *Федонников А.С.* – финальное утверждение рукописи; *Супонева Н.А.* – координация исследования, финальное утверждение рукописи.

Transcranial Direct Current Stimulation for Improvement of Neurotransplantation Outcomes in Rats with 6-Hydroxydopamine-Induced Parkinsonism

Alla V. Stavrovskaya[1], Dmitry N. Voronkov[1], Ivan A. Potapov[1], Daniil S. Titov[2], Artem S. Olshansky[1], Anastasiia K. Pavlova[1], Olga S. Lebedeva[3], Sergey N. Illarioshkin[1]

[1]Research Centre of Neurology, Moscow, Russia;
[2]Bauman Moscow State Technical University, Moscow, Russia;
[3]Lopukhin Federal Research and Clinical Center of Physical-Chemical Medicine, Moscow, Russia

Abstract

Introduction. *With the number of patients with Parkinson's disease steadily growing, the need for novel treatment approaches is increasing. Combining transplantation of neuronal progenitors derived from induced pluripotent stem cells and transcranial direct current stimulation (tDCS) is among the promising methods.*

Aim*: to examine the effect of tDCS on the cell graft condition and motor symptoms of Parkinson's syndrome in rats.*

Materials and methods. *Parkinson's syndrome was modeled in Wistar rats by the unilateral intranigral injection of 6-hydroxydopamine (6-OHDA; 12 μg in 3 μL) The model rats underwent neurotransplantation (3×10^5 cells in 10 μL) into the caudate nuclei on the affected side. The animals underwent tDCS for 14 days. Behavioral changes were analyzed by open field and beam-walking tests. Development and morphological characteristics of the graft were assessed by the morphochemical study.*

Results. *Neurotransplantation had no significant effect on the behavior of rats with parkinsonism; however, combined with tDCS, it increased motor activity during the open field tests compared with the group of model rats ($p=0.0014$) and mitigated their anxiety-related behaviors ($p=0.048$) in tests at 3 weeks after the transplantation. These effects were not observed in tests at 3 months. The morphochemical study revealed larger graft sizes in the animals that underwent tDCS compared with the controls and cell shift to the marginal zone of the graft. Stimulation was also shown to induce division of a part of cells at early stages of differentiation and promote active synaptogenesis.*

Conclusion. *Combining neurotransplantation and tDCS in the 6-OHDA-induced model of parkinsonism demonstrated its potential to manage both motor and non-motor symptoms. Optimizing protocols of transplantation and tDCS and evaluating their long-term efficacy and safety are required to successfully implement this method into clinical practice.*

Keywords: *Parkinson's disease; animal models; neurotransplantation; transcranial direct current stimulation*

Ethics approval. The study protocol was approved by the Ethics Committee of the Research Center of Neurology (Protocol No. 10-7/20, 27 November, 2020).

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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.

For correspondence: 5, build. 2, Obukha lane, Moscow, 105064, Russia. Brain Institute, Research Center of Neurology. E-mail: alla_stav@mail.ru. Stavrovskaya A.V.

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Опыт применения транскраниальной электростимуляции постоянным током с целью улучшения исходов нейротрансплантации у крыс с паркинсонизмом, индуцированным 6-гидроксидофамином

А.В. Ставровская[1], Д.Н. Воронков[1], И.А. Потапов[1], Д.С. Титов[2], А.С. Ольшанский[1], А.К. Павлова[1], О.С. Лебедева[3], С.Н. Иллариошкин[1]

[1]Научный центр неврологии, Москва, Россия;
[2]Московский государственный технический университет имени Н.Э. Баумана, Москва, Россия;
[3]Федеральный научно-клинический центр физико-химической медицины имени академика Ю.М. Лопухина, Москва, Россия

Аннотация

Введение. *Неуклонно растущее число пациентов с болезнью Паркинсона диктует необходимость поиска новых терапевтических подходов к её лечению. Одним из перспективных методов представляется сочетание трансплантации нейрональных предшественников, полученных из индуцированных плюрипотентных стволовых клеток, и транскраниальной электростимуляции (ТЭС).*

Цель *исследования: изучить влияние ТЭС постоянным током на состояние клеточного трансплантата и моторные симптомы паркинсонического синдрома у крыс.*

Материалы и методы. *Паркинсонический синдром у крыс Вистар моделировали односторонним интранигральным введением 6-гидроксидофамина (6-ГДА; 12 мкг на 3 мкл). Нейротрансплантацию (3×10^5 клеток в 10 мкл) осуществляли в хвостатые ядра мозга животных-моделей на стороне повреждения. ТЭС постоянным током проводили в течение 14 дней. Изменения поведения животных анализировали в тестах «открытое поле» и «сужающаяся дорожка». В морфохимическом исследовании оценивали развитие и морфологические характеристики трансплантата.*

Результаты. *Нейротрансплантация не оказала значимого влияния на поведение крыс с паркинсонизмом, однако в сочетании с ТЭС привела к увеличению двигательной активности крыс в тесте «открытое поле», по сравнению с группой крыс-моделей ($p = 0,0014$), и ослаблению у них неврозоподобного состояния ($p = 0,048$) в тестах через 3 нед после введения трансплантата. В тестах, проведённых через 3 мес, эти эффекты не наблюдались. Морфохимическое исследование выявило бóльшие размеры трансплантата у животных, подвергнутых ТЭС, по сравнению с контролем, и смещение клеток в краевую зону трансплантата. Показано также, что стимуляция провоцирует деление части клеток, находящихся на ранних стадиях дифференцировки, и способствует активному формированию синаптических контактов.*

Заключение. *Сочетание нейротрансплантации и ТЭС на 6-ГДА-индуцированной модели паркинсонизма демонстрирует потенциал данной технологии для коррекции как двигательных, так и недвигательных проявлений заболевания. Для успешной трансляции метода в клинику необходимы дальнейшая оптимизация протоколов трансплантации и ТЭС, оценка долгосрочной эффективности и безопасности.*

Ключевые слова: *болезнь Паркинсона; модели на животных; нейротрансплантация; транскраниальная электростимуляция*

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Адрес для корреспонденции: 105064, Россия, Москва, пер. Обуха, д. 5, стр. 2. Институт мозга Научного центра неврологии. E-mail: alla_stav@mail.ru. Ставровская А.В.

Для цитирования: Ставровская А.В., Воронков Д.Н., Потапов И.А., Титов Д.С., Ольшанский А.С., Павлова А.К., Лебедева О.С., Иллариошкин С.Н. Опыт применения транскраниальной электростимуляции постоянным током с целью улучшения исходов нейротрансплантации у крыс с паркинсонизмом, индуцированным 6-гидроксидофамином. *Анналы клинической и экспериментальной неврологии.* 2024;18(4):44–54.

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Introduction

Parkinson's disease (PD) is one of the most common neurodegenerative disorders that leads to severe disability [1]. PD pathogenesis is still poorly understood. Main motor symptoms of PD are known to be caused by the death of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and the subsequent nigrostriatal pathway degeneration and striatal dopamine deficiency. Nigral neurodegeneration is often linked to the accumulation of aggregated forms of the phosphorylated α-synuclein protein, which form Lewy bodies and neurites. Apart from α-synuclein accumulation, affected dopaminergic neurons are observed to have signs of mitochondrial dysfunction [2]. Numerous current studies on animal models and in patients indicate that neuroinflammation has a key role in the initiation and progression of neurodegeneration in the SNpc [3], as well as in oxidative stress development in the affected brain tissue [4, 5].

To date, there is no effective treatment that halts PD progression. Current treatment options can only alleviate numerous PD symptoms, which are classified into motor and non-motor. Non-motor manifestations tend to occur long before motor impairments, and their diagnosis can facilitate timely treatment [6, 7].

A wide range of animal models is used to elucidate causes of PD development and search for new treatment options. The most common PD model is the stereotaxic injection of neurotoxins into certain brain structures, thus avoiding their systemic effects [8].

Unilateral stereotaxic injection of 6-hydroxydopamine (6-OHDA) into the SNpc, which selectively affects dopaminergic neurons, is an optimal model to test neurotransplantation (NT) methods in PD [9]. Transplantation of dopaminergic neuronal progenitor cells into the caudate nuclei allows to replenish the dopamine deficiency in this structure, which may affect the neurodegenerative process to some extent. Transplantation of induced pluripotent stem cells (iPSC) and their derivatives, including autologous ones, reduces the recipient's immune response, eliminates ethical concerns, and has no limitation on the number of transplanted cells [10]. It should be noted that iPSC transplantation increases the percentage of progenitor cells that adapted and differentiated into healthy dopaminergic neurons. However, the issue of transplanted cell survival and function has not been fully addressed [10–13].

Transcranial direct current stimulation (tDCS) is a non-invasive and safe neuromodulation technique, which is successfully used in neurology to manage some pathologies [14]. The literature data confirm that various types of electrical stimulation can alleviate motor and non-motor symptoms of PD and also demonstrate that tDCS has a beneficial effect on differentiation and survival of transplanted cells [15, 16]. Thus, combining tDCS and NT may be a promising approach for PD therapy.

To expand the range of experiments with tDCS, we had to develop and build a multichannel electrical stimulator for small laboratory animals. The staff of the Laboratory of Experimental Nervous System Pathology and Neuropharmacology (Brain Science Institute, Research Center of Neurology) and engineers from the Bauman Moscow State Technical University jointly designed and engineered a multichannel prototype for tDCS, which operates in different modes.

The study **aims** to examine the effect of tDCS on the cell graft condition and motor symptoms of 6-OHDA-induced Parkinson's syndrome in rats that underwent NT using human iPSC derivatives.

Materials and Methods

Animals

All experiments were conducted in line with bioethical standards for proper handling of laboratory animals, including minimizing the number of animals used. The study was approved by the ethics committee of the Research Center of Neurology (Protocol No. 10-7/20 dated November 27, 2020).

Male Wistar rats ($n = 40$) 3.5 months old and weighing 300–350 g at the beginning of the experiment were taken from the Stolbovaya Branch of the Scientific Center for Biomedical Technologies of the Federal Medical-Biological Agency.

Animal procedures were conducted in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (CETS No. 170), Order of the Ministry of Health of the Russian Federation No. 119H dated April 1, 2016 "On Approval of the Rules of Laboratory Practice", and the national standard "Species-Specific Provisions for Laboratory Rodents and Rabbits" (GOST 33216-2014). The animals were kept under standard vivarium conditions, with a 12-hour light/dark cycle and ad libitum access to food and water. The rats were quarantined for 14 days before the beginning of the experiment.

Surgical Procedures

For stereotactic surgery, the animals were secured in a stereotaxic frame (Stoelting Co., RWD Life Science Co. Ltd.); the scalp was incised, and burr holes were drilled in the skull using a portable drill to access specific brain structures. A cotton gauze pad was placed between the work surface and the animal to prevent hypothermia during and after surgery.

Zoletil 100 (Valdepharm; solvent, Delpharm Tours) at 3 mg/100 g and xyla (Interchemie werken 'De Adelaar' B.V.) at

3 mg/kg were administered intramuscularly to maintain anesthesia. Atropine (Dalkhimpharm) at 0.04 mg/kg was given subcutaneously 10–15 minutes before xyla administration.

For a model of Parkinson's syndrome, the animals ($n = 32$) were injected with 6-OHDA (Sigma), a selective toxin for dopaminergic neurons, at a dose of 12 μg in 3 μL of 0.05% ascorbic acid solution in the right SNpc (Paxinos Atlas coordinates [17]: AP = –4.8; L = 1.9; V = 8.0) (Fig. 1). The same volume of the solvent was administered in the left substantia nigra. Sham-operated (control) animals ($n = 8$) were injected with the same volume of the solvent bilaterally.

On day 25 after the 6-OHDA injection, the animals ($n = 24$) underwent transplantation of neural progenitor cells into the caudate nuclei (Paxinos Atlas coordinates: AP = 1.5; L = 2.2; V = 4.5). The anesthesia technique was described above. The control animals that did not receive the neurotoxin (group C1; $n = 8$) and a part of the 6-OHDA-injected animals did not undergo transplantation; group C2 ($n = 8$) was bilaterally injected with the same volume of the normal saline into the caudate nuclei.

Cell transplantation was performed unilaterally, on the affected side. A suspension (3×10^5 cells in 10 μL of the normal saline) was injected at a constant rate for 5 minutes via the Hamilton microliter syringe into the caudate nuclei. After the injection, the syringe was left in place for 2 minutes and then slowly withdrawn. The same volume of the normal saline was injected in the left caudate nuclei. The animals received 12 mg/kg of cyclosporine one day before cell transplantation and then daily during the entire experiment.

Cell cultures were obtained in the cell biology laboratory of the Lopukhin Federal Research and Clinical Center of Physical-Chemical Medicine. Neurons were differentiated from iPSCs, which were derived from skin fibroblasts and obtained from a healthy donor (a 60-year-old man without any neurological pathology) after the informed consent. The iPSC line IPSRG4S was characterized according to generally accepted standards [18]. The cell line has a normal karyotype. IPSRG4S pluripotency was confirmed at molecular and functional levels. The iPSCs were directed to differentiate into early neuronal progenitors that were later differentiated into ventral mesencephalic neuronal progenitors, which were used for transplantation on day 24 of differentiation. The method of IPSC differentiation and media composition are available upon request.

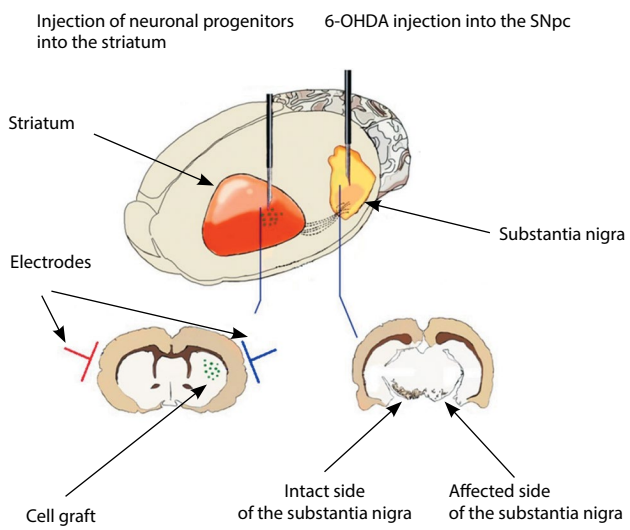

Fig. 1. Schematic representation of modeling Parkinson's syndrome and subsequent NT.

The 6-OHDA-injected rats that underwent NT were divided into 3 groups (8 animals each). The rats from group T + tDCS underwent tDCS with the new stimulator; group T + S underwent sham stimulation and sedation, and group T had neither stimulation nor sedation.

Bilateral tDCS

tDCS began on day 5 after the transplantation of ventral mesencephalic neuronal progenitors into the dorsolateral caudate nucleus.

The designed autonomous electrical stimulator is a microprocessor-based programmable device, which can be considered a generator of various stable current types used for tDCS in laboratory animals. The device consists of a programmable master oscillator, a multichannel voltage-to-current converter, a power supply, and control hardware. The master oscillator, based on a microprocessor of the selected series, uses software to generate a pulse-code modulation data stream, describing the current's waveform, amplitude, and time characteristics (frequency and duration). All stimulation parameters are set via the control panel and displayed on the screen.

Data are transferred between the device blocks and circuit elements via a common I2C interface, an industry-standard solution with low cost but sufficient speed and reliability. Then the data stream through the galvanic isolation based on ADuM microcircuits goes to the MCP4725 digital-to-analog converter. Galvanic isolation is needed to ensure the electrical safety of the device and improve noise immunity.

The digital-to-analog converter converts the data stream into an analog signal, a voltage that varies with the data stream and is used as the control signal for the stable current generator. Then the signal is fed to the input of the stable current generator, designed to form the actuating signal, a time-varying current of the parameters set by an experimenter.

The use of a microprocessor enabled to flexibly change the stimulation current parameters according to the experiment aims.

Device specifications:
- up to 16 channels;
- frequency range of 0 (DC) to 80 Hz;
- current range of 0 to 1 mA;
- various pulse waveforms, including rectangular, triangular, sinusoidal, and noise-like signals.

Prior to tDCS the rat was immobilized by the intramuscular injection of 0.5 mL/kg of 0.5% dexmedetomidine solution (Dexdomitor, Orion Pharma) and placed on a pad with thermal insulation properties to prevent hypothermia. We used a 0.5% solution of hypromellose (Iskusstvennaya sleza, Firn M) to prevent damage to the cornea. The fur from the temporal regions was carefully removed to improve adhesion and reduce electrical resistance; the skin of the temples was degreased, and a part of MedTab electrodes (23×34 mm, Ceracarta) was symmetrically placed on the temporal regions so that an imaginary line through their centers intersected the geometric center of the cell graft (Fig. 2). The anode was placed on the contralateral side of the graft and the cathode on the ipsilateral side. Using a stimulating device, a direct current of 0.5 mA was applied to the electrodes for 20 minutes; then the electrodes were disconnected, and the residual adhesive layer of the electrodes was removed from the temporal regions with water.

The rat was returned to its home cage and 30 minutes later injected with 0.2 mL/kg of a 0.5% solution of atipamezole (Antisedan, Orion Corporation) intramuscularly to accelerate recovery from sedation. The time between the end of the stimulation session and the atipamezole injection is needed to prevent rats from scratching the skin under the electrodes, which may be caused by paresthesia at the electrode sites and is a very common adverse effect [19].

The stimulation sessions were performed once a day at the same time for 14 consecutive days. During the first stimulation procedures, the temperature of the electrodes and surrounding skin was monitored by an infrared pyrometer (Raytek).

Fig. 2. Simultaneous tDCS in 4 rats.

In sham tDCS, all procedures were performed similarly to those described above, but no electric current was applied to the electrodes.

Behavioral effects

Behavioral effects of exposure to a toxicant followed by NT and tDCS were assessed by changes in the rats' motor activity during open field (OF) and beam-walking (BW) tests. The OF test duration was 3 minutes, and the test was performed three times: before cell injection, at 3 weeks, and at 3 months. Rat behavior was recorded using the ANY-maze video tracking system (Stoelting Inc.).

In the BW test, the animal had to walk across an elevated beam from one end to the home cage. We recorded the walking time and the percentage of slips in relation to the total number of steps to cross the beam. In this experiment, we also assessed the psychoemotional state of the rats with an anxiety scale [20, 21]. We recorded non-standard behavioral activities that could be attributed to external signs of anxiety-related behaviors: compulsive head turns, chewing movements, active sniffing and licking of the beam, circling, backward gait, grooming, diaphragm contractions, ptosis, etc. The rats were trained to perform the BW test for 3 days, 2 sessions per day, 1 hour apart, before stereotactic brain surgery. The maximum test time was 100 seconds.

Factorial analysis of variance (ANOVA) was used to determine the statistical significance of differences, and Fisher's post hoc test was employed to compare the groups. The differences were considered significant at $p<0.05$. The data are presented as the mean ± standard error of the mean.

At 3 months after NT and at the end of physiologic examination, half of the rats from each group were decapitated, and the brains were extracted for immunohistochemistry.

Immunohistochemistry and Morphometry

The brain specimens of 4 rats from each group were used in the immunomorphologic study. For morphologic evaluation of the graft 3 months after the cell injection, the animals were decapitated. The brains were fixed for 24 hours in 10% formalin. Frozen frontal sections (10 μm thick) were used for the study. Antigen retrieval was performed by heating in citrate buffer (0.01 M, pH 6.0). The sections were incubated with primary antibodies for 18 hours at room temperature, and corresponding secondary antibodies labeled with Atto 488 or Atto 555 fluorochromes (Invitrogen) were used to detect binding. The sections were further stained with DAPI. Antibodies against human nuclear antigen (HNA) and species-specific antibodies against human neuron-specific enolase (NSE) were used to de-

tect graft cells. Furthermore, antibodies against synaptophysin (SYP) were used to assess graft integration. Transplantation outcomes were previously characterized using an expanded panel of neuronal and glial marker proteins [13].

On the frontal sections using a ×4 objective, we estimated the cross-sectional area of the graft in the striatum by NSE detection. We selected at least 3 sections that showed the needle track at the full depth of insertion. The NIS-Elements software was used to calculate the area in the images.

The data are presented as median and interquartile ranges. The Mann–Whitney test was used to compare the groups.

Results

All the animals tolerated the surgical procedures and tDCS well, and their condition was satisfactory throughout the study. Regular daily examinations by a veterinarian did not reveal any changes in bowel and bladder functions, porphyrin discharge around the eyes and nose, or alopecia. No neoplasms were found during autopsy after the decapitation.

Behavioral tests were performed before the administration of ventral mesencephalic neuronal progenitors (test 1: 25 days after the 6-OHDA administration in the SNpc), at the end of the tDCS course (test 2: 3 weeks after NT), and at 3 months after NT of ventral mesencephalic neuronal progenitors (test 3).

Fig. 3 shows the distance traveled in the OF test by the control animals from groups C1 and C2 that did not receive a cell graft. 6-OHDA administration resulted in a statistically significant decrease in motor activity, which was observed in all the tests: 13.990 ± 0.881 and 6.387 ± 1.112 (ANOVA, p (pA) = 0.0005) in test 1, 13.469 ± 1.572 and 6.439 ± 1.406 (pA = 0.0007) in test 2, and 13.076 ± 1.406 and 6.404 ± 1.575 (pA = 0.0013) in test 3 in groups C1 and C2, respectively.

Fig. 4 shows changes in motor activity of the model rats following neuronal progenitor transplantation into the dorsolateral caudate nucleus. It should be noted that by the time of test 2, a part of the rats (group T+tDCS) had undergone a tDCS course. Fig. 4 shows that locomotor activity remained at the level recorded before cell administration in group T+S receiving dexdomitor for sham tDCS (5.946 ± 1.011 and 5.233 ± 1.229; pA = 0.9436), in contrast to the significantly decreased in the rats without sedation (3.006 ± 0.601 and 6.996 ± 1.178; pA = 0.0227). In the rats after tDCS, the distance traveled in the OF test more than doubled: 14.069 ± 1.094 and 5.635 ± 1.511 (pA = 0.0014). The motor activity at 3 months after NT remained unchanged in all the groups with the graft compared with the test 1 results.

The BM test at 3 weeks after NT also revealed significant differences between groups C1 and C2, most of which were the refusal of the 6-OHDA-injected rats to walk along the beam and

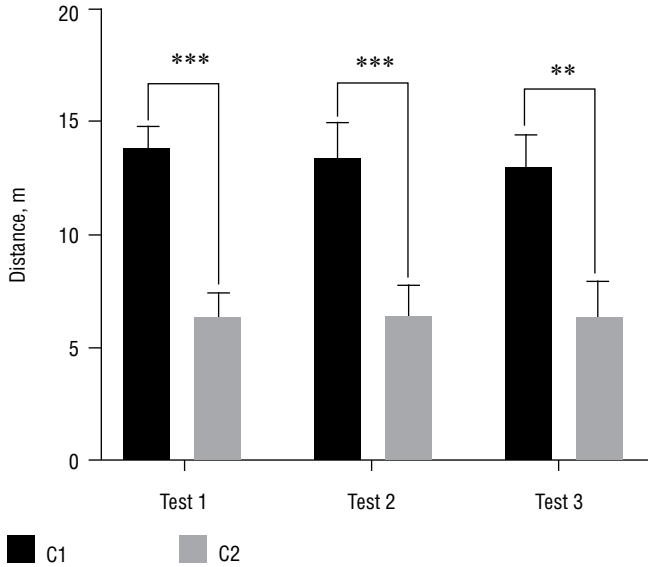

Fig. 3. Motor activity assessment by the OF test in the rats. *p_A < 0.05 compared with group C2.

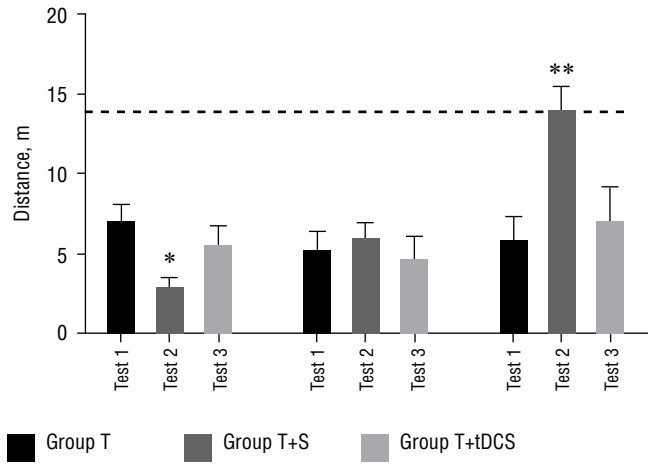

Fig. 4. Distance traveled in the OF test by the model animals after NT. *p_A < 0.05 compared with test 1.

distinct (pA = 0.01) signs of anxiety-related behaviors (Fig. 5, *A*). Therefore, it was not possible to process the numerical values of the number of stumbles using statistical methods. The ventral mesencephalic neuronal progenitor administration and tDCS course had no effect on the rats' movement along the beam. However, while groups T and T+S exhibited anxiety-related behaviors, which scores were significantly different compared with group C1 (8.00 and 7.83 vs 2.67 scores; pA = 0.0005 and pA = 0.001, respectively), group T+tDCS had significantly lower scores and no statistically significant differences with group C1 (4.71 and 2.67; pA = 0.139). This parameter was also significantly different compared with groups T and T+S (pA = 0.017 and pA = 0.029, respectively). In test at 3 months after NT, the difference in these parameters between the groups leveled off, which is consistent with the data of the OF test (Fig. 5, *B*).

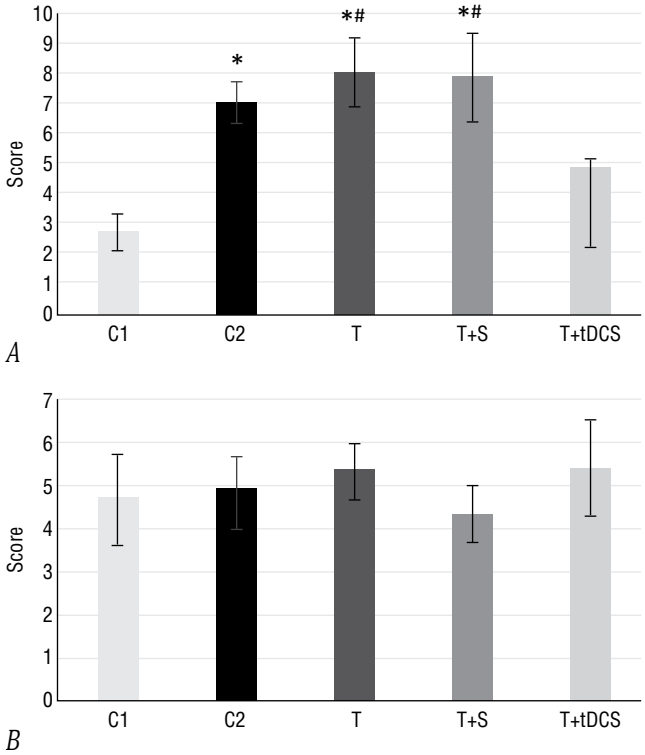

Fig. 5. Anxiety-related behavior score in the BW tests at 3 weeks (*A*) and 3 months (*B*) after NT.
***$p_A < 0.05$ compared with group C1; #$p_A < 0.05$ compared with group T+tDCS.**

Previous morphologic studies of grafts have shown decreased staining for tyrosine hydroxylase on the side of 6-OHDA injection [12, 13], indicating damage to SNpc neurons. Also, by month 3, 3% to 5% of dopaminergic neurons were detectable in the graft, and we did not observe migration of cells expressing mature neuronal markers outside the graft area.

The animals subjected to tDCS had larger graft sizes compared with the controls (Fig. 6). Previously we showed the zonal structure of grafts in animals without tDCS exposure [13] with predominant localization of NSE+-cells (mature neurons) in the central zone and formation of glial sheath around the graft. Due to tDCS the graft morphology changed: after the stimulation there was a shift of NSE staining to the marginal zone of the graft, which was located outward. The graft size in the striatum was significantly greater ($p = 0.002$, Mann-Whitney test) after tDCS. In the control group, the median area of NSE^+ staining was 1.695 [1.45; 1.89] mm^2, and it was 4.04 [3.08; 6.03] mm^2 as a result of tDCS. The central regions in the group after tDCS comprised HNA^+ cells with low NSE expression. The stimulation was likely to provoke division of some cells in the early stages of differentiation, which should be further investigated. The detection of SYP may indicate the synaptogenesis in the graft by month 3. We have previously shown an increase in SYP expression as neurons mature [13]. The more pronounced staining for SYP

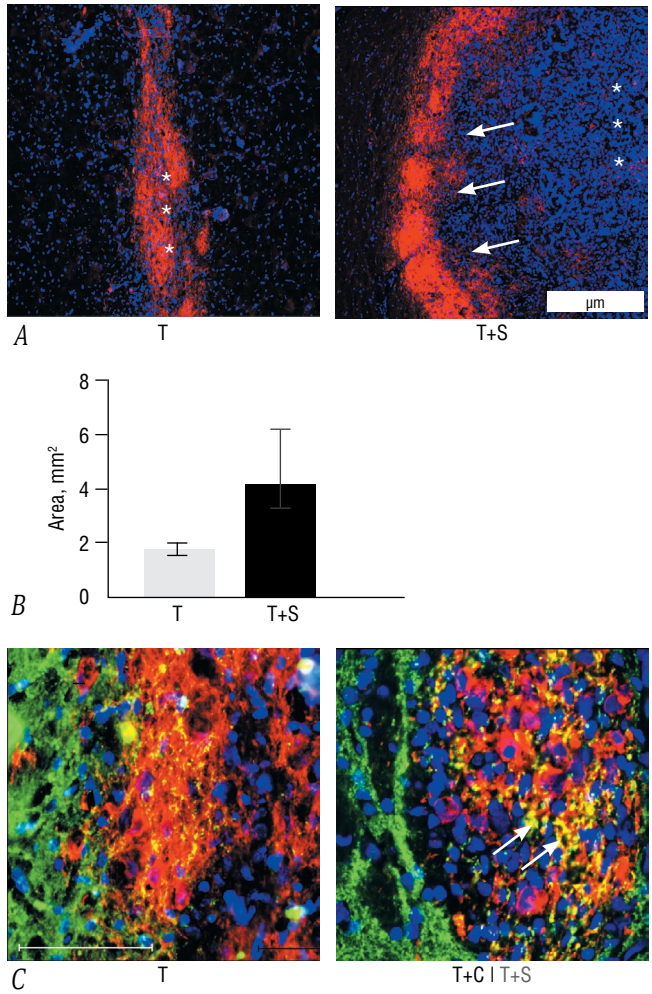

Fig. 6. Localization of transplanted neurons in the control group (day 24 of differentiation) and after tDCS at 3 months following the transplantation.
***A* – shift of NSE^+ cells (shown in red) to the marginal zone of the graft (arrows), the central zone is indicated by asterisks; *B* – graft size; *C* – increase in SYP (shown in green) and NSE (shown in red) colocalization areas caused by tDCS (arrows). Cell nuclei were counterstained with DAPI (shown in blue).**

and overlap with NSE+ structures may reflect the effect of tDCS on the formation of synaptic contacts with transplanted neurons.

Thus, the morphologic study showed the effect of tDCS on development and morphologic characteristics of the graft and cell migration within the graft area. No pathologic changes in the structures surrounding the graft were detected. tDCS appears to have an effect on both differentiation and migration as well as integration of graft neurons, which should be further studied.

Discussion

NT is one of the promising therapies for PD. Alleviation of motor symptoms in PD is its main expected behavioral ef-

fect. This effect has been mostly shown in studies on NT of embryonic ventral mesencephalic dopaminergic neurons [22, 23]; however, the injection of such cells raised ethical concerns and caused severe graft-induced dyskinesias. Another source of cell grafts with autologous dopaminergic neurons is iPSCs obtained via reprogramming fibroblasts using expression of peptide pluripotency factors in them [24], followed by in vitro differentiation of iPSCs into neurons according to different protocols [11]. The following parameters serve as criteria of morphofunctional correspondence of dopaminergic neurons differentiated from iPSCs to native dopaminergic neurons: survival of transplanted neurons, intensity of neurite growth from the graft, formation of a diffuse network of dopaminergic terminals in the striatum, dopamine release, their bioelectrical activity, as well as recovery of lost motor functions in animals with a PD model [25]. Our studies using a similar differentiation protocol have previously shown the development of dopaminergic neurons and the formation of their outgrowths in the graft by month 3-6 [12, 13]. Transplantation of neuronal progenitors in animals with PD models has shown certain advantages over fetal cell transplantation, but the positive results achieved are still not well reproducible [25, 26] due to a number of factors: the type and quality of transplanted cells, the PD model used, and individual characteristics of recipient animals. Optimizing these factors will improve treatment efficacy and stability of behavioral effects.

An independent promising therapeutic approach in neurodegenerative diseases is the use of non-invasive neuromodulation methods [14, 27]. They include various forms of low-intensity transcranial electrical stimulation; direct current stimulation is the most studied, and its effects on neuroplasticity in the motor cortex are polarity-dependent. In this study, we focused on cathodal polarity, in which the resting membrane potential is hyperpolarized (in contrast to anodal polarity, in which the resting membrane potential is depolarized) [28]. Cathodal tDCS using standard protocols reduces cortical excitability and can induce homosynaptic long-term depression in case of sufficiently long stimulation duration. Apart from duration and intensity, the stimulation repetition is a crucial factor in cathodal tDCS efficacy, affecting the duration of the neuroplastic effect. The mechanisms underlying the beneficial effects of tDCS are not yet fully understood; animal models, especially those involving rodents, facilitate their studying, testing the method safety, and optimizing stimulation parameters [29–31]. When selecting stimulation parameters, we were guided by the literature data because we have not previously conducted such study [15, 16, 32].

tDCS was shown to have a beneficial effect on differentiation and survival of transplanted cells [15, 16]. In our previous studies [12, 13] we found that functional maturation of transplanted neurons occurred within 3 months after transplantation, and the greatest changes in the expression of cell differentiation proteins were observed within 1 month and continued up to 3 months after transplantation, which determines the possible time frame of the tDCS effect on the graft in terms of neuronal maturation improvement. It should be noted that in some experiments [12], the graft contained a mixed glioneuronal culture, and part of the cells yielded an astrocyte population. Researchers discuss possible mechanisms for the tDCS effect on astrocytes [33], which may have a significant impact on both the host astroglia response in transplantation and donor astrocytes when mixed cultures are used. Thus, combining tDCS and NT may be a promising approach for PD therapy.

The observed increase in graft size and changes in graft morphology may indicate the direct effect of tDCS on the transplanted cells, their maturation and integration into the recipient's striatum. That being said, the tDCS effects on the graft behavior and development may be caused by a number of factors: the effect of the striatum, neocortex, and other brain structures, involved in the motor activity regulation in animals, on neurons via changing the balance between excitatory and inhibitory inputs [34], the effect on glial cells, including anti-inflammatory effects [35], increased expression of BDNF [36] involved in plastic changes in the nervous system, etc.

Although animal models are a powerful tool in identifying neurobiological mechanisms of tDCS action, finding a current generator, which is easy to use and allows for a wide range of stimulation parameters, can be challenging and/or expensive [37]. In most cases, Russian researchers use foreign devices, for example, Alpha-Stim (Electromedical Products International, Inc.), when studying the effects of tDCS. Such devices are designed for tDCS procedures to treat anxiety, insomnia, depression, and pain. They are effective, safe, easy to use, and received necessary regulatory approvals. However, some design features limit their use in laboratory setting: the waveform of the generated pulses, frequency range, current range, and pulse duration.

Experimental conditions for studying transcranial electrical stimulation require a much wider range of stimulating current parameters: eg, a current in the form of sinusoidal pulses with a constant component or a noise-like signal. The technical limitations of transcranial electrical stimulation devices dictated the need to develop an original device designed primarily for laboratory use and free from the disadvantages of existing and commercially available devices. Specialists from the Bauman Moscow State Technical University designed and engineered a prototype of such stimulator. The Beta-Stim device is a programmable stable current generator with the frequency range from DC to 80 Hz, an arbitrary (set by the experimenter) form of the signal, and a current range from 1 μA to 1 mA. It was designed for experiments on small rodents. The device is easy to operate. It is built with many freely available Russia-produced components, free from license and patent restrictions.

In this study, 6-OHDA-induced Parkinson's syndrome was modeled in Wistar rats. This model is most convenient for studying the potential of NT of dopaminergic neuronal progenitors into the caudate nuclei of the rat brain. NT is known to temporarily worsen symptoms in the early postoperative period [38], which seems to explain the decreased motor activity in group T detected 3 weeks after reoperation. The motor activity later returned to the preoperative levels. The maintained level of motor activity in group T + S might be linked to the anti-inflammatory effect of dexdomitor used for sedation. NT was combined with a tDCS course. We observed positive effects of tDCS on motor activity and emotional state in group T + tDCS. The rats from groups T + S, C2, and T showed signs of anxiety-related behavior, which allows to rule out the possibility of a dexdomitor effect on this parameter. Behavioral testing 3 months after NT did not reveal any differences between the groups, which may indicate that our chosen mode for tDCS has short-term effects.

Conclusion

The model rats with PD tolerated well tDCS and transplantation of dopaminergic neuronal progenitors using the developed specialized laboratory stimulator.

The findings of physiologic and morphochemical studies indicate the tDCS effects on graft development and structure, as well as on changes in motor and non-motor symptoms in rats after NT.

Thus, the combination of NT and tDCS in PD models, particularly those induced by the 6-OHDA injection into the SNpc, demonstrates the potential to manage both motor and non-motor symptoms. However, further optimizing protocols of transplantation and tDCS and evaluating their long-term efficacy and safety are required to successfully implement this method into clinical practice.

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Information about the authors

Alla V. Stavrovskaya – leading researcher, Head, Laboratory of experimental pathology of the nervous system and neuropharmacology, Brain Institute, Research Center of Neurology, Moscow, Russia, https://orcid.org/0000-0002-8689-0934
Dmitry N. Voronkov – senior researcher, Laboratory of neuromorphology, Brain Institute, Research Center of Neurology, Moscow, Russia, https://orcid.org/0000-0001-5222-5322
Ivan A. Potapov – junior researcher, Laboratory of experimental pathology of the nervous system and neuropharmacology, Brain Institute, Research Center of Neurology, Moscow, Russia, https://orcid.org/0000-0002-7471-3738
Daniil S. Titov – postgraduate student, Bauman Moscow State Technical University, Moscow, Russia, https://orcid.org/0000-0002-3290-0367
Artem S. Olshansky – senior researcher, Laboratory of experimental pathology of the nervous system and neuropharmacology, Brain Institute, Research Center of Neurology, Moscow, Russia, https://orcid.org/0000-0002-5696-8032
Anastasiia K. Pavlova – laboratory research assistant, Laboratory of experimental pathology of the nervous system and neuropharmacology, Brain Institute, Research Center of Neurology, Moscow, Russia, https://orcid.org/0009-0006-5653-5524
Olga S. Lebedeva – senior researcher, Laboratory of cell biology, Lopukhin Federal Research and Clinical Center of Physical & Chemical Medicine, Moscow, Russia, https://orcid.org/0000-0003-0767-5265
Sergey N. Illarioshkin – Dr. Sci. (Med.), Prof., RAS Full Member, Director, Brain Institute, Deputy Director, Research Center of Neurology, Moscow, Russia, https://orcid.org/0000-0002-2704-6282

Authors' contribution. *Alla V. Stavrovskaya* – conceptualization, data analysis, conducting research, supervision; *Dmitry N. Voronkov* – conceptualization, data analysis, conducting research; *Ivan A. Potapov* – conceptualization, data curation; *Daniil S. Titov* – methodology, model creation, data curation; *Artem S. Olshansky*–conducting research; *Anastasiia K. Pavlova* – conducting research, data analysis; *Olga S. Lebedeva* – conceptualization, methodology; *Sergey N. Illarioshkin* – supervision.

Информация об авторах

Ставровская Алла Вадимовна – в. н. с. лаб. экспериментальной патологии нервной системы и нейрофармакологии Института мозга Научного центра неврологии, Москва, Россия, https://orcid.org/0000-0002-8689-0934
Воронков Дмитрий Николаевич – с. н. с. лаб. нейроморфологии Института мозга Научного центра неврологии, Москва, Россия, https://orcid.org/0000-0001-5222-5322
Потапов Иван Александрович – м. н. с. лаб. экспериментальной патологии нервной системы и нейрофармакологии Института мозга Научного центра неврологии, Москва, Россия, https://orcid.org/0000-0002-7471-3738
Титов Даниил Сергеевич – аспирант МГТУ им. Н.Э. Баумана, Москва, Россия, https://orcid.org/0000-0002-3290-0367
Ольшанский Артём Сергеевич – с. н. с. лаб. экспериментальной патологии нервной системы и нейрофармакологии Института мозга Научного центра неврологии, Москва, Россия, https://orcid.org/0000-0002-5696-8032
Павлова Анастасия Кирилловна – лаборант-исследователь лаб. экспериментальной патологии нервной системы и нейрофармакологии Института мозга Научного центра неврологии, Москва, Россия, https://orcid.org/0009-0006-5653-5524
Лебедева Ольга Сергеевна – с. н. с. лаб. клеточной биологии ФКНЦ физико-химической медицины им. акад. Ю.М. Лопухина, Москва, Россия, https://orcid.org/0000-0003-0767-5265
Иллариошкин Сергей Николаевич – д. м. н. профессор, академик РАН, зам. директора Научного центра неврологии по научной работе, директор Института мозга Научного центра неврологии, Москва, Россия, https://orcid.org/0000-0002-2704-6282

Вклад авторов: *Ставровская А.В.* – создание концепции исследования, анализ данных, проведение исследования, руководство научно-исследовательской работой; *Воронков Д.Н.* – создание концепции исследования, анализ данных, проведение исследования; *Потапов И.А.* – создание концепции исследования, курирование данных; *Титов Д.С.* – разработка методологии, создание моделей, курирование данных; *Ольшанский А.С.* – проведение исследования; *Павлова А.К.* – проведение исследования, анализ данных; *Лебедева О.С.* – создание концепции исследования, разработка методологии; *Иллариошкин С.Н.* – руководство научно-исследовательской работой.

A Genetic Perspective on Ischemic Stroke: Recent Advances and Future Directions

Praveen Kumar Chandra Sekar, Ramakrishnan Veerabathiran

Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Kelambakkam, Tamil Nadu, India

Abstract

Objective. *This narrative review aimed to explore the multifaceted nature of ischemic stroke (IS) and its underlying genetic factors, emphasize the role of genetics in early detection and prevention, and acknowledge the complex influences on stroke prevalence across various countries.*

Methods. *An extensive overview of the causes, mechanisms, and genetics of IS was conducted by reviewing several studies and recent findings. The role of specific genes in monogenic stroke disorders, implications of polygenic influences, recent advances in genetic evaluation, and methods for early IS detection were synthesized and discussed.*

Results. *IS was influenced by genetics, underlying medical conditions, and lifestyle. Specific genes, including NOTCH3, HTRA1, COL3A1, and mtDNA, are involved in monogenic stroke syndromes and predominantly affect younger populations. Polygenic disorders, studied using genome-wide association study and sequencing techniques, play a prominent role in susceptibility to IS. Genetic evaluation has become instrumental in risk prediction, influencing clinical practices and potential therapeutic interventions. Early detection methods, such as enhanced imaging techniques and blood biomarkers, are crucial for managing IS outcomes.*

Conclusion. *Ischemic stroke is a complex disorder with a significant global impact. Understanding its genetic basis promises to improve early detection and effectively establish preventative measures. Although genetic evaluation and innovative detection techniques offer promise, focusing on lifestyle modifications and managing underlying health conditions remains paramount for reducing the incidence and severity of IS. Continuous research and technological advancements are essential for developing personalized medical approaches and improving global healthcare strategies.*

Keywords: *ischemic stroke; genetics; therapeutic; pathways; pathophysiology*

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For correspondence: 603103, India, Tamil Nadu, Kelambakkam, Chettinad Academy of Research and Education.
E-mail: rkgenes@gmail.com. Veerabathiran R.

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Генетические аспекты ишемического инсульта: последние достижения и направления исследований

Praveen Kumar Chandra Sekar, Ramakrishnan Veerabathiran

Больница и научно-исследовательский институт Четтинада, Научно-образовательная академия Четтинада, Келамбаккэм, Тамилнад, Индия

Аннотация

Цель *данного нарративного обзора – описать многофакторность ишемического инсульта (ИИ) и генетические факторы его развития, подчеркнуть роль генетики в ранней диагностике и профилактике ИИ, а также осветить комплексное влияние на распространённость инсульта в разных странах.*

Проведён обзор исследований и последних данных для всестороннего освещения причин, механизмов и генетических аспектов развития ИИ. В статье обобщаются и обсуждаются роль специфических генов в развитии моногенных заболеваний, связанных с ИИ, последствия полигенных заболеваний, последние достижения генетических исследований и методы ранней диагностики ИИ.

На развитие ИИ влияют генетические факторы, сопутствующие заболевания и образ жизни. Специфические гены (NOTCH3, HTRA1, COL3A1) и гены митохондриальной ДНК задействованы в моногенных заболеваниях, связанных с ИИ и поражающих преимущественно молодых людей. Полигенные заболевания, изученные посредством полногеномного поиска ассоциаций и секвенирования, играют важную роль в предрасположенности к развитию ИИ. Генетические исследования становятся эффективными инструментами прогнозирования рисков, влияя на клиническую практику и потенциальные терапевтические вмешательства. Такие методы ранней диагностики, как специализированные модальности нейровизуализации и исследование биомаркеров крови, играют ключевую роль в улучшении исходов ИИ.

Заключение. *ИИ – комплексное заболевание, несущее значительное глобальное бремя. Понимание генетических факторов, влияющих на его развитие, поможет улучшить раннюю диагностику и эффективно внедрить профилактические меры. Несмотря на то что генетические исследования и инновационные методы диагностики вселяют надежду, коррекция образа жизни и лечение основных заболеваний сохраняют своё первостепенное значение в снижении частоты и тяжести ИИ. Непрерывная исследовательская деятельность и технологические достижения – ключ к разработке индивидуальных подходов к лечению и улучшению глобальных стратегий здравоохранения.*

Ключевые слова: *ишемический инсульт; генетика; лечение; сигнальные пути; патофизиология*

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

Адрес для корреспонденции: 603103, India, Tamil Nadu, Kelambakkam, Chettinad Academy of Research and Education. E-mail: rkgenes@gmail.com. Veerabathiran R.

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Introduction

Stroke is a multifaceted disorder with a range of contributing factors, including lifestyle choices such as smoking, underlying conditions such as dyslipidemia, hypertension, and diabetes; and a genetic component [1]. The most prevalent form of stroke is ischemic stroke (IS), which occurs when the blood supply to the brain is obstructed, often due to blood clots. It is the second leading cause of death and disability, with a substantial global impact [2]. Particularly in economically disadvantaged nations such as India, IS exerts a heavy toll [3]. Worldwide, stroke remains a significant cause of mortality and morbidity, with staggering statistics indicating that 1.5 million lives are claimed by stroke annually, while 2.5 million new cases are documented in China alone [4]. Among these, IS was predominant, accounting for 62.4% of all stroke cases.

One notable characteristic of IS is the development of arterial plaques, which may result in severe complications, including heart attack and stroke. However, genetic factors have often been overlooked. However, they are crucial for human development, particularly when discussing early-onset stroke [5]. Specific genetic abnormalities follow Mendelian inheritance patterns, leading to monogenic diseases often associated with distinct and uncommon stroke subtypes [6]. IS comprises various subtypes, including small-vessel disease, cardioembolism, and large-vessel atherosclerotic IS [7]. Among these subtypes, large artery stroke exhibits the highest hereditary factor, accounting for nearly 40% of cases, with cardioembolic IS closely trailing in 33% of the ISs.

In contrast, only 16% of strokes originating from small vessel disease are hereditary [8, 9]. Genetic factors are more likely to play a role in the development of diseases affecting both small and large blood vessels rather than being significant contributors to the causes of cardioembolic IS. However, the prevalence of single-gene disorders related to stroke is estimated at approximately 1%. These disorders predominantly affect younger individuals. However, the accuracy of these data cannot be guaranteed. Several monogenic disorders involve specific genes, including *NOTCH3*, *HTRA1*, *COL3A1*, *mtDNA*, and *TREX1*, as well as interleukins (IL), such as tumor necrosis factor-α (TNF-α), IL-1β, and IL-6, all of which can contribute to the occurrence of IS. As research advances, polygenic disorders are emerging as a significant area of interest in IS genetics, accounting for approximately 38% of all ISs [10]. Studies conducted in previous years has provided valuable insights, but current research focuses on identifying modern risk factors and genetic markers that can facilitate early stroke risk detection and implementing preventive strategies.

Furthermore, when considering age-standardized mortality rates due to IS for the mentioned countries between 2020 and 2030, it is evident that some countries, such as South Africa (2.64%), Cyprus (4.16%), China (2.19%), Portugal (7.18%), Ethiopia (1.83%), Mongolia (2.38%), Ecuador (5.43%), Cabo Verde (8.17%), and Mauritius (10.90%) have experienced an increase in mortality rates, while Comoros (9%) has seen a decrease [11]. These trends highlight the intricate interplay of factors, such as evolving lifestyles, disparities in socioeconomic status, healthcare accessibility, and demographic shifts in shaping the prevalence of IS in different regions.

This narrative review **aimed** to explore the multifaceted nature of IS and its underlying genetic factors, emphasize the role of genetics in early detection and prevention, and acknowledge the complex influences on stroke prevalence across various countries.

Causes and mechanisms of ischemic stroke

Thrombotic IS is a common subtype of stroke that arises when a clot forms within an artery inside the brain. This blockage can be triggered by a thrombus or an embolus [12]. A significant contributor to thrombotic IS is atherosclerosis, which is characterized by plaque buildup along blood vessel walls. The rupture of this plaque can lead to the formation of a blood clot at the rupture site, culminating in thrombotic IS [13].

On the other hand, embolic ISs arise when a blood clot or other debris (embolus) forms elsewhere in the body, typically in the heart or major arteries. These emboli can travel through the bloodstream and eventually reach the brain, where they may become lodged in a small blood vessel, impeding blood flow and causing embolic IS. Familiar sources of emboli include blood clots originating in the heart, often associated with conditions such as atrial fibrillation or detachment of plaque particles from larger arteries [14].

In IS, reactive oxygen species (ROS) are responsible for the cerebral damage because they can deplete adenosine triphosphate and interfere with the ability to produce energy [15]. It involves a series of biochemical reactions that lead to neuronal death, disintegration of cell membranes, and ionic imbalance, which causes cell depolarization and glutamate release. These reactions are caused by disrupted levels of Ca^{2+}, K^{+}, and Na^{+} [16]. Excess levels of glutamate trigger the activation of N-methyl-D-aspartate receptors. This activation can be detrimental to cell health and cause damage to the central nervous system.

Furthermore, it can also lead to the release of more glutamate, which leads to excitotoxicity and activates the death signalling pathway through oxidative and nitrosative stress, mitochondrial dysfunction, and blood–brain barrier dysfunction. A subsequent cascade of injuries occurs as a result of ischemia-induced injury [17]. Figure 1 illustrates the IS mechanism.

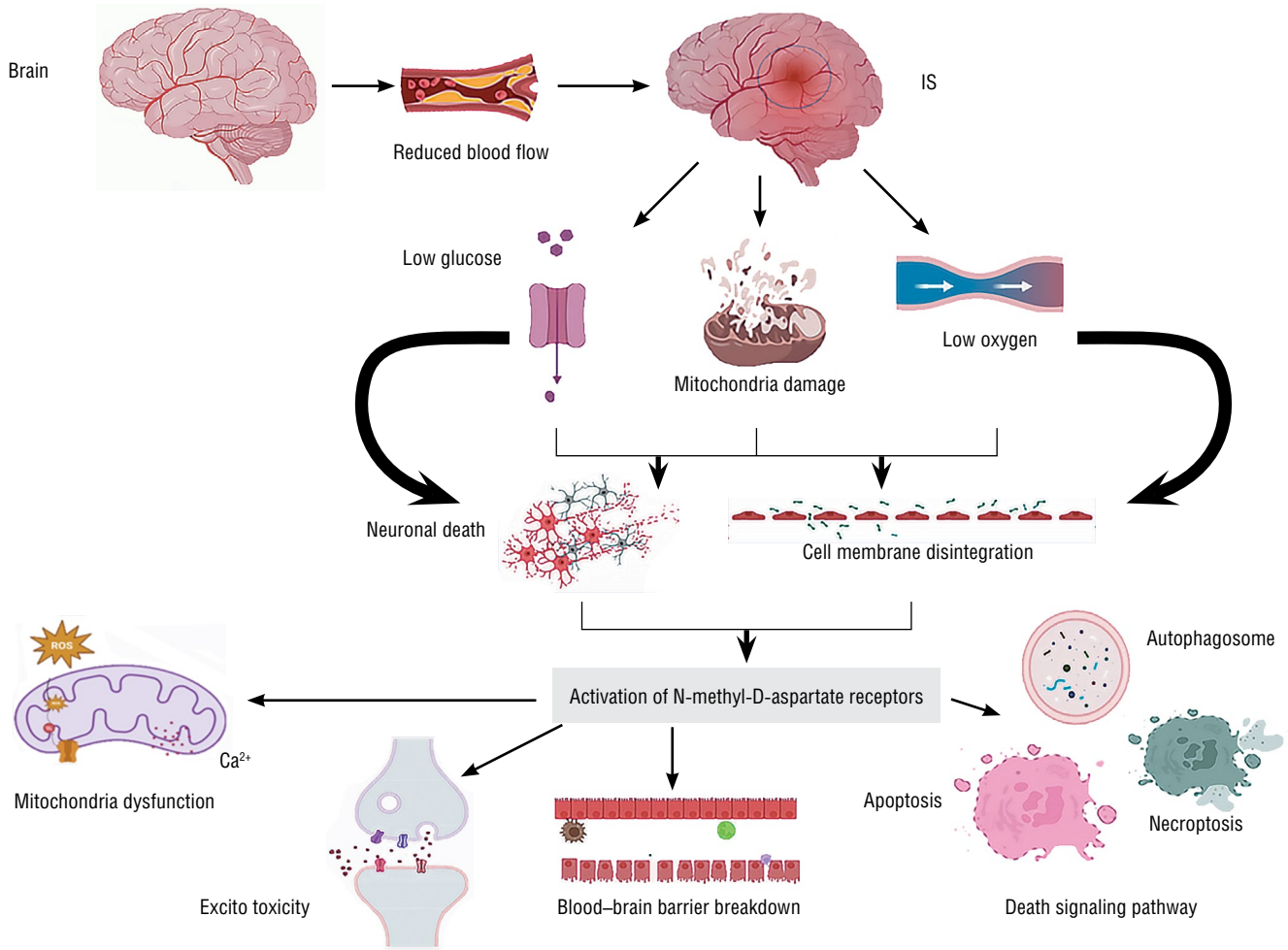

Fig. 1. Mechanism of IS.

Genetics of ischemic stroke

Notably, in cases of early onset, genetic factors are important in the development of IS. Several genes have been linked to IS, including *NOTCH3*, *HTRA1*, *COL3A1*, and some genes of mitochondrial DNA (*mtDNA*). The hereditary form of IS known as CADASIL is associated with the *NOTCH3* gene. Additionally, IS has been linked to mutations in *HTRA1*. Moreover, TNF-α polymorphisms and a range of tandem repeats in the IL-1 receptor antagonist gene have been linked to an increased risk of IS. Moreover, there is evidence of a genetic predisposition to small-vessel IS. Multiple environmental and genetic factors contribute to the development of IS. However, the precise genetic mechanisms underlying IS and its subtypes remain to be elucidated.

Monogenic disorders

Monogenic disorders are a class of hereditary illnesses caused by mutations in a single gene. These disorders are often referred to as Mendelian. Inheritance of a mutant or faulty copy of a particular gene from one or both parents often defines these illnesses.

NOTCH 3

NOTCH3 (Notch Receptor 3) is a protein-coding gene located in Chr19 with a 33-exon count and codes for 2321 amino acids. Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), a genetic disorder, is the most frequent monogenic trigger for IS. Autosomal dominant inheritance and *NOTCH3* gene mutations are the causes of CADASIL [18]. Consequently, the walls of the tiny blood vessels in the brain accumulate the protein NOTCH3. This can cause vessels to become narrow and stiff, leading to an increased risk of IS. It is diagnosed using molecular genetic testing or skin biopsy, with immunohistochemistry and electron microscopy revealing the typical features [19]. The NOTCH3 protein is a transmembrane protein with a single pass. Most vascular smooth muscle cells express this protein. The NOTCH3 protein comprises an extracellular domain (NOTCH3ECD) and an intracellular

domain. The protein undergoes three cleavage stages when a specific ligand (either Jagged or Delta) binds to NOTCH-3ECD. The intracellular domain eventually reaches the nucleus, which functions as a transcription factor. *NOTCH3* mutations in CADASIL, a genetic disorder affecting the brain and blood vessels, can lead to an unequal number of cysteine residues in the mutant EGFr proteins. This typically results in 5 or 7 cysteine residues that can significantly affect protein function and cause symptoms of illness. There may be an issue with the formation of disulfide bridges in EGFr, which could lead to protein misfolding. Additionally, this issue may be related to increased multimerization of NOTCH3 with cysteine. A.P. Pan et al. reported 914 patients (median age of 60 years) with CADASIL of whom 65.2% had documented cerebrovascular events (i.e., CADASIL-Stroke patients) between September 2018 and April 2020. It is essential to investigate these findings further to understand their underlying mechanisms and potential implications for human health [20].

HTRA1

HTRA1 (High-Temperature Requirement A Serine Peptidase 1), situated on chr10q26, comprises nine exons and encodes the p-serine protease HTRA1, which consists of 480 amino acids. Cerebral autosomal recessive artery disease with subcortical infarcts and leukoencephalopathy is a rare hereditary condition among the general population. It primarily affects the brain and the blood vessels. Patients with CARASIL typically experience difficulty in walking, early onset dementia, hair loss, and lower back pain. CARASIL: besides the symptoms above, patients with CARASIL may experience visual problems, muscle weakness, and urinary incontinence. CARASIL has been reported in three cases: a mutation in the *HTRA1* gene, p. Arg302end, and the p. Ala252Thr mutation in a sibling [21, 22]. It is a serine protease essential for several cellular functions, including transforming growth factor (TGF) signaling [23]. Abnormal TGF-β signalling is thought to play a role in the emergence of CARASIL. Studies have shown that when mutations occur in CARASIL, the function of *HTRA1* is often disturbed.

TGF-binding protein-1 (LTBP-1) is a matricellular factor that plays a significant role in TGF-bioactivation and acts as a physiological substrate for HTRA1. This discovery may have important implications for our understanding of this complex biological process [24]. Down-regulation of the TGF pathway is essential for CARASIL development. It has also been found that LTBP-1 is a significant substrate of HTRA1 in this process. Furthermore, it as been shown that LTBP-1 is a crucial protein in this pathway, and there is currently no cure for CARASIL. Treatment aims to reduce symptoms and enhance quality of life. Individuals with a family history of CARASIL must seek genetic counseling before planning to have children [25].

COL3A1

The *COL3A1 (Collagen Type III Alpha 1 Chain)* gene on Chr2q32.2 comprises 51 exons and codes for a protein pro-alpha1 chain of type III collagen consisting of 1466 amino acids. Vascular Ehlers–Danlos syndrome (EDS), also known as EDS type IV, is caused by *COL3A1* mutation. Individuals with EDS type IV often exhibit unique facial features and premature aging of the limb extremities (acrogeria), making them susceptible to vascular and digestive ruptures and uterine perforations during pregnancy. This condition is caused by a genetic mutation that affects collagen III production. Collagen III is a vital protein that helps regulate the behavior and function of cells by binding to integrated cell surface receptors. In addition, they provide structural support to tissues and play crucial roles in angiogenesis, cell differentiation, and wound healing. Collagen III is critical for maintaining healthy cellular function and promoting optimal physiological processes. Heterozygous mutations in *COL3A1* give rise to this condition, resulting in compromised connective tissues, particularly the skin, blood vessels, and organs, which may lead to potential weakness. The pro1(III) triple-helix domain is often altered by substituting glycine residues with conventional triplet repeats.

Another typical mutation is in-frame exon skipping, which occurs in approximately 25% of cases owing to splice site changes. Insertions or small in-frame deletions can occur in rare cases. Procollagen III is a homotrimeric protein synthesized from regular and mutant 1(III) chains. As a result, 1, 2, or 3 mutant chains were found in approximately 88% of homotrimers. Thirteen distinct subtypes of EDS and mutations in 20 genes have been identified. This highlights the genetic complexity of this condition and the need for further research to understand and treat each subtype [26]. Although there is no cure for EDS type IV, patients with this condition can benefit from regular medical monitoring and symptom management.

TREX1

The *TREX1 (Three Prime Repair Exonuclease 1)* gene on chr3.48 consists of three exons that encode a protein. Three-prime repair exonuclease 1 comprises 314 amino acids. Retinal vasculopathy with cerebral leukodystrophy (RVCL) is inherited in an autosomal dominant manner, affecting both the retina and the central nervous system [27]. It is a minor hereditary vascular disease affecting the cerebral cortex. Hereditary vascular retinopathy, Cerebroretinal vascular disease, and hereditary endotheliopathy, retinopathy, nephropathy, and stroke are the three significant illnesses covered by RVCL [28]. Heterozygous frameshift mutations affecting the C terminus of *TREX1*, which encodes a 3'-5' exonuclease, lead to renal failure, vascular retinopathy, and focal neurologic symptoms, including ischemia events and cognitive deterioration. *TREX1* mutations result in RVCL and systemic manifestations (RVCL-S) [29].

A malfunctioning gene leads to the production of a truncated form of TREX1. Typically located within cells in the endoplasmic reticulum (ER), a network of membranes crucial for protein synthesis and release, the normal TREX1 protein is affected in RVCL-S due to one mutant copy of the *TREX1* gene, with mutations occurring in the gene's final quarter. This region encodes a portion of the protein required to maintain it in the ER compartment. Mutations in this region allow the protein to escape the ER and become mislocalized throughout the cell. Mislocalized TREX1 protein specifically affects and eventually destroys the lining of blood vessels, disrupting brain and ocular function in an unknown way. According to the autosomal dominant inheritance pattern, most individuals inherit RVCL-S from an affected parent. Disease onset and severity can differ significantly even within the same family. A 50% chance exists for an individual with RVCL-S to pass on the *TREX1* pathogenic variant to their progeny [30].

Both males and females are equally susceptible to RVCL-S, typically in middle-aged individuals (35–50 years). Initial signs often involve eye issues such as increased 'floaters' or 'blind patches.' Cases of RVCL-S have been identified in various countries, including Spain, Turkey, the United Kingdom, the United States, Australia, Japan, the Netherlands, China, France, Germany, Italy, Mexico, Switzerland, and Taiwan [31].

mtDNA

Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) Mitochondrial disorders, a diverse group of illnesses, result from gene mutations producing the proteins necessary for proper mitochondrial function. These disorders vary significantly in clinical presentation, biochemical features, and challenging diagnosis and management [32]. Among the mitochondrial diseases with the highest prevalence are those caused by mutations in the mtDNA, including MELAS. They may not necessarily represent genuine ISs but may arise from mitochondrial cytopathy, mitochondrial angiopathy, or a combination of both. Human mtDNA comprises two strands of DNA and contains 37 genes. These genes encode 13 structural peptide subunits of the oxidative phosphorylation system and numerous other molecules, making mtDNA an essential component of cellular respiration and energy production [33]. This medical condition is characterized by various symptoms, including episodic headaches, stroke-like episodes, short stature, lactic acidosis, vomiting, seizures, and skeletal myopathy. These symptoms typically occur before the age of 40 years and can be debilitating [34]. mtDNA has a single-base pair mutation rate that is much higher than that of the nuclear DNA. Therefore, mtDNA is more susceptible to changes in its genetic code over time, which has important implications in genetic and evolutionary research. Although this difference in mutation rates is not yet fully understood, scientists believe it may be due to the lack of efficient DNA repair mechanisms in the mitochondria compared to the nucleus.

ROS damage is widely accepted as the main factor leading to mtDNA mutagenesis. Continued mutations increase mitochondrial dysfunction and ROS generation, perpetuating a harmful cycle [35]. Additionally, mtDNA is vulnerable to chemical damage because histone proteins do not shield it. Furthermore, mutations in polymerase are the most common cause of inherited mitochondrial diseases [36]. MELAS disorders caused by mutations in oxidative phosphorylation components impair adenosine triphosphate synthesis and increase ROS generation. Mutations in mtDNA can have severe consequences for cellular health. Mutations in mitochondrial DNA can result in reductions in transfer RNA (tRNA) and proteins involved in oxidative phosphorylation. This may result in reduced adenosine triphosphate production, which may increase ROS production and cause oxidative stress. Oxidative stress can result in apoptosis, tissue damage, and mutations in mtDNA. Mitochondrial dysfunction can also disrupt calcium ion (Ca^{2+}) metabolism, leading to cell enlargement and death. Therefore, it is essential to understand the mechanisms underlying mtDNA mutations and their effects on cell health [37].

Hereditary hemorrhagic telangiectasia

People with Osler–Weber–Rendu disease, an autosomal dominant condition that does not occur very often, tend to bleed, and abnormal blood vessel growth and development can be seen all over the body. It can affect the arms, fingers, conjunctiva, trunk, and gastrointestinal tract. Symptoms may not appear until the late stages of life or later (approximately 90% of patients show signs by age 40) [38]. Patients with Osler–Weber–Rendu disease and their relatives can undergo genetic testing to determine whether the relevant genes, such as the chromosome 12 activin receptor-like kinase type I (*ALK-1*) gene and the endoglin gene (*ENG*) in chromosome 9, have mutations [39], both of which encode TGF-beta (TGF-β) superfamily receptors that are essential for the healthy formation of blood vessels. The *ALK-1* gene, associated with hereditary hemorrhagic telangiectasia (HHT) type 2, and the *ENG* gene, linked to HHT type 1, are commonly recognized as the most frequently implicated genes in HHT. Other genes are less regularly associated [40]. Both type III and type I TGF receptors, ALK-1 and endoglin, occur only in vascular endothelial cells. Endoglin enables the phosphorylation of type I TGF-β receptors, specifically ALK-5 and ALK-1, when TGF binds to type II TGF-β receptors on endothelial cells. Endoglin and ALK-1 directly bind to BMP-9 and BMP-10, and their interaction with the type II BMP receptor results in aberration [41]. Phosphorylation of ALK-5 and ALK-1 activates the downstream proteins Smad2/3 and Smad1/5, respectively. Subsequently, these activated Smad proteins detach from

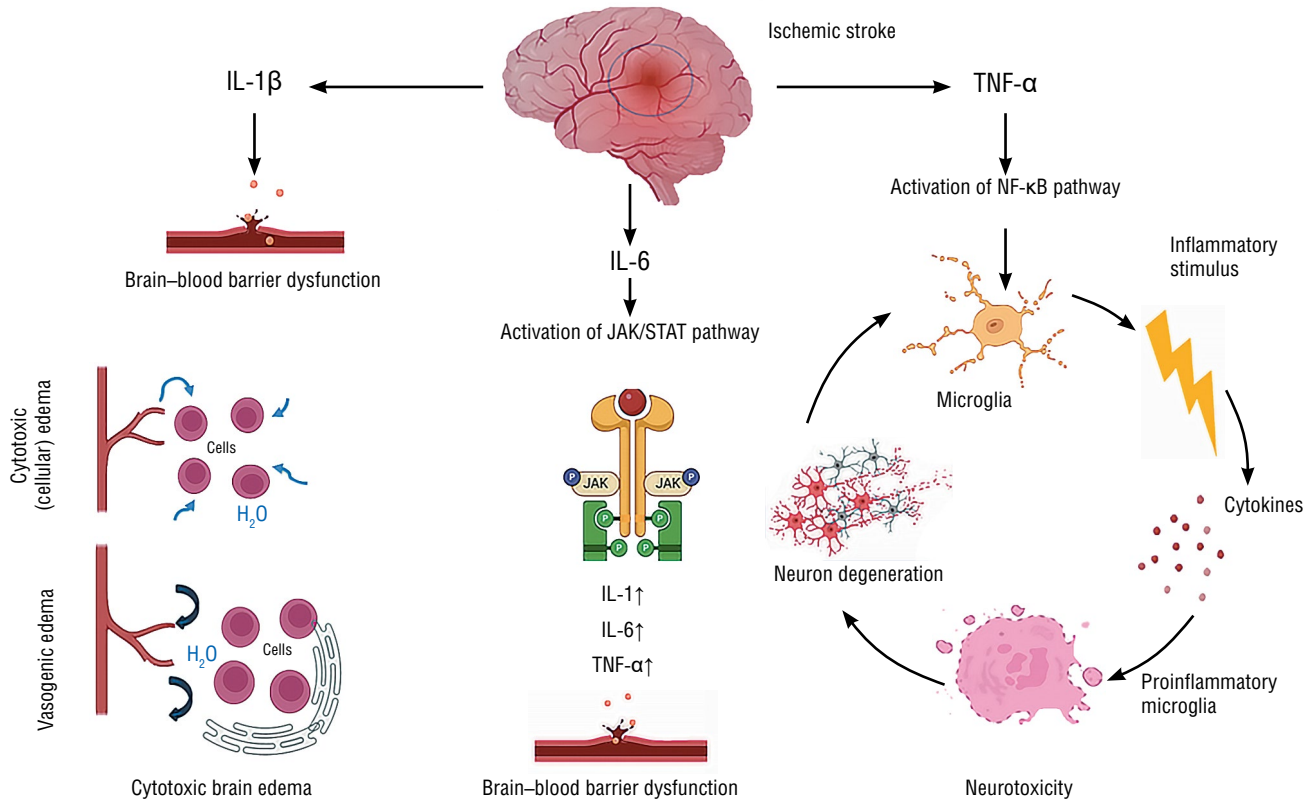

Fig. 2. Role of interleukin gene in IS.

the type I TGF receptor, combine with Smad4, and enter the nucleus to modulate the transcription of specific gene promoters associated with angiogenesis.

Genes involved in the mechanisms of IS

Interleukin genes

Research has indicated that proinflammatory cytokines play a vital role in the development of atherosclerosis. A lymphocyte medium termed an IL, mediates the connection between immunological cells and white blood cells. It belongs to the same class of cytokines as the blood cell growth factors. It is also essential for information transmission, immune cell activation and control, T and B cell activation, multiplication, differentiation, and inflammatory response [42]. Some major interleukins associated with IS are *IL-6*, *IL-1β*, and *TNF-α*. Figure 2 shows the involvement of the interleukin genes in IS.

IL-1β causes a variety of biological reactions that help the body respond to damage or infection, such as fever, sleepiness, appetite loss, acute phase protein synthesis, vasodilatation, adhesion, generation of chemokines, molecular upregulation, and the pro-coagulant condition, as well as the creation and release of growth factors, matrix metalloproteinases, and hematopoiesis. *IL-1*, *IL-1a*, and *IL-1b* contain 2 molecules. The *IL-1* gene complex, which contains three related genes: *IL1a*, *IL1b*, and *IL-b*, is found on chromosome 14. Five alleles exist within IL-1: IL-1RN1, IL-1RN2, IL-1RN3, IL-1RN4, and IL-1RN5. Among these, IL-1RN2 is considered a genetic risk factor for IS, a condition closely associated with coronary artery disease, and polymorphism is considered a genetic risk factor. Reduced blood flow in IS can cause nerve cell damage and inflammation. IL-1β, a potent proinflammatory cytokine, causes blood–brain barrier dysfunction by activating enzymes that break down arachidonic acid and release prostaglandins and leukotrienes. These compounds increase the blood–brain barrier permeability, causing vasogenic brain edema, potentially leading to pressure buildup and brain damage. They also cause cytotoxic brain edema through reduced oxygen and glucose after stroke, leading to cytotoxic brain edema [43]. The interaction between vasogenic and cytotoxic edema can increase cranial pressure, harm brain tissue, and cause cerebral herniation. *IL-1β* promotes the expression of adhesion molecules in endothelial cells. Ischemia triggers an inflammatory response by attracting immune cells to the affected area, causing them to migrate to this area [44].

IL-6-driven inflammation is a mechanism that drives various types [45]. *IL-6* mediates cellular communication via two different mechanisms: classic and trans-signalling [46].

The conventional *IL-6* signaling pathway involves binding of *IL-6* to its transmembrane receptor, IL-6R. The cleavage of transmembrane IL-6R gives rise to a naturally occurring soluble form known as sIL-6R. sIL-6R can bind to IL-6, enabling IL-6 responsiveness in cells lacking the transmembrane IL-6R. Upon binding to membrane-bound IL-6R or sIL-6R, *IL-6* induces the oligomerization of gp130, initiating the Janus kinase/signal transducer and activator of transcription pathway. Numerous cytokines and growth factors alter gene expression by sending signals via Janus kinase and signal transducer and activator of transcription pathways from the cell surface to the nucleus. A soluble antagonist of gp130 effectively inhibits IL-6/sIL-6R activity by binding to the IL-6/sIL-6R complex. This antagonist specifically discriminates between trans-signaling, where *IL-6* affects cells lacking IL-6R, and the conventional signaling pathway involving membrane-bound IL-6R, as it only interferes with trans-signaling and does not affect the traditional signaling pathway [47].

Macrophages in the immune system produce *TNF-α*, which plays a role in various physiological functions. *TNF-α* is located in the primary histocompatibility complex class III region of Chromosome 6. Depending on the situation and the specific pathways activated, TNF-α can exhibit both pro- and anti-inflammatory effects [48]. Studies have mainly concentrated on the G308A mutation in the etiology of IS concerning TNF-α gene polymorphisms. TNF-α mutations in the gene's promoter region may alter the transcriptional activity of the gene [49]. This genetic variation can increase the activity of the TNF-α gene, resulting in the excessive production of TNF-α within the body.

Increased concentrations of TNF-α in blood have significant implications. It disrupts the blood flow to the brain, leading to damage and inflammation. Microglia are the brain cells involved in this condition, and activated astrocytes release significant amounts of TNF-α. Excessive TNF-α is deemed harmful because it affects transmission and plasticity, contributing to the core symptoms observed in patients with IS. TNF-α plays an essential role in the brain; on one hand, when it binds to its receptors, it triggers a pathway called NF-κB activation. This activation can lead to neurotoxic and neuroprotective responses. G308A variation in the promoter region of TNF increased TNF production and promoted IS progression. Elevated TNF-α levels can affect transmission and plasticity, leading to cognitive impairment in IS patients. Furthermore, the interaction of TNF-α with its receptors can trigger NF-κB activation, which has complex and context-dependent effects on brain cells and influences neurotoxicity and neuroprotective responses. The diverse outcomes of TNF-α signaling in the brain depend on factors such as the stage of neuronal development, the type of brain cell involved, and the specific receptor subtypes engaged [50].

Table 1 illustrates the functions of all the genes related to the disorder.

Polygenic disorder

IS is significantly influenced by polygenic disorders that arise from the interaction of multiple genes. Various sequencing techniques, including Mendelian sequencing, genomewide association studies (GWAS), whole-exome sequencing (WES), and whole-genome sequencing (WGS), play a crucial role in studying IS. Mendelian sequencing is used to detect monogenic disorders that can result in recognizable clinical manifestations, including IS. Researchers are making rapid advancements in identifying polygenic variations associated with these conditions. One of the primary tools employed in this pursuit is GWAS [51]. Uncovering the connections between genetic variations and complex traits or disorders is invaluable. GWAS is essential for understanding the genetic underpinnings of IS in the context of polygenic diseases. A study found a correlation between hereditary imbalances detected through GWAS and unfavorable outcomes three months after a IS [52]. Although monogenic disorders contribute to only approximately 7% of IS cases, they can lead to recognizable clinical symptoms, including IS. GWAS can assist in identifying the genetic elements responsible for these disorders and their association with IS [53]. Thousands of genetic variations that affect human disease susceptibility and its characteristics have been revealed. Nonetheless, understanding how these genetic variations, particularly those in non-coding regions, influence the development of related diseases and traits continues to pose a substantial challenge [54].

A compelling discovery in IS genetics is the link between the 7q21 region near the histone deacetylase 9 (*HDAC9*) gene (polymorphism rs12524866) and the LAA subtype of IS. This finding marked a significant breakthrough as it was the first widely replicated genetic association with this particular IS subtype [55]. Subsequent studies and additional patient data from Europe, America, and Australia consistently confirmed this genetic relationship. Furthermore, ongoing GWAS investigations have uncovered additional genetic variations associated with LAA stroke. Notably, variations on chromosome 6p21.1 and genes *MMP12* (rs12122539) and *TSPAN2* (rs13107327) have been associated with this IS subtype [45]. Furthermore, a genetic variation located on chromosome 14q13.3 in proximity to *PTCSC3* has been documented to be associated with LAA stroke in the Chinese Han population [56]. Genetic variation near *ABCC1* (rs74475935) has also been linked to IS in European and African populations [57], as shown in Table 2. These findings collectively demonstrate the power of GWAS in unraveling the intricate genetic components that increase the risk of IS within the realm of polygenic disorders.

WES and WGS are increasingly employed in daily diagnostics and are more efficient and promising techniques. WES has been applied to investigate young IS patients with familial clustering of stroke, whereas WGS has been applied to analyze families where a monogenic cause of stroke seems likely [58]. According

Table 1. IS-associated genes related to disorder and their function

Ген Gene	Gene name	Chromosome number	Number of exons	Amino acid	Role	Disorder	Mutation	Source
NOTCH3	Notch receptor 3	19	2321	33	Receptor for membrane-bound ligands	CADASIL	*De novo* mutation	[19]
HTRA1	HtrA serine peptidase 1	10	480	9	Stimulate synovial cells to increase the production of MMP1 and MMP3	CARASIL	Homozygous mutation	[24]
COL3A1	Collagen type III alpha 1 chain	2	1466	51	Participates in the control of cortical development	EDS	Autosomal dominant mutations	[26]
TREX1	Three prime repair exonuclease 1	3	314	3	Inhibits the autonomous triggering of autoimmunity within cells	Retinal vasculopathy with cerebral leukodystrophy	«Truncating mutation	[31]
IL-1β	Interleukin-1β	2	7	269	Potent proinflammatory cytokine	Cytotoxic brain edema	Chronic deletion	[43]
IL-6	Interleukin-6	7	5	212	Potent inducer of the acute phase response	Blood–brain barrier dysfunction	Gain-of-function mutations	[45]
TNF-α	Tumor necrosis factor-α	6	4	233	Stimulate cell proliferation and induce cell differentiation	Neurotoxicity	*G-A* mutation	[48]

to a recent article, the introduction of massive parallel sequencing methods, such as WES and WGS, has led to the detection of an increasing number of gene-disease associations. Regularly reassessing data, updating gene panels, and incorporating recent detailed information on the phenotype can improve the diagnostic yield of WES and WGS tests in stroke patients [59].

Genetic disorder related to stroke

A genetic syndrome is a collection of distinct genetic disorders or medical conditions that exhibit shared characteristics due to abnormalities or mutations in one or more genes. Typically, these syndromes affect various organ systems, leading to physical, developmental, and intellectual difficulties. These genetic syndromes may either be inherited from parents or arise from spontaneous genetic mutations.

Familial moyamoya

Moyamoya disease is an uncommon genetic disorder that affects the blood vessels of the brain, particularly the internal carotid arteries and their branches. This disorder can result in symptoms such as transient ischemic attacks (mini-strokes), strokes, and seizures. Occasionally, delicate blood vessels that form as a response to narrowed arteries can develop protrusions (aneurysms) or bursts, leading to brain bleeding [60]. Moyamoya's disease can affect individuals across various age groups, and the types of ischemic events experienced may vary by age. This condition predominantly results in brain ischemic events in children, whereas it can give rise to both ischemic and hemorrhagic events in adults [61].

Ehlers–Danlos syndrome

EDS encompasses a spectrum of inherited connective tissue disorders that can potentially affect various organ systems, including blood vessels. Among the different subtypes of EDS, the vascular subtype is notably associated with an elevated risk of cerebrovascular complications such as stroke and intracranial aneurysms. These neurological aspects of EDS, including IS and cerebrovascular disease, have garnered increased attention in recent years [62]. Although the primary molecular defect in EDS does not typically target the nervous system directly, there has been a growing focus on

Table 2. IS

Gene	Chromosome	Polymorphism	*p*	Odds ratio (95% CI)	Association	Source
HDAC9	7q21	rs12524866	1.47E-08	1.11 (1.08–1.14)	Associated with LAA	[55]
MMP12	6p21.1	rs12122539	1.54E-08	1.09 (1.06–1.12)	Associated with LAA	[45]
TSPAN2	6p21.1	rs13107327	8.75E-09	1.10 (1.07–1.12)	Associated with LAA	[45]
ABCC1	16p13.11	rs74475935	3.01E-05	1.373 (1.182–1.594)	Associated with CG-type IS stroke	[56]
PTCSC3	14q13.3	rs2415317	1.37E-05	1.394 (1.199–1.620)	Associated with LAA	[57]

its neurological symptoms, encompassing conditions such as IS and cerebrovascular disease. EDS is also associated with neurological issues such as musculoskeletal pain, fatigue, headaches, muscle weakness, and paresthesias [62].

Methylmalonic acidemia

Methylmalonic acidemia is a genetic disorder that follows an autosomal recessive pattern and affects amino acid metabolism. This genetic disorder is marked by a deficiency in the conversion of methylmalonyl-coenzyme A (CoA) to succinyl-CoA. It has been associated with an increased risk of IS, particularly affecting the bilateral globus pallidi [63]. Methylmalonic acidemia is a hereditary disorder transmitted within families and falls into the "inborn errors of metabolism." Typically, this condition is identified during the initial years of a person's life[1].

Fabry disease

Fabry disease is associated with an increased risk of IS, particularly in younger individuals. The disease is an X-linked lysosomal storage disorder caused by a mutation in the α-galactosidase A enzyme, leading to a deficiency of this enzyme. Progressive accumulation of globotriaosylceramide within the endothelium of blood vessels is thought to play a primary role in vasculopathy and the risk of IS in patients with Fabry disease. It accounts for approximately 1–5% of all stroke cases, with stroke being a primary manifestation of some hereditary disorders [64]. Males affected by the classic phenotype experience acroparesthesia, hypohidrosis, and corneal opacities as children, and in their third or fifth decade of life, they may experience stroke, cardiac hypertrophy, or renal failure. Certain female heterozygotes exhibited no symptoms, whereas others experienced symptoms comparable to those of males. Regardless of sex, individuals with Fabry disease may experience transient cerebral ischemia and stroke as part of the natural progression of the illness, even at a young age [65].

Vascular diseases related to stroke

Cerebrovascular diseases encompass a spectrum of conditions that affect blood flow to the brain, with notable associations with atrial fibrillation, hypertension, and diabetes. Both hypertension and IS are strongly associated with an increased risk for cognitive impairment. Hypertensive individuals face a 3.9 times higher risk of cerebral hemorrhage than non-hypertensive individuals, with a 2.8 times higher relative risk in the case of aneurysmal subarachnoid hemorrhage [66]. Chronic hypertension exerts detrimental effects on cerebral vessels and tissues, leading to atrophy, silent infarctions, micro hemorrhages, and white matter lesions [67]. This physiological mechanism reinforces a correlation between hypertension and cognitive impairment. Diabetes, as an independent risk factor for atherothrombotic IS, mainly affects women and contributes significantly to the risk of IS, second only to hypertension. It is a primary risk factor for cerebral small vessel disease, demonstrating a substantial association with symptomatic recurrence after a first lacunar-type cerebral infarction [68, 69]. Atrial fibrillation and atrial flutter, often linked to cardioembolic IS, are crucial and controllable risk factors. Cardioembolic infarction is recognized as the most severe subtype of IS [70]. In individuals over 70 years of age, approximately 5% show atrial fibrillation, and in the absence of organic heart disease, there is a 3- to 4-times higher risk of IS [71].

[1]Noonan syndrome.
URL: https://medlineplus.gov/genetics/condition/noonan-syndrome (accessed: 20.11.2023).

Impact of genetic evaluation

Genetic evaluation influences multiple facets of IS, including drug discovery, risk prediction, and clinical practice. Across diverse ancestries, distinct genetic regions associated with stroke have been identified, providing pivotal insights for future biological investigations of IS etiology and proposing potential therapeutic targets for intervention. The primary role in vasculopathy and risk of IS in patients with Fabry disease is believed to be due to the progressive accumulation of globotriaosylceramide within the endothelium of blood vessels. Fabry disease accounts for approximately 1–5% of all stroke cases, and in some hereditary disorders, stroke is a primary manifestation [72].

Polygenic scores derived from genetic variations associated with vascular risk factors accurately predict outcomes across individuals with diverse ancestries. These scores are valuable in forecasting genetic susceptibility to IS and demonstrate predictive efficacy independent of clinical risk variables [73]. Genetic studies have successfully established connections between monogenic disorders and stroke, prompting some experts to advocate the incorporation of IS gene panels in risk assessment and broader stroke research. Identifying new biomarkers for the genetic basis of IS and distinctive targets for gene therapy may advance gene therapy and enhance tailored IS care [74].

Advances in stroke genetics

Exploration of the genetic underpinnings of stroke has undergone significant advancements in recent years, particularly in the domains of hemoglobinopathies, thrombophilia, and disorders affecting small vessels and cardioembolic pathways. Researchers have identified genetic variations associated with increased susceptibility to cardioembolic IS, shedding light on the fundamental processes underlying clot formation in the heart and subsequent embolism in the brain [75]. There is growing emphasis on understanding the impact of genetic variables on cerebral microvascular function and small vessel integrity in minor vessel disorders. This focus has yielded crucial insights into the genetic susceptibility of minor artery disorders and the intricate pathophysiology governing them [76]. Notable strides have been made in genetic research related to hemoglobinopathies to elucidate the connection between abnormal hemoglobin variations and the risk of stroke. For individuals with hemoglobinopathies predisposed to stroke, the identification of specific genetic markers has facilitated risk assessment and formulation of tailored treatment plans [77]. Similarly, substantial progress has been made in understanding the genetic components contributing to thrombophilia, a condition characterized by abnormal blood clotting that can lead to stroke. This knowledge directly informs therapeutic strategies and preventive measures, enabling healthcare professionals to better manage individuals at risk of stroke due to thrombophilia [78].

Early detection of IS

Several promising approaches have emerged for IS detection and diagnosis. A groundbreaking, nonlinear, modified Laplacian pyramid technique was introduced to improve the early identification of IS in computerized tomography scans. This approach plays a crucial role in facilitating accurate and prompt IS detection, thereby expediting the initiation of appropriate treatment [79]. Furthermore, there has been a significant exploration of blood biomarkers for their potential utility in the early detection of IS. One notable example is the stroke chip study, which rigorously assessed a panel of biomarkers. It aimed to differentiate between genuine strokes and conditions that mimic strokes and distinguish among various stroke subtypes [80]. Additionally, the application of computer-aided detection techniques, such as segmentation and texture feature analysis, has shown promise for the early identification of IS. When applied to magnetic resonance imaging, these methods aid in recognizing stroke lesions and determining the necessity for thrombolysis [12]. Together, these innovative approaches and studies contribute coherently to ongoing efforts to enhance IS's early detection and management of IS.

Conclusions and future prospects

In conclusion, IS remains a significant cause of mortality and morbidity worldwide, and distinct genetic and environmental factors underlie its development. Several polygenic and monogenic disorders have been associated with an increased risk of stroke, and current research continues to identify new genetic markers and risk factors. Genetic evaluation has emerged as a critical tool in facilitating early detection and prevention strategies for IS. As the understanding of the genetic underpinnings of IS progresses, the focus on genetic syndromes associated with stroke and the exploration of innovative techniques continue to offer possibilities for early detection and targeted treatments. These advances hold significant promise for globally reducing IS's incidence and adverse impact. However, while improvements in diagnosis and treatment have shown promising results, preventative measures such as lifestyle modifications, early risk factor identification, and timely intervention remain crucial in reducing the global burden of IS. Therefore, continued research efforts to uncover the multifaceted interactions and mechanisms underlying IS are essential for developing effective preventive and therapeutic strategies.

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Information about the authors

Praveen Kumar Chandra Sekar — Dr. Sci (Med.), Human cytogenetics and genomics laboratory, Faculty of allied health sciences, Chettinad Academy of Research and Education, Kelambakkam, Tamil Nadu, India, https://orcid.org/0009-0008-5346-9597
Ramakrishnan Veerabathiran — Cand. Sci (Med.), Human cytogenetics and genomics laboratory, Faculty of allied health sciences, Chettinad Academy of Research and Education, Kelambakkam, Tamil Nadu, India, https://orcid.org/0000-0002-9307-5428

Authors' contribution. *Praveen Kumar Chandra Sekar* — writing the text of the manuscript, edition of the figures and tables; *Ramakrishnan Veerabathiran* — design of the study, correction and approving of the manuscript for submission.

Информация об авторах

Praveen Kumar Chandra Sekar — д-р мед. наук, лаборатория цитогенетики и геномики человека, факультет смежных медицинских наук, Академия исследований и образования Четтинада, Келамбаккам, Тамилнад, Индия, https://orcid.org/0009-0008-5346-9597
Ramakrishnan Veerabathiran — канд. мед. наук, лаборатория цитогенетики и геномики человека, факультет смежных медицинских наук, Академия исследований и образования Четтинада, Келамбаккам, Тамилнад, Индия, https://orcid.org/0000-0002-9307-5428

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Primary Progressive Aphasia: Variants and Main Language Domains

Diliara R. Akhmadullina, Ekaterina Yu. Fedotova

Research Center of Neurology, Moscow, Russia

Abstract

Language is one of the higher brain functions and the primary method of communication, so it plays a key role in human social functioning. Primary progressive aphasia, as a slowly progressive neurodegenerative disease with a clinical predominance of different speech and language disorders, is a promising model for a more detailed study of topographic distribution of language disorders. This review presents data on different clinical variants of primary progressive aphasia and the corresponding clinical and neuroanatomical correlates that have significantly expanded the modern understanding of the neural network language organization.

Keywords: *primary progressive aphasia; language; neurodegeneration; frontotemporal dementia; Alzheimer's disease*

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For correspondence: 80 Volokolamskoye shosse, Moscow, 125367, Russia. Research Center of Neurology.
E-mail: fedotova@neurology.ru. Fedotova E.Yu.

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Первичная прогрессирующая афазия: варианты и основные речевые домены

Д.Р. Ахмадуллина, Е.Ю. Федотова

Научный центр неврологии, Москва, Россия

Аннотация

Речь является одной из высших корковых функций и представляет собой основной метод коммуникации, из-за чего сложно переоценить её значимость для полноценного функционирования человека в социуме. Первичная прогрессирующая афазия, будучи медленно развивающимся нейродегенеративным заболеванием с преобладанием в клинической картине разнообразных речевых нарушений, является перспективной моделью для более подробного исследования топографического распределения расстройств речи. В обзоре приводятся данные о различных клинических вариантах первичной прогрессирующей афазии, а также соответствующие им клинико-нейроанатомические корреляции, благодаря которым в значительной степени расширилось современное понимание нейросетевой организации речи.

Ключевые слова: *первичная прогрессирующая афазия; речь; нейродегенерация; лобно-височная деменция; болезнь Альцгеймера*

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Адрес для корреспонденции: 125367, Россия, Москва, Волоколамское шоссе, д. 80. Научный центр неврологии.
E-mail: fedotova@neurology.ru. Федотова Е.Ю.

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Introduction

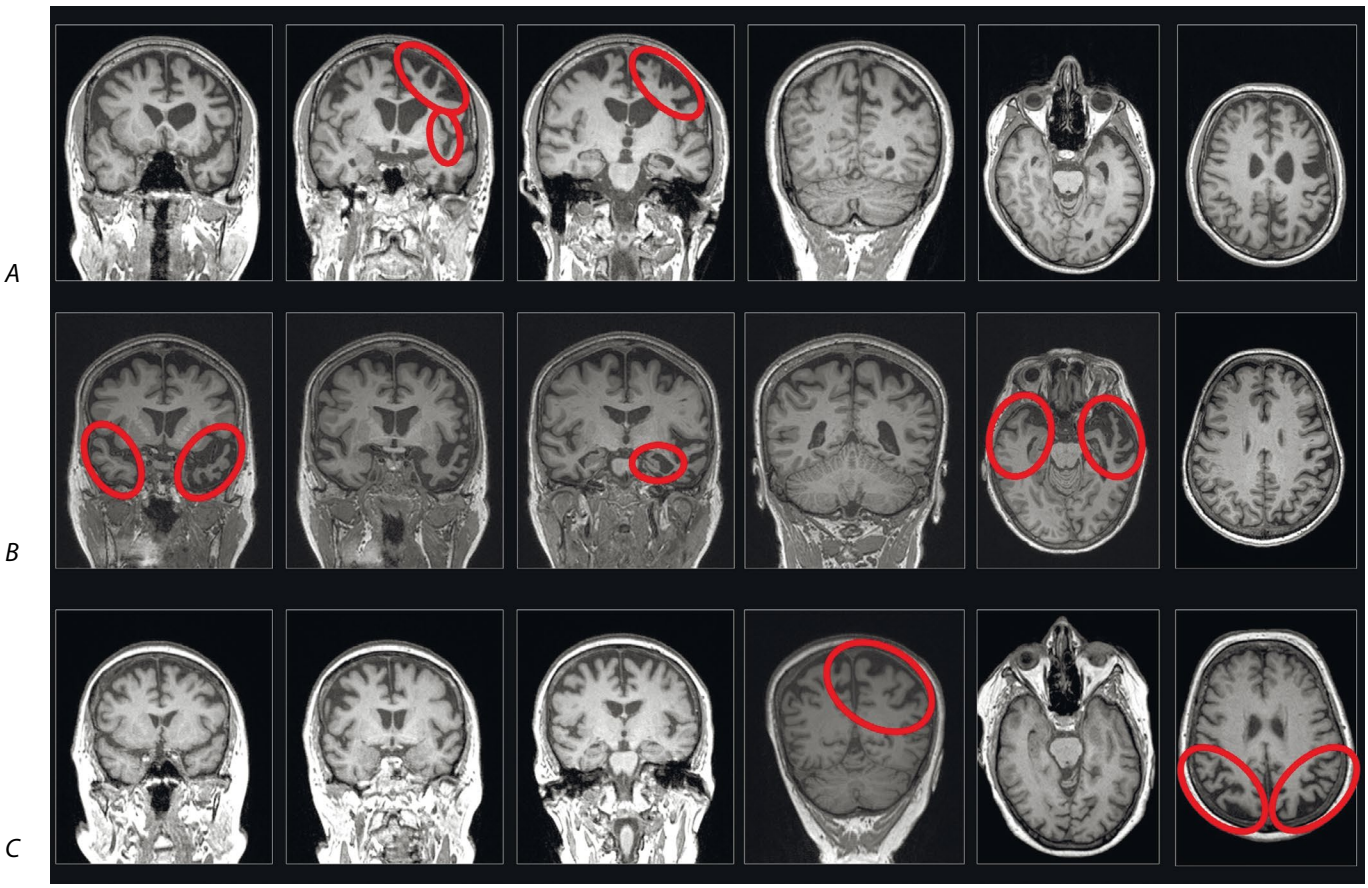
Primary progressive aphasia (PPA) is a group of neurodegenerative disorders characterized by predominantly progressive language impairment. PPA usually manifests at the age of 50–60 years and affects both men and women equally. In addition to language disorders, other cognitive, behavioral, and motor disorders may develop over time. However, aphasia is the most disabling symptom, at least during the first two years of the disease [1].

Neurodegenerative aphasia was first described by Arnold Pick in 1892 [2], but the term PPA was introduced only in 1987, when it was proposed to differentiate PPA from Alzheimer's disease (AD) as a distinct disease entity [3]. Initially, PPA was considered to be a single syndrome with a central symptom of anomia, but over time, three clinical syndromes have been identified such as non-fluent (nfPPA), semantic (svPPA), and logopenic (lvPPA) variants. This classification has been used since 2011, when the current diagnostic criteria for PPA were established, describing specific clinical and neuroimaging signs of each variant [1]. The Figure shows examples of PPA from the authors' clinical practice.

In recent decades, there have been significant advances in our understanding of the neurobiological mechanisms underlying language behavior and diseases that manifest with language impairment. At the same time, the study of PPA has played an important role in developing new concepts of language, initially and primarily based on studying stroke and other focal lesions. The localization of neurodegeneration and atrophy in PPA is unique and significantly different from the anatomical distribution of lesions in vascular disease. The speech deficits in PPA are also very diverse. Together, these findings identify novel correlations between brain regions and cognitive impairment and provide new directions for studying the neuroanatomical basis of language.

Non-fluent variant

NfPPA is characterized by apraxia of speech and agrammatism with relatively preserved understanding of single words and functions of objects [1]. Patients' speech becomes laconic and interrupted, with frequent stumbling, fluctuating rate, and decreased speaking activity. Patients with this variant tend to use simple sentences with minimal words. Spoken language understanding is less affected.



Atrophy localization in primary progressive aphasia variants.
A – non-fluent variant (patient age 49 years, disease duration 5 years); *B* – semantic variant (patient age 72 years, disease duration 6 years); *C* – logopenic variant (patient age 64 years, disease duration 4 years).
The most characteristic areas of atrophy for each of the variants are outlined in red.

Neuroimaging of nfPPA is primarily characterized by atrophy of the left posterior frontal/insular regions (Figure, *A*), although the process starts affecting other brain regions over time [1, 4]. NfPPA usually develops in patients with frontotemporal degeneration, more commonly with 4R tau inclusions, less commonly with 3R tau or TDP-43, while up to 10% of cases may have an atypical presentation of AD [5, 6]. A positive family history is reported in one third of cases. There are nfPPA cases with mutations across all three major frontotemporal dementia genes such as *C9orf72, MAPT, GRN* [6].

Agrammatism is characterized by misused prepositions, inconsistent endings or verb forms. This affects not only the grammatical structure of single words, but also that of sentences that are misconstrued by using nouns instead of adjectives, participles, or verbal adverbs. As the disease progresses, patients start using more laconic sentences and eventually develop so-called telegraphic speech. Agrammatism is found in both spoken and written language and include transposed letters and syllables, incorrect use of endings, and joined-up spellings of prepositions. Despite preserved comprehension of isolated words, patients have difficulty understanding syntactically complex structures such as passive voice or complex sentences [6]. Overall speech rate is reduced by more than 3 times compared to healthy controls [7].

Apraxia of speech is associated with poor complex motor planning and manifests as a disruption in speech rhythm and intonation. The disorder is most evident when people are asked to repeat a complex word or a phrase several times; people with apraxia repeat the word or phrase differently each time. The nfPPA is often associated with hypokinetic or mixed hypokinetic-spastic dysarthria [8]. It should be noted that in addition to nfPPA, there is a condition called primary progressive apraxia of speech (PPAS), in which patients present with an isolated apraxia of speech. In PPAS, apraxia of speech is the only manifestation without signs of aphasia, which is the main feature that distinguishes it from nfPPA. However, in some cases PPAS may progress to nfPPA over time.

In addition to language disorders, the clinical picture may include motor, behavioral, and other cognitive disorders. In nfPPA, behavioral disturbances occur less frequently and later than in other types of PPA, but symptoms such as apathy, agitation, depression, reduced empathy, eating disorders, and disinhibition may develop over time [9, 10]. Among non-speech cognitive impairments, impaired regulatory functions are more typical for nfPPA, with memory and visuospatial functions remaining intact for a long time [6].

Atrophy in nfPPA most commonly affects the opercular part of the left inferior frontal gyrus and left premotor cortex, with possible involvement of associated cortical and subcortical regions such as the anterior insula, prefrontal cortex, supplementary motor area (SMA), basal ganglia and supramarginal gyrus [1, 10].

In addition, nfPPA is characterized by the involvement of the left inferior frontal gyrus, which plays an important role in regulating the motor-phonological network and ensuring the correct use and comprehension of grammatically or syntactically complex structures in spoken and written language. Atrophy of this region correlates with the overall severity of aphasia and agrammatism in patients with nfPPA [11–13]. In addition to the left inferior frontal gyrus, the severity of agrammatism and aphasia in nfPPA is also associated with damage to thalamus and putamen [11], while a decrease in fluency is associated with the volume of the anterior insula [14].

In contrast to agrammatism, apraxia of speech most often correlates with damage to the left premotor cortex and SMA. The SMA plays a role in movement initiation and speech motor control, and its atrophy is associated with reduced articulation rate in PPA [15]. The left superior lateral premotor cortex is involved in planning complex actions and is thought to determine the sequence of syllables in speech. In nfPPA patients, its atrophy is associated with the severity of speech impairment based on the Apraxia of Speech Rating Scale [13]. Focal atrophy of the left superior lateral premotor cortex is associated with PPAS. PPAS conversion to nfPPA is attributed to atrophy involving the left inferior frontal gyrus and subcortical structures [11, 16]. Some studies show that atrophy of the regions associated with agrammatism occurs before its clinical manifestation and may predict which proportion of PPAS patients will progress to nfPPA over time.

In addition to apraxia of speech, patients with PPAS and nfPPA develop nonverbal oral apraxia. In this type of apraxia, people are unable to consciously purse their lips, blow, or cough. In nfPPA and PPAS, nonverbal oral apraxia is associated with bilateral involvement of prefrontal and premotor cortex and SMA [17]. Ideomotor apraxia, which is common in nfPPA, is also associated with left premotor cortex atrophy [18].

Predominant damage to certain regions and the connections between them appear to explain the clinical heterogeneity of nfPPA; for example, predominant damage to the motor component is reported in premotor cortex atrophy and agrammatism in prefrontal cortex degeneration, whereas simultaneous damage to all regions results in early mutism [19–22].

Genetic variants of nfPPA may differ from sporadic variants in the pattern of atrophy. A small study showed that nfPPA patients with *GRN* mutations had a more posterior pattern of atrophy with bilateral involvement of the lateral parietal lobe, less involvement of the left frontal lobe, and more extensive atrophy of the frontoinsular regions of the right hemisphere [23].

In addition to grey matter, nfPPA also involves degeneration of white fibers connecting the frontal lobes, subcortical structures, and parietal regions. Atrophy is shown to progress from the inferior frontal gyrus to the SMA via the frontal oblique fasciculus. The severity of its damage is associated

with the severity of apraxia of speech [24], as well as with impaired speech fluency, while damage to the arcuate and superior longitudinal fasciculi is associated with impaired syntactic structure of speech [25, 26].

Semantic variant

Compared to other variants of PPA, svPPA is characterized by the greatest clinical, pathomorphological, genetic, and neuroimaging homogeneity. Anomia and impaired semantic knowledge of objects are the main clinical features of svPPA. In addition, patients may have dyslexia, dysgraphia, and impaired object knowledge. However, fluency and repetition are spared [1]. The atrophy affects the temporal pole, usually the left one (Figure, *B*). However, in about one-third of cases, atrophy of the right hemisphere predominates, with more severe behavioral disturbances and prosopagnosia in the clinical picture [6]. Pathological studies show that the vast majority of svPPA patients have a frontotemporal degeneration with an abnormal accumulation of TDP-43 type C [5, 6]. Cases with TDP-43 type A or B, tauopathy, or Alzheimer's degeneration are less common. Hereditary forms are less typical for svPPA than for nfPPA. Genetic mutations are found in approximately 2–4% of cases, a positive family history is reported in 2–17% of patients, and most cases are sporadic [27, 28].

Although naming disorders are present in all variants of PPA, they are most severe in svPPA patients. The diagnosis of svPPA should consider that at early stages, knowledge of less common concepts and objects may be impaired, while knowledge of more familiar and general concepts may be spared. As svPPA progresses, the comprehension of familiar and routine words is also impaired. Patients often tend to replace rare words with familiar terms (e.g., they may say *an animal* when presented with a picture of a giraffe) or use nonspecific words (*this, thing, something*). In contrast to nfPPA, nouns present the greatest difficulty. Since the anomia is based on a loss of word knowledge, a choice of 4 prompt words usually does not help patients with naming tests, in contrast to other PPA variants. In addition to verbal deficits, patients also have impaired ability to perform nonverbal semantic tasks such as identifying colors, sounds, smells, celebrity faces, and object functions, suggesting a widespread loss of semantic knowledge in this variant [29–31]. In addition to anomia, patients may have mild dysgraphia and dyslexia, manifested by misspelled words or unpronounceable consonants. In contrast, impaired repetition, syntax, or grammar are not typical for svPPA, and sentence comprehension is usually better than that of single words (due to additional context).

Despite severe semantic deficit, episodic and autobiographical memory are usually spared or relatively spared, although they are often difficult to test because of language and speech disorders. Behavioral disturbances are more typical for svPPA than for other variants, occur earlier, and are more severe [6]. Disinhibition, egocentrism, loss of empathy for close ones, compulsive behavior, and personality changes are frequently observed. Reduced insight is often reported.

In most cases, svPPA begins with atrophy of the left temporal pole [1], which is a semantic hub for storing, processing, and retrieving verbal semantic information. Interestingly, the study of svPPA has greatly aided in the identification of this function of the left temporal pole. Structural and functional neuroimaging data showed that disruption of multiple connections of the left temporal pole with regions of the semantic evaluation network and other cortical regions is associated with anomia and impaired single word comprehension due to semantic deficit, and that left temporal pole atrophy correlates with the severity of naming disorders [32–34].

As noted above, approximately 30% of svPPA patients have atrophy that develops from the right rather than the left temporal pole [32]. In this case, the first symptoms include non-verbal semantic impairment, such as the inability to recognize familiar faces, images, and objects [35], since the right temporal pole is responsible for encoding predominantly non-verbal semantic stimuli and contributes to recognizing familiar visual images [36]. In addition, verbal semantic functions may be spared in such patients at the early stages of the disease, or such disorders may be less severe than in those with left-sided onset of the disease. Right-sided svPPA is characterized by more severe and earlier behavioral disturbances, which may complicate differential diagnosis with the behavioral variant of frontotemporal dementia. This is because the right temporal pole and right fusiform gyrus play an important role in the perception of emotions, empathy, and recognition of familiar faces [29, 37, 38], and atrophy of the right fusiform gyrus, the right inferior temporal gyrus, and bilateral atrophy of the temporal poles and amygdalae correlate with the impaired model of perception of other mind [39].

As the disease progresses, the atrophy of the temporal poles becomes more symmetrical, leading to a decrease in differences in clinical manifestations between different forms of svPPA. Left-sided svPPA gradually presents with behavioral and non-verbal semantic deficits, and right-sided svPPA develops language and speech disorders [32, 40]. In addition to contralateral progression, atrophy also affects regions associated with the anterior temporal pole, extending to more posterior parts of the temporal lobe and orbitofrontal areas [41]. Damage to the middle parts of the left superior and middle temporal gyri in svPPA, which are thought to be responsible for connecting semantic regions with parietal and posterior temporal regions, correlates with the severity of anomia, impaired single word comprehension, and dyslexia [32, 33]. Moreover, svPPA is characterized by atrophy of the hippocampus and parahippocampal gyri [4], which may complicate the differential diagnosis with AD. However, in contrast to the latter, hippocampal atrophy is asymmetric

and predominantly affects anterior rather than posterior regions [42]. In addition to the right temporal lobe, other brain regions are involved in the development of emotional disturbances in svPPA. Impaired emotion recognition correlates with atrophy of the orbitofrontal cortex, which is normally involved in processing emotional information from the temporal poles and regulating complex social behavior [38]. In addition to the right temporal lobe and orbitofrontal cortex, the decline in emotional memory also correlates with decreased volume of the right frontal pole [43]. Structural abnormalities are not restricted to the grey matter of the brain, as in nfPPA. In svPPA, lesions are found in the ventral white matter tracts of the frontotemporal regions, usually the anterior part of the inferior longitudinal fasciculi and uncinate fasciculi [25, 44].

Logopenic variant

Key features of lvPPA include impaired word selection in spontaneous speech and naming, and impaired repetition of long phrases and sentences, while the motor component of speech and semantic knowledge are spared and no agrammatism is observed [45]. In addition, phonological speech errors are typical for lvPPA. Histologically, in contrast to the other two variants, the vast majority of lvPPA patients have Alzheimer's degeneration; less frequently (in 5–40% of cases), variants of frontotemporal degeneration with accumulation of tau protein or TDP-43 can be observed [5, 46–48]. In lvPPA associated with frontotemporal degeneration, hereditary forms can also occur, most commonly with mutations in the *GRN* gene [49, 50].

Although naming disorders are important clinical manifestations of both svPPA and lvPPA, their underlying mechanisms are very different. Anomia and impaired repetition of long phrases in lvPPA develop as a result of atrophy of other brain regions such as posterior parts of the temporal lobes, middle temporal gyrus, angular gyrus and the precuneus of the left hemisphere [51, 52]. This is because semantic information is transmitted from the anterior temporal pole to the left temporoparietal junction, where it is re-coded into phonological form and sent to the motor speech areas of the frontal lobes. This region is also responsible for short-term phonological storage (i.e., the "phonological loop") [53]. Therefore, despite the presence of anomia due to the disrupted connection between motor and semantic language areas, in lvPPA, unlike svPPA, single word comprehension is spared and no atrophy is observed in the left temporal pole responsible for the storage of verbal semantic information [52]. Impaired phonological memory leads to the main clinical manifestations of lvPPA, such as speech pauses when searching for words, phonological paraphasias when naming words (especially long ones), difficulty repeating long unfamiliar sentences or a series of numbers or words. However, repetition of single words or short phrases is usually unaffected or less affected.

Differential diagnosis between lvPPA and nfPPA tends to be the most difficult because of the similarity in clinical presentation (impaired fluency, pronunciation errors). Speech pauses in lvPPA are caused by difficulty finding words and alternate with fluent speech. Speech production rate is moderately reduced, not markedly as in nfPPA; patients can replace a forgotten word with its description; and they are well helped by prompts to the first syllable of the word, both in oral speech and in naming tasks [45, 54]. Sound errors in lvPPA consist mainly of substitution, deletion, or insertion of single existing phonemes, whereas in nfPPA they are phonetic, with initial mispronunciation of sounds [55]. As with nfPPA, there may be a slight decrease in the understanding of complex syntactic structures, but this is due to impaired phonological storage rather than a lack of understanding of grammatical structures [56]. Some studies suggest that difficulties in the diagnosis of lvPPA and in the differential diagnosis between lvPPA and nfPPA are due to drawbacks of existing diagnostic criteria [57, 58]. For example, nfPPA patients without apraxia of speech may meet the core criteria for both nfPPA and lvPPA. In this regard, it is proposed to slightly modify the existing criteria so that one of the mandatory signs of lvPPA would be the lack of agrammatism and language comprehension disorders [59]. Other researchers point to the lack of sensitivity of the criteria at the early stages and suggest to make them less stringent, allowing for some difficulty with single word comprehension and emphasizing the absence of any decline in fluency, except for isolated pauses for searching for words [60].

In addition to aphasia, lvPPA is characterized by mild to moderate non-verbal cognitive impairment, such as visuospatial impairment, dyscalculia, executive function and memory impairment, which may be primarily due to Alzheimer's degeneration [61–63]. Over time, global aphasia with significant impairment of other cognitive functions is common and, in the absence of a medical history, differential diagnosis with the amnestic variant of AD may be challenging [56, 62, 64]. Slow progression is less common, with minimal increase in symptoms and grey matter atrophy [65].

In lvPPA, various behavioral disturbances may also develop, although they are less severe than in svPPA and nfPPA. Anxiety, irritability, depression, and apathy are more typical, while disinhibition or decreased empathy are less common [10, 66]. Some studies show that behavioral disturbances in lvPPA occur only at the late stages, when the severity of aphasia reaches a high level [67].

LvPPA is characterized by atrophy of the left posterior perisylvian and parietal regions such as the inferior parietal lobule, the temporoparietal junction, and the posterior parts of the temporal lobe (Figure, *C*), which play an important role in phonological storage [1]. Over time, atrophy may progress to the frontal areas of the brain and the hippocam-

pus, which is associated with additional symptoms of other variants of PPA [68]. The pattern of atrophy also seems to depend on the pathomorphological variant of the disease. In one of the studies, atrophy was more severe in lvPPA with positive AD markers compared with lvPPA without AD markers in the left superior parietal region, inferior temporal gyrus, and more ventral parts of the superior and middle temporal gyri [46]. In general, less studies have been performed to evaluate neuroimaging markers of lvPPA compared with other variants because lvPPA is often considered together with other variants of AD without focusing on language disorders. Available studies of clinical and neuroimaging correlations have shown that naming disorders are associated with atrophy of the middle parts of the left temporal gyrus [33, 52], repetition disorders correlate with damage to the left angular, supramarginal gyri, and posterior parts of the superior temporal gyrus [51], and the rate of phonological speech errors increases with the severity of atrophy of the supramarginal gyrus and inferior parietal lobule [69]. In lvPPA, white matter damage is less severe than in nfPPA and svPPA, affecting only the temporoparietal regions [25].

Conclusion

Different variants of PPA represent a unique model to study speech function because their clinical diversity affects almost all speech domains. The selectivity of focal network damage allows a more precise determination of the structures and functionally related regions involved in different aspects of speech. Current insights on the function of the language network not only contribute to fundamental ideas about how information is processed in the central nervous system, but also have immediate practical implications. A deep understanding of the neuroanatomy of language and speech is necessary to perform neurosurgical procedures in functionally significant regions, such as tumor removal. The network model of language can also be used to rehabilitate aphasic patients by selecting the optimal target for navigated transcranial magnetic stimulation or transcranial direct current stimulation. Despite significant advances in the understanding of the neuroanatomy of language in recent years, further comprehensive studies of the language network using the latest neuroimaging and neurophysiological techniques are needed to identify alternative compensatory options for patients with language disorders.

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Information about the authors

Diliara R. Akhmadullina – postgraduate student, Research Center of Neurology, Moscow, Russia,
https://orcid.org/0000-0001-6491-2891
Ekaterina Yu. Fedotova – Dr. Sci. (Med.), leading researcher, Head, 5th Neurology department, Institute of Clinical and Preventive Neurology, Research Center of Neurology, Moscow, Russia,
https://orcid.org/0000-0001-8070-7644

Authors' contribution. *Akhmadullina D.R.* – study conceptualization, literature review and analysis, original draft preparation; *Fedotova E.Yu.* – study conceptualization, study supervision, manuscript review and editing.

Информация об авторах

Ахмадуллина Диляра Робертовна – аспирант Научного центра неврологии, Москва, Россия, https://orcid.org/0000-0001-6491-2891
Федотова Екатерина Юрьевна – д-р мед. наук, в. н. с., зав. 5-м неврологическим отделением Института клинической и профилактической неврологии Научного центра неврологии, Москва, Россия,
https://orcid.org/0000-0001-8070-7644

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Aerobic Exercise in Rehabilitation of Patients with Parkinson's Disease

Olga V. Guseva

Siberian State Medical University, Tomsk, Russia

Abstract

Disability due to Parkinson's disease (PD) is increasing faster than for any other neurodegenerative disorder. A multidisciplinary approach to rehabilitation of patients with PD is recommended including various types of physical training. Because of its general beneficial effect, aerobic endurance training is necessary for all people to maintain their health. Aerobic exercise in PD is also used for rehabilitation of motor and non-motor symptoms. This article justifies the choice of aerobic exercise intensity, shows challenges in selecting intensity based on maximum oxygen consumption due to the influence of clinical and behavioral factors, difficulties in assessing the effectiveness of therapy due to the wide range of training intensity and amount in the studies. The article summarizes types of exercises used in rehabilitation of patients with PD (walking, Nordic walking, training with a bicycle ergometer and treadmill, aquatic exercises) and their benefits for patients with different courses of the disease. For patients with freezing of gait, bicycle ergometer is a piece of equipment of choice for aerobic stationary training, and Nordic walking is a preferred type of outdoor training. The author shows the role of aerobic training in the treatment of non-motor symptoms such as depression, cognitive changes, and sleep disorders. A question about the use of aerobic training in patients with Hoehn–Yahr grade 4–5 of PD remains open. Further studies are needed to evaluate training protocols, assess rehabilitation effectiveness and evaluate physical training in the advanced PD.

Keywords: *Parkinson's disease; aerobic exercise; intensity; effectiveness; general beneficial effect; motor and non-motor symptoms*

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For correspondence: 2 Moscow tract, Tomsk, 634050, Russia. Siberian State Medical University. E-mail: gusolvl@gmail.com. Guseva O.V.

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Аэробная физическая нагрузка в реабилитации пациентов с болезнью Паркинсона

О.В. Гусева

Сибирский государственный медицинский университет, Томск, Россия

Аннотация

Болезнь Паркинсона (БП) является заболеванием с самым высоким приростом инвалидности среди нейродегенеративной патологии. Рекомендован мультидисциплинарный подход к реабилитации пациентов с БП, включающий различные типы физических тренировок. Аэробная тренировка на выносливость, вследствие общеукрепляющего действия, необходима для поддержания здоровья всем людям. Аэробную нагрузку при БП применяют также для реабилитации моторных и немоторных симптомов. В статье обоснован выбор интенсивности аэробной нагрузки, показаны трудности в подборе интенсивности при определении максимального потребления кислорода из-за влияния клинических и поведенческих факторов пациентов, сложности оценки эффективности терапии вследствие широкого диапазона представленных в исследованиях показателей интенсивности и объёма нагрузки. В статье обобщены типы спортивно-прикладных упражнений на выносливость, встречающихся при реабилитации пациентов с БП (ходьба, скандинавская ходьба, тренировки на велоэргометре и тредмиле, упражнения в воде), и их преимущества при разном течении болезни. Например, для пациента с застыванием аппаратурой выбора для аэробной стационарной тренировки является велоэргометр, а тренировки на улице предпочтительны в виде скандинавской ходьбы. Автором показано значение аэробной нагрузки для терапии немоторных симптомов: депрессии, когнитивных изменений и нарушения сна. Открытым остаётся вопрос о применении аэробной нагрузки у пациентов с БП 4–5-й стадии по Hoehn–Yahr. Требуются дальнейшие исследования по протоколу нагрузки, оценке эффективности реабилитации и применению нагрузки на развёрнутой стадии БП.

Ключевые слова: *болезнь Паркинсона; аэробные упражнения; интенсивность; эффективность; общеукрепляющий эффект; моторные и немоторные симптомы*

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Конфликт интересов. Автор заявляет об отсутствии явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Адрес для корреспонденции: 634050, Россия, Томск, ул. Московский тракт, д. 2, Сибирский государственный медицинский университет. E-mail: gusolvl@gmail.com. Гусева О.В.

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Introduction

Parkinson's disease (PD) is a chronic neurodegenerative disease the pathogenesis of which is based on progressive degeneration of dopamine-producing cells in the substantia nigra of the brain. Excessive accumulation of a protein called α-synuclein with the formation of Lewy bodies is the cause of the pathological changes at the cellular level [1, 2]. Familial PD accounts for 5–15% of all cases but genetic studies in these families helped understand the pathogenesis of the disease [3–5].

Clinically, PD is manifested by motor symptoms such as tremor, rigidity, slowness of movements, and postural instability. The resulting motor disturbances make the patient to consult a doctor. Dystonia and dyskinesia are associated with further speech and motor impairment. Patients with advanced PD develop freezing of gait [6]. Apart from motor symptoms, patients may have non-motor dysfunctions, which can bother them long before the onset of the motor symptoms[1]. These include depression, orthostatic hypotension, constipation, urinary disorders, weight loss, fatigue, sleep disorders, smell and taste disorders, delirium, hallucinations, pain, etc. [6–8]. The incidence of PD increases with age. PD occurs in 1.6–1.8% of people 65 years of age and older. The average age of onset is 61 years. However, 13% of cases are diagnosed before the age of 50 years [1, 9]. In all countries, disability due to PD is increasing faster than for any other neurodegenerative disorder.

In 2022, World Health Organization (WHO) launched Parkinson's disease technical brief justifying the relevance of increasing PD morbidity and disability and necessary solutions[2]. The technical brief includes respecting the patient's rights to timely diagnosis and treatment and an integrated approach to symptom management. Disease management is considered from a multidisciplinary perspective. Physical rehabilitation is an important part of the treatment. Various techniques are used for rehabilitation such as endurance, strength, balance, and flexibility training, external signal stimulation, dual tasks, etc. The superiority of any method has not been shown. Physical activity is also considered a preventive factor for PD, which reduces the risk of its occurrence, along with the use of tobacco, coffee, or calcium channel blockers [1, 10].

This review **aimed** to assess the effects of aerobic training and different types of aerobic physical exercise on health of PD patients.

Methodology

The search for article titles and abstracts was conducted in two open databases (i. e. PubMed and eLIBRARY.RU) and included available free full-text articles published before 06/07/2023 in English or Russian without restrictions on publication date. Literature search strategy is presented in the table below.

Search in eLIBRARY.RU database gave no articles for these keywords; search in PubMed database with literature references gave 115 articles with subsequent exclusion of non-relevant articles.

Aerobic exercise

The 2020 WHO guidelines for patients with neurodegenerative disease, including PD, define a beneficial minimum amount of physical activity [11]. People with PD, like all other patients and healthy people, need 75 to 150 minutes of vigorous-intensity regular aerobic physical activity per week or 150 to 300 minutes of moderate-intensity regular aerobic physical activity per week. To provide additional

[1] The American Parkinson Disease Association. Common Symptoms of Parkinson's Disease [updated 15 Apr 2023].
URL: https://www.apdaparkinson.org/what-isparkinsons/symptoms/#nonmotor (assessed on 11/19/2023).

[2] WHO. Launch of WHO's Parkinson disease technical brief. 2022 June 14 [updated 02 May 2023]. URL: https://www.who.int/news/item/14-06-2022-launch-of-who-s-parkinson-disease-technical-brief (assessed on 11/19/2023).

Characteristics of a literature search

Keyword	Parkinson's disease AND aerobic load OR aerobic exercises OR endurance
Databasa	PubMed, eLIBRARY.RU
Language	English, Russian
Document type	Peer-reviewed empirical and theoretical papers
Inclusion criteria	Population: patients with Parkinson's disease; Intervention: aerobic physical activity; Comparison is necessary for empirical papers
Exclusion criteria	Thesis papers, conference materials, articles in other languages

health benefits, moderate-intensity aerobic physical activity may be increased to more than 300 minutes per week or vigorous-intensity aerobic physical activity may be increased to more than 150 minutes per week. This amount of physical activity is necessary to strengthen the cardiopulmonary system, bones and muscles and decrease the risk of non-infectious disease and depression [11]. In this context, aerobic training in patients with PD is considered general beneficial physical activity. Required intensity of aerobic exercise is most often determined by heart rate (HR): 60–75% and 75–90% of the maximum heart rate correspond to medium (moderate) and vigorous intensity of physical activity, respectively. In several studies, intensity was determined by calculating necessary percentage of maximum heart rate [12, 13]. In other studies, heart rate reserve or maximum oxygen consumption (Vo_2max) were used [14]. In some studies, intensity varied during the training course from medium to high, which makes it difficult to assess the effectiveness of therapy [14].

Measuring oxygen consumption during exercise is the gold standard for determining endurance exercise intensity. During physical exercise on a treadmill or bicycle ergometer, oxygen consumption is linearly related to load power until Vo_2max is reached. Further increase in power is maintained for a short period due to anaerobic metabolism, which is caused by the accumulation of lactate. However, the linear relationship is conditional due to the influence of a person's gender, height, and age on Vo_2max. Several corrections are used for these factors [15]. The influence of common external factors such as lack of training and insufficient muscle mass is obvious; however, they cannot be taken into account by introducing one or another correction into the formula.

Given that patients with PD suffer from motor impairments, often do not have training skills and, like most people today, lead a sedentary lifestyle, determining Vo_2max to calculate the intensity of aerobic activity in this population is challenging. The "gold criterion" for diagnosing the required intensity of aerobic exercise may be applicable mainly for professional sports. The key benefits of aerobic exercise include cardiovascular fitness, both for primary and secondary prevention of cardiovascular disease. The more time a person engages in moderate-intensity and high-intensity aerobic exercise, the better their cardiovascular prognosis [16]. Interpretation of results and data on the effectiveness of aerobic exercise therapy for PD are inconsistent, since the studies were performed with different amounts of physical activity (i.e. course duration and frequency of training per week) and a wide range of training intensity (60–80% of maximum heart rate, 50–80% of reserve heart rate or 60–80% of Vo_2max). Exercise with too high intensity, i. e. greater than 11 MET (1200 kgm/min, or 200 W[3]), is not recommended for people who use exercise solely for health maintenance and disease prevention.

Aerobic training has a general beneficial effect on metabolism. Aerobic exercise was shown to reduce postprandial lipogenesis, muscle insulin resistance, high blood pressure, and metabolic syndrome [17]. The effect of aerobic exercise on metabolism is important for patients with PD because concomitant arterial hypertension and metabolic syndrome can lead to rapid PD progression [17]. Regular aerobic exercise were shown to improve calcium metabolism and bone mineral density. The prevalence of osteoporosis in patients with PD is high. The pathogenesis of osteoporosis is associated with inhibition of osteoclast differentiation, decreased mineralizing ability of osteoblasts with high doses of levodopa, decreased muscle mass due to age-related sarcopenia and insufficient physical activity due to hypokinesia and physical inactivity, as well as low levels of vitamin D due to intestinal dysfunction, decreased sense of smell, taste and appetite [18].

Moderate to high intensity aerobic endurance exercises are used in patients with PD. According to the 2022 Report of American Physical Therapy Association, this intensity has high strength and quality of evidence for aerobic training in patients with PD [19]. In addition to its general effect on the body, training of this intensity has a specific effect. Aerobic physical activity alleviates disease symptoms in patients with PD. This physical activity is associated with improved Vo_2max, motor skills, functional parameters, and quality of life [20]. Studies are being conducted to investigate effects of physical activity on motor and non-motor symptoms of PD and elucidate possible mechanisms underlying these effects. Study results showed improvements in motor symptoms,

[3] 1 Watt is equivalent to 6.1 kilogram meters per minute (kgm/min), 1 kgm = 1 J.

mobility, decreased freezing of gait, improved forward and backward walking, and positive effects on cognitive function [19–21].

A study in patients with PD was conducted to evaluate the effectiveness of aerobic exercise with a bicycle ergometer with increasing exercise intensity from medium to high during the course [21]. Intensity was assessed by heart rate reserve. The reference group of patients with PD performed stretching exercise. The authors evaluated the effect of physical activity on the brain using magnetic resonance imaging (MRI) data. During the study, a decrease in brain atrophy rate and an improvement in cognitive function were seen in the main group. Therefore, aerobic exercise was shown to support the stimulation of functional and structural neuroplasticity. MRI, clinical and psychological testing data indicated a slowdown in PD progression.

A similar conclusion was reached in laboratory studies. An experimental study of neurotoxin-induced parkinsonism in animals indicated a specific targeted effect of aerobic exercise on the brain [22]. Physical exercise increased dopamine release, affected synaptogenesis, improved regional cerebral circulation, and increased endogenous levels of neurotrophic substances in the brain (brain and glial neurotrophic factors), which may reduce striatal dopamine loss [21, 23, 24]. Studies of continuous moderate-intensity aerobic exercise were published, indicating an increase in dopaminergic stimulation after a treatment course [25].

Epidemiological and experimental data based on immune markers suggest that aerobic exercise reduces disease progression rate. However, there are still no valid immune biochemical markers for PD progression, so there is no evidence to support the modifying effects of aerobic exercise on the brain of patients with PD. Possible modification effects remain incredibly complex but future studies of aerobic activity are planned to elucidate them and consider aerobic exercise as replacement therapy during the washout period of specific dopaminergic medications or in the delayed treatment initiation setting [26].

Exercises for rehabilitation of motor functions

To perform the required amount of physical activity, different types of exercises can be used. Usually, walking of necessary intensity is used. For the purpose of the studies, physical activity intensity during walking is measured by Vo_2max or heart rate.

In walking training, additional equipment is used such as a treadmill or a modified treadmill with no load to lower body [26]. In addition to improving aerobic capacity, treadmill training improved balance and strength [26, 27]. A modified treadmill with no load to lower body is used for patients with severe pain, which is one of non-motor PD symptoms, and in patients with mental changes such as excessive fear. This equipment is used at the advanced stage of PD and in patients with orthostatic hypotension [10, 28].

In PD patients, gait impairment has dopaminergic origin, non-dopamine origin, and causes directly related to walking [29]. The latter type of disorders includes freezing of gait, i. e. a disabling phenomenon when the patient is in a state of short-term episodic absence or noticeable decrease in the forward movement of their legs, despite their intention to walk. For patients with freezing of gait, bicycle ergometer is a piece of equipment of choice for aerobic stationary training [30]. In addition, despite postural instability, PD patients demonstrate stability when riding a bicycle or exercising on a bicycle ergometer [29, 30].

At early stage of the disease, aerobic exercise is prescribed regardless of the time when the patient takes drug therapy. As the disease progresses, with many years of levodopa use it becomes impossible to complete the task, and training is carried out in the "on" state [21]. When the effect of high-intensity training on a treadmill and a bicycle ergometer were compared in the general population of patients with PD, no significant differences were found in improvement of aerobic abilities using different equipment [10].

A comparison of physical exercises carried out over a year and aimed at developing various physical parameters showed the superiority of aerobic exercise with a treadmill over dance therapy (tango) and stretching in motor symptoms and walking speed forward and backward, with the results maintaining for 3 months after therapy. Stretching therapy was less effective, although it improved motor function and backward movement speed. No changes in any parameters were reported after tango classes [27]. Another study compared the effectiveness of general and specific aerobic treadmill training, resistance training, and stretching. Only stretching and strength training increased muscle strength. Aerobic capacity assessed by Vo_2max increased only after a course of treadmill training; 6-minute walk test results improved after low- and high-intensity treadmill training, stretching, and strength training [31].

Walking is the most common type of aerobic activity. Walking training in patients with PD has general beneficial and specific effects. Walking is considered an independent rehabilitation method as it reduces the severity of motor symptoms and improves step length, walking speed, mobility, and balance. Modified Scandinavian (Nordic) walking can be a walking option for patients with PD [32]. The exercise power for Nordic walking is 6.6–7.7 MET (700 kgm/min, or 110 W) compared with 3.3–5.0 MET (450 kgm/min, or 75 W) for simple walking. A comparative study that involved stretching, Nordic walking, and simple walking showed the best rehabilitation results with Nordic walking, which was associated with an improvement in motor characteristics such as step length,

speed, gait variability, and postural stability [33]. Another study compared Nordic walking training in healthy people and patients with PD. Gait variability was assessed using a wearable accelerometer-based device placed on the ankle of the patient's most affected lower extremity in 60–120 min after administration of a dopaminergic agent vs. the ankle of the non-dominant leg of a healthy control subject. A course of training was associated with an improvement in spatiotemporal characteristics of walking. Step length and rhythmicity in the main and control groups became almost similar [34].

Freezing of gait impairs patients' mobility, significantly increases the risk of falling, and interferes with daily activities, reducing patients' quality of life. It occurs more often during step initiation and turning [35, 36]. In physical therapy management of PD, specific rehabilitation options such as external signal stimulation (sound, light, tactile) can be used [21]. During Nordic walking, impacts of poles on the ground or floor act as an element of stimulation, thus enhancing aerobic exercise [37]. Use of Nordic walking poles moves the muscles of the upper half of the body, which makes it easier to initiate a step due to better coordination.

Aquatic exercises are another aerobic activity option for patients with PD. M. Avenali et al. compared the effectiveness of deep-water exercises, Nordic walking, and dance therapy (samba) in patients with PD [36]. After a course of deep-water exercises, walking function in the 6-minute walk test and quality of life improved with, however, no effect on motor performances assessed by means of MDS-Unifed Parkinson's Disease Rating Scale, part III (UPDR-SIII). Deep-water exercises were effective in most severe patients at the advanced stage of the disease, although in patients with freezing of gait better results were shown with dance and Nordic walking.

Rehabilitation of non-motor functions

Besides motor symptoms, patients are also bothered by non-motor symptoms of PD, which have a negative impact on their quality of life. Unfortunately, not all authors assessed this important aspect of the patient's health when conducting aerobic training. The effects of aerobic training on sleep quality, cognitive level, and depression were evaluated most frequently [37–39]. In addition, non-motor symptoms were not planned as a primary endpoint in the studies [26]. Therefore, patients were not included in the studies based on the severity of their non-motor symptoms. Therefore, it is difficult to compare rehabilitation results for non-motor symptoms in the main and control groups. The motor rehabilitation course may have been not long enough to alleviate some non-motor symptoms, and the study results may have erroneously suggested that physical therapy had no effect on non-motor symptoms.

Depression is a very common symptom in various stages of PD. Its incidence rate is 2–90% [40]. Patients with PD and depression have worse quality of life. However, they are usually not screened for depression and do not receive treatment [41]. An analysis of depression treatment in patients with PD over the past 10 years showed that exercise is the most popular method of therapy [42]. Results of studies to evaluate aerobic exercises in PD with depression were inconsistent: some studies showed a positive effect of aerobic exercises, while others found no effect [14, 24, 42, 43].

Cognitive impairment in patients with PD is heterogeneous in severity and rate of progression. The symptoms range from cognitive deficits and mild cognitive impairment to dementia. Authors showed improvements in attention, memory, conscious actions, and information processing speed in healthy adults after 4 months of aerobic training with intensity Vo_2max = 70% [44]. In patients with PD and mild cognitive impairment, memory and conscious actions improved only after 2 years of aerobic exercise therapy [45]. Other authors found no benefit from aerobic exercise in improving cognitive symptoms in PD [14, 46, 47].

Sleep disorders are another common symptom in patients with PD. This non-motor symptom is seen in 40–80% of patients. Sleep disorders in patients with PD have a complex nature [48–50]. Early PD symptoms such as interrupted sleep and difficulty falling asleep are common in the general elderly population. These disturbances may be related to normal aging processes. PD is characterized by more pronounced sleep fragmentation and daytime sleepiness. Drug treatment for insomnia has limited options in PD. Transcranial magnetic stimulation is not effective [26]. Exercise has a positive effect on sleep; however, it is difficult to evaluate the effectiveness of aerobic exercise therapy [14, 51]. Patients with sleep disturbances are usually administered with mixed physical rehabilitation programs. Therefore, a positive effect (if any) may be related to an improvement of other training qualities but not endurance [51].

Time of initiation is important for physical therapy. Patients often already have Hoehn–Yahr grade 2–3 of PD at first presentation, and, in this case, they are late with starting physical therapy. In early stages of PD (Hoehn–Yahr grade 1–2), physical exercise as a treatment option has a pronounced protective effect with a significant slowdown in neurodegenerative process rate [1]. Individual training programs are an option of choice for all patients. The predictive model for aerobic exercise selection includes patient phenotype and exercise parameters. A common limitation of all studies examining the effects of physical exercise was the exclusion of patients with Hoehn–Yahr stages 4 to 5 [19].

Conclusion

Aerobic training for patients with PD is recommended as a general beneficial activity, used along with other types of physical exercises for rehabilitation of patients with motor

and non-motor symptoms. Further studies on rehabilitation protocols and evaluation are needed. A question about the use of aerobic training in patients with Hoehn–Yahr grade 4–5 of PD remains open; however, aerobic exercise of proven required intensity is unlikely to be performed by this category of patients. The amount of aerobic exercise to achieve a modifying effect on motor symptoms remains unclear. It is not known whether high-intensity aerobic training has benefits compared with moderate-intensity training, and there is a lack of studies on the treatment of non-motor symptoms [20].

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Information about the author

Olga V. Guseva – Cand. Sci. (Med.), exercises treatment doctor, Physiotherapy department, assistant professor, Division of advanced-level therapy with a course in rehabilitation, physiotherapy and sports medicine, Siberian State Medical University, Tomsk, Russia,
https://orcid.org/0000-0001-8659-9832

Информация об авторе

Гусева Ольга Владимировна – канд. мед. наук, врач по лечебной физкультуре отделения физиотерапии, доцент каф. госпитальной терапии с курсом реабилитации, физиотерапии и спортивной медицины Сибирского государственного медицинского университета, Томск, Россия,
https://orcid.org/0000-0001-8659-9832

Sensitivity and Specificity of the Diagnostic Method for Detecting α-Synuclein as a Histological Marker for Parkinson's Disease in Salivary Gland Tissues: a Systematic Review and Meta-analysis

Kristina K. Khacheva, Sergey N. Illarioshkin, Alexey V. Karabanov

Research Center of Neurology, Moscow, Russia

Abstract

Immunohistochemistry of α-synuclein (α-syn), a marker for Parkinson's disease, in salivary gland (SG) biopsy specimens has been actively studied as a method of verification and early diagnosis. This systematic review and meta-analysis ***aim*** *to analyze characteristics of study designs and evaluate pooled sensitivity and specificity.*

The review included publications that were found by keyword search and met inclusion criteria. The meta-analysis of comparative studies was conducted using a univariate random-effects model to calculate pooled specificity and sensitivity.

The systematic review and meta-analysis included 16 and 13 clinical studies, respectively. Antibodies against modified α-syn, double detection, and incisional biopsy specimens of SGs were the most common approaches used in the studies. There is a need for clinical studies with quantitative data analysis. Approximately 15% of patients experienced adverse events, which were more common in case of fine-needle aspiration biopsy specimens of SGs. Pooled sensitivity and specificity (regardless of the anti-α-syn antibody type and SG size) were 76.6% and 98.0%, respectively. Sensitivity (76.3%) and specificity (99.3%) were higher when antibodies against phosphorylated α-syn and major SGs were used.

The most promising variant of the method involved double detection using antibodies against modified α-syn and markers of nerve fibers in incisional biopsy specimens of major SGs and quantitative data analysis. The meta-analysis revealed a possibility of developing this diagnostic method and implementing it into routine practice owing to its high sensitivity and specificity. Further studies employing quantitative data analysis are required to gain deeper insight into the method's role in verifying Parkinson's disease and informing the severity of neurodegeneration and disease prognosis.

Keywords: *Parkinson's disease; salivary gland biopsy; immunohistochemistry; α-synuclein; meta-analysis; systematic review*

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For correspondence: 80 Volokolamskoye shosse, Moscow, 125367, Russia. Research Center of Neurology.
E-mail: christina.khacheva@gmail.com. Khacheva K.K.

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Анализ чувствительности и специфичности метода детекции α-синуклеина в ткани слюнных желёз в качестве диагностического гистологического маркера болезни Паркинсона: систематический обзор и метаанализ

К.К. Хачева, С.Н. Иллариошкин, А.В. Карабанов

Научный центр неврологии, Москва, Россия

Аннотация

Иммуногистохимическое исследование маркера болезни Паркинсона α-синуклеина (α-syn) в биоптатах слюнных желёз (СЖ) – один из активно изучаемых методов верификации и ранней диагностики заболевания. ***Цель*** *систематического обзора и метаанализа – проанализировать особенности дизайнов клинических исследований (КИ) и оценить объединённую чувствительность и специфичность метода.*

В обзор включались публикации, найденные по заданным ключевым словам и соответствующие критериям включения. Метаанализ проводился только для сравнительных КИ с использованием универсантной модели случайных эффектов с целью вычисления объединённой специфичности и чувствительности.

В систематический обзор включены 16 КИ, в метаанализ – 13 КИ. Наиболее часто в КИ использовали антитела (АТ) к модифицированному α-syn и двойную детекцию, а также инцизионные биоптаты СЖ. Выявлена необходимость проведения КИ с количественной оценкой результатов. Доля пациентов с нежелательными явлениями составила около 15%, они чаще отмечались при использовании тонкоигольной биопсии СЖ. Объединённая чувствительность и специфичность метода (без учёта вида АТ к α-syn и размера СЖ) составили 76,6 и 98,0% соответственно. При использовании АТ только к фосфорилированному α-syn и крупных СЖ показаны бóльшие чувствительность (76,3%) и специфичность (99,3%).

Наиболее перспективным вариантом методики является двойная детекция с АТ к модифицированному α-syn и маркерам нервных волокон в инцизионном материале крупных СЖ с количественной оценкой результатов. Метаанализ продемонстрировал возможность развития и внедрения метода в клинику как диагностического из-за его высокой чувствительности и специфичности. Необходимы дальнейшие КИ с количественной оценкой для получения полного представления о значимости метода не только для верификации болезни Паркинсона, но и для получения представлений о выраженности нейродегенеративного процесса и прогнозе течения заболевания.

Ключевые слова: *болезнь Паркинсона; биопсия слюнной железы; иммуногистохимия; α-синуклеин; метаанализ; систематический обзор*

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

Адрес для корреспонденции: Россия, Москва, Волоколамское шоссе, д. 80. Научный центр неврологии.
E-mail: christina.khacheva@gmail.com. Хачева К.К.

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Introduction

Postmortem histology with α-synuclein (α-syn) detection in the substantia nigra is the gold standard in diagnosing Parkinson's disease (PD) [1]. The α-synuclein protein is predominantly expressed in the nervous system and located in presynaptic terminals, where it is involved in vesicle transport, regulation of dopamine release, and intracellular calcium homeostasis [2].

Exogenous and endogenous factors that trigger α-syn modification and aggregation, as well as molecular processes directly causing accumulation of α-syn aggregates have yet to be elucidated. To date, there have only been assumptions about potential effects of polymorphisms in risk genes, chemicals, physical forces (eg, brain injuries), and radiation on the activation of oxidative stress and neuroinflammation, inducing α-syn aggregation [3]. Modified (phosphorylated or nitrosylated) α-syn disrupts intracellular transport and neurotransmission, increases oxidative stress due to mitochondrial dysfunction, and causes microglial activation. These processes underlie neuronal dysfunction, which results in clinical manifestations of PD as the α-syn aggregates continue to accumulate [4]. Thus, despite a large number of unexplored factors and processes contributing to the PD pathogenesis, a key role in PD development was attributed to modified α-syn that can be used as a histological marker for PD [4, 5].

More than 20 years ago H. Braak et al. hypothesized the gut-brain axis in PD and formation of α-syn aggregates in the peripheral nervous system long before their appearance in the substantia nigra and typical motor symptoms of PD [6]. Clinical studies showed that such aggregates are detected by histological evaluation of specimens not only of the brain but also of other organs with abundant peripheral innervation, which explains non-motor symptoms of PD that are associated with peripheral nervous system dysfunction and tend to predate motor impairments [5, 7]. Findings of these studies suggest a possibility of antemortem histological diagnosis of sporadic PD by detecting its main marker, pathological α-syn, in biopsy specimens of skin, intestine, and salivary glands (SG) [5, 8, 9].

A systematic review of English-language articles came to the conclusion that biopsy and histology of the skin and SGs could be potential diagnostic tools in PD, whereas intestinal biopsy and histology were excluded due to the low rate of aggregate detection, safety concerns, and high complexity of sampling [9]. However, other researchers highlight a number of limitations to skin biopsy as a diagnostic tool [10, 11]. A relatively high rate of detecting α-syn aggregates during skin examinations is highly dependent on sampling sites and requires examining multiple biopsy sites to obtain adequate results. Furthermore, most skin examinations use a frozen section technique, a less common and accessible laboratory procedure worldwide compared with paraffin sections [10].

Some researchers believe that main issues of using SG specimens for α-syn detection can be linked to frequent sampling of surrounding tissues (muscles, adipose tissue, lymph nodes) in addition to SG tissues [9]. It should be noted that the mentioned drawbacks are associated with fine-needle aspiration biopsy (FNAB) of minor SGs, which is a minimally invasive procedure that obtains only a small volume of tissue (7–34 mm^3 according to the studies) [12, 13]. However, utilization of major SGs and incisional biopsy (IB), which obtains an SG tissue volume of 84–390 mm^3 via a small (< 1 cm) incision, significantly mitigates the issue of insufficient study material [14–17]. Currently, there are no Russian meta-analyses of studies examining characteristics of various immunohistochemistry (IHC) techniques for SG biopsy specimens and safety data. The only similar meta-analysis was conducted by foreign researchers approximately 5 years ago and did not include Russian studies [9].

Our systematic review and meta-analysis of Russian and foreign clinical studies **aimed** to evaluate pooled sensitivity and specificity of IHC in terms of detecting modified α-syn in SG tissues. Furthermore, we assessed safety and analyzed characteristics of study designs to determine the most promising technique.

Methodology

This systematic review included all clinical studies (in English and Russian) on α-syn detection in SG tissues of patients with verified PD (both antemortem and postmortem). PubMed and Google Scholar were searched using the following keywords: "болезнь Паркинсона," "биопсия," "слюнная железа," "синуклеин," "Parkinson's disease," "biopsy," "salivary gland," and "synuclein". We did not consider articles without full-text access due to the inability to analyze study designs and findings. We did not include duplicate publications and articles concerning only histological examination of SG biopsy specimens in PD patients that did not have statistical analysis or data analysis. For the meta-analysis we pooled articles that met the above criteria and selected comparative studies involving PD patients and controls.

We took into account data on the number of participants and their diagnoses, methods of diagnosis verification, use of various tests to assess the condition of PD patients, studied specimens and sampling techniques, IHC markers, data analysis (qualitative/semiquantitative/quantitative), number and characteristics of adverse events (AE) caused by an intervention, as well as data to evaluate sensitivity and specificity. We disregarded data concerning α-syn aggregates in specimens of patients with other neurodegenerative disorders to calculate pooled sensitivity and specificity because such patients could not be assigned either to a control group (due to the high likelihood of α-syn detection in their specimens compared with healthy volunteers) or a group of PD patients.

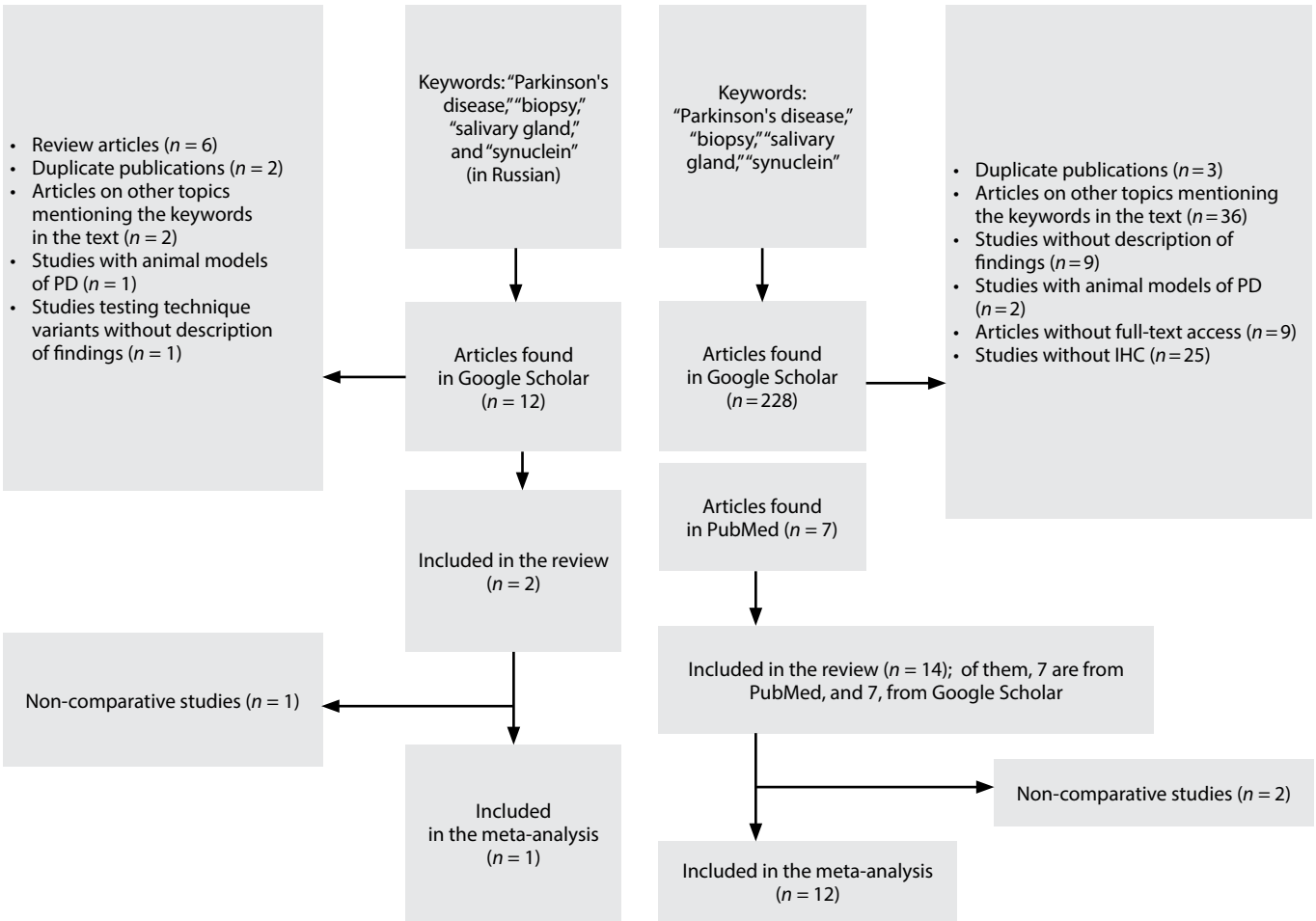

Fig. 1. Study selection for systematic review and meta-analysis.

Statistical analysis

Statistical analysis and data processing were performed using the Python programming language, SciPy module[1], and MetaDiSc 2.0, a software to perform test accuracy meta-analysis [18]. We calculated medians, interquartile ranges (IQR), and percentages. We used a univariate random-effects model, which is suitable for analyzing a small number of heterogeneous studies, to calculate pooled specificity and sensitivity, diagnostic odds ratio (DOR), false positive rate (FPR), positive likelihood ratio (PLR), and negative likelihood ratio (NLR). Heterogeneity was measured using the I^2 test (range from 0% to 100%) that describes the percentage of variability between the studies.

General characteristics of study designs and participants

We analyzed 16 publications that met the inclusion criteria: of them, 14 articles (87.5%) were published in English between 2010 and 2023 and included in PubMed [14–17, 19–28] (Table, Fig. 1). All the studies were open and non-randomized. The comparative studies involving controls who underwent the same procedures as a study group accounted for 81.2% ($n = 13$) [13–15, 17, 19–27]. Their findings were used for 2×2 tables and meta-analysis of sensitivity and specificity. The findings of non-comparative studies were used to analyze safety and examine characteristics of different techniques.

The total number of participants in 16 clinical studies was 762. Of them, 712 underwent all examinations, including SG biopsy with subsequent IHC. Clinically verified diagnosis (PD) was made in 288 patients, of whom 260 underwent all examinations. In 15 studies, PD was sporadic (idiopathic), while in one study, it was genetic, which was confirmed by genetic testing. In 6 studies, PD diagnosis was made only by the Movement Disorder Society diagnostic criteria, whereas in 5 studies, by findings of dopamine transporter single-photon emission computed tomography. In 3 studies, patients underwent brain magnetic resonance imaging (MRI) and transcranial sonography of the

[1] Python SciPy scientific computation library. URL: https://scipy.org

Data from the articles meeting the inclusion criteria

No.	Source	Number of patients	Number of patients undergoing biopsy and immunohistochemistry	Number of PD patients undergoing biopsy and immunohistochemistry	PD diagnosis verification (beyond the Movement Disorder Society diagnostic criteria)	Hoehn and Yahr stage	PD etiology	Number of controls undergoing biopsy and IHC	Sampling site	Sampling technique	Ab	Ab for double detection	True-positive result	False-positive result	Data analysis
1	et al. (2011)	6	6	3	NA	2	Idiopathic	3	Minor SGs	IB	α-syn	Neurofilaments	2	0	Scale +/–
2	Adler C.H. et al. (2014)	15	12	12	NA	NA	Idiopathic	NA	Submandibular/ minor SGs	FNAB	pS129-α-syn	NA	9	NA	Scale +/–
3	Adler C.H. et al. (2016)	35	28	19	DAT scan	NA	Idiopathic	9	Submandibular SG	FNAB	pS129-α-syn	NA	14	2	Scale +/–
4	Beach T.G. et al. (2016)	228	228	46	NA	NA	Idiopathic	79	Sublingual SG	IB	pS129-α-syn	NA	42	0	Scale +/–
5	Folgoas E. et al. (2013)	27	27	16	NA	NA	Idiopathic	9	Minor SGs	IB	pS129-α-syn	NA	3	2	0–5 scale
6	Gao L. et al. (2015)	26	26	13	DAT scan	1,7	Idiopathic	13	Minor SGs	IB	α-syn	NA	9	0	Scale +/–
7	Vilas D. et al. (2016)	71	47	12	NA	2	Idiopathic	26	Submandibular SG	FNAB	α-syn/pS129-α-syn	Tyrosine hydroxylase	8	0	Scale +/–
8	Carletti R. et al. (2017)	14	14	7	NA	NA	Idiopathic	7	Minor SGs	IB	α-syn/pS129-α-syn	NA	5	0	Quantitative
9	Shin J. et al. (2019)	16	16	16	NA	1,8	Idiopathic	14	Submandibular SG	FNAB/IB	pS129-α-syn	S100	8	0	Scale +/–
10	Iranzo A. et al. (2018)	118	118	13	Transcranial sonography of the substantia nigra	2,4	Idiopathic	33	Minor SGs	IB	α-syn/pS129-α-syn	Tyrosine-hydroxylase/ PGP 9.5 Iodotyrosine deiodinase	7	1	0–4 scale

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No.	Source	Number of patients	Number of patients undergoing biopsy and immunohistochemistry	Number of PD patients undergoing biopsy and immunohistochemistry	PD diagnosis verification (beyond the Movement Disorder Society diagnostic criteria)	Hoehn and Yahr stage	PD etiology	Number of controls undergoing biopsy and IHC	Sampling site	Sampling technique	Ab	Ab for double detection	True-positive result	False-positive result	Data analysis
11	Fernández-Espejo E. et al. (2021)	80	75	45	DAT scan	2,1	Idiopathic	30	Parotid SG	FNAB	Nitrosylated α-syn	Iodotyrosine deiodinase	45	0	0–5 scale
12	Ma L.-Y. et al. (2019)	26	15	8	DAT scan	1,6	Idiopathic	7	Minor SGs	IB	Nitrosylated α-syn	NA	8	0	Scale +/–
13	Mangone G. et al. (2022)	61	61	27	Magnetic resonance imaging	2	Idiopathic	18	Minor SGs	FNAB	α-syn	NA	15	7	Scale +/–
14	Shin J.H. et al. (2020)	3	3	3	NA	NA	Familial	NA	Minor SGs	FNAB	pS129-α-syn	Neurofilaments	0	NA	0–4 scale
15	Khudoerkov R.M. et al. (2016)	10	10	8	NA	2,1	Idiopathic	2	Major SGs	IB	pS129-α-syn/α-syn	β-Tubulin/ tyrosine hydroxylase	6	1	0–4 scale
16	Khacheva K.K. et al. (2023)	12	12	12	Transcranial sonography of the substantia nigra	2,3	Idiopathic	NA	Sublingual gland	IB	pS129-α-syn	PGP 9,5	12	NA	Quantitative
	Всего	748	698	260	NA	2 [1,85; 2,1]		236							

substantia nigra [22, 25, 28]. Hoehn and Yahr stages of PD were described in 10 (62.5%) publications (median, 2 [IQR, 1.85–2.10]) [17, 21, 23, 24, 28]. The mean PD duration (6.51 ± 3.70 years) was indicated in 15 studies [12, 13, 15, 19–21, 23–28].

Characteristics of immunohistochemistry techniques for assessment of salivary glands specimens

Minor SGs were sampled in a half of the studies [15, 16, 20, 22, 23, 25–27] (Table). Among the studies with major SG specimens, submandibular (4 studies), sublingual (2), and parotid (1) SGs were examined. In one study, the exact sampling site of major SGs was not indicated [12–14, 17, 19, 21, 24, 28].

In 15 (93.7%) studies, antemortem biopsy specimens were used, whereas in 1, postmortem specimens. In 10 studies, SG tissues were sampled by IB; in 6 studies, by FNAB. The publications with FNAB (especially without ultrasound guidance) demonstrated a high likelihood of sampling material unrelated to SGs, thus causing to repeat a procedure or exclude a patient from the study [13, 21]. The small volume of collected tissues usually allowed only to determine the presence of α-syn inclusions, and authors could not fully assess the extent or characteristics of their spread [15, 16, 20, 23, 25, 27]. IB did not have such drawbacks and allowed for sufficient amount of SG specimens.

Two (11%) studies additionally compared findings of SG histology and neuroimaging (MRI and transcranial sonography of the substantia nigra) [26, 28]. Both studies did not find any correlation between the neuroimaging and histology findings.

In 8 studies, motor and non-motor functions of PD patients were additionally assessed by various tests and questionnaires: the Unified Parkinson's Disease Rating Scale, Non-Motor Symptoms Scale, Non-Motor Symptoms Questionnaire, Parkinson's Disease Quality of Life Questionnaire, Montreal Cognitive Assessment, Mini-Mental State Examination, Epworth Sleepiness Scale, and University of Pennsylvania Smell Identification Test [13, 16, 20, 21, 23, 25, 26, 28]. Only one study investigated a potential relationship between clinical symptoms and histology findings and demonstrated a correlation between the spread of inclusions of α-Syn phosphorylated at serine 129 (pS129-α-syn) and severity of non-motor symptoms, sleep disorders, and emotional disorders [28]. This study did not find any correlation with Hoehn and Yahr stages.

In all the studies, IHC examination for various α-syn forms in SG tissues was performed: pS129-α-syn (11 studies; 68.7% of all the studies), nitrosylated α-syn (2 studies; 12.5%), and α-syn without posttranslational modifications (7 studies; 43.7%).

Seven (43.7%) studies used double detection with antibodies (Ab) against α-syn and markers of nerve fibers: tyrosine hydroxylase (3 studies), PGP 9.5 (2 studies), neurofilaments (2 studies), and β-tubulin (1 study) [14, 15, 17, 21, 23, 27, 28]. In all the studies, paraffin-embedded tissues were used for IHC. The studies in which pS129-α-syn was detected used 3 types of Abs: anti-pS129-α-syn mouse monoclonal Abs (WAKO, clone No. pSyn#64; Abcam, clone No. 81A, ab184674) and/or anti-pS129-α-syn rabbit monoclonal Abs (Abcam, clone No. EP1536Y). The studies were characterized by high variability of histology and IHC techniques and Ab clones. The highest number of true-positive results (>70% of positive results in all PD patients) was observed in the studies using double detection and nitrosylated α-syn and pS129-α-syn.

In 5 (31.2%) studies, semiquantitative analysis was used to assess the spread of α-syn inclusions; in 9 (56.2%) studies, qualitative analysis was used, taking into account only the presence or absence of inclusions. Only 2 (12.5%) studies employed quantitative analysis [22, 28]. In the former study, ImageJ software was used to measure the spread, and the authors calculated ratios of pS129-α-syn and unmodified α-syn inclusions to nerve fibers labeled with anti-S100 Abs and to the total specimen area. Calculations revealed that the ratio of unmodified α-syn to nerve fibers was lower in PD patients compared with controls due to nerve fiber degeneration, whereas the presence of pS129-α-syn and its high ratio relative to nerve fibers was characteristic of PD patients only [22]. In the latter study, measurement was performed with a software written in Python 3.9 using the Open CV library for calculations and scikit-image for image processing. The authors calculated the absolute value of the pS129-α-syn inclusion area and the ratio of the inclusion area to the area of nerve fibers labeled with anti-PGP 9.5 Abs. pS129-α-syn was detected in all PD patients, and the ratio directly correlated with the results of the clinical condition assessment and severity of non-motor symptoms [28].

In all the studies, α-syn inclusions in various modifications were detected in SG specimens of PD patients. PD patients with true-positive results (ie, inclusions of modified α-syn) accounted for more than 70% in 8 (50%) studies, 6 of which (46.1%) were comparative [12, 13, 17, 19, 22, 24, 25, 28]. Among these 8 studies, the studies with IB and IHC of major SG specimens accounted for 62.5% and 75%, respectively.

There were 100% of positive results in 3 (18.75%) studies, 2 of which (15% of all comparative studies) were comparative [24, 25, 28]. Among these 3 studies, 2 studies were conducted using major SG specimens [24, 28].

In 5 (31.2%) studies, rare α-syn inclusions were detected in controls without PD. In half of these studies, unmodified α-syn without any phosphorus or nitrogen residues was used as the main marker [13, 17, 20, 23, 26]. Such cases were attributed to potential early (preclinical) PD or defects of IHC staining [13, 17, 22]. Such assumption is supported by the fact that Lewy bodies are found during postmortem examination in approximately 10% to 20% of people older than

60 years without any signs of parkinsonism or dementia [29]. Unmodified α-syn detected in controls (2 studies) is shown to be a relatively common and normal finding in the peripheral nervous system of healthy individuals without neurodegeneration [17, 23].

Accumulation of modified α-syn was observed in some patients with other neurodegenerative disorders. Positive results (presence of pS129-α-syn and nitrosylated α-syn) were characteristic of patients with Alzheimer's disease and dementia with Lewy bodies in 4 studies [16, 21, 23, 26].

In 3 studies, α-syn aggregates were found in 43.8%, 50%, and 89% of patients with idiopathic rapid eye movement sleep behavior disorder [21, 23, 26]. Idiopathic rapid eye movement sleep behavior disorder without other symptoms is considered a prodromal phase of neurodegeneration and eventually progresses into clinically established PD in 80% of cases [21]. Thus, positive results in this group are likely to be caused by an early stage of synucleinopathy.

Safety analysis was based on 15 studies with in vivo assessment of α-syn inclusions in SG specimens. AEs were reported in 4 (26.6%) studies, 3 of which were conducted using FNAB (50% of all studies with FNAB), and 1 of which, using IB (10% of all studies with IB). The total number of patients with AEs was 77 (14.8% of all the participants [$n = 520$] who underwent IB in 15 studies). No severe AEs were observed. The most common AEs were transient edema, minor hemorrhage, and pain at the biopsy site. Moderate throat pain and minor hemorrhage at the biopsy site (in case of FNAB) were less common. All the AEs were transient and mild and resolved without any medical or surgical intervention.

Sensitivity and specificity of immunohistochemistry techniques for α-syn detection in salivary glands

Of 16 clinical studies for the meta-analysis, we selected 13 comparative studies that compared the rates of detecting modified and unmodified α-syn using Abs in minor and major SG specimens of patients with PD and controls without neurodegenerative disorders [13–17, 19–26]. The total number of participants undergoing biopsy was 685; of them, PD was clinically diagnosed in 223 patients.

Pooled sensitivity across the 13 comparative studies with a control group, regardless of anti-α-syn Abs and specimens (i. e., all the studies using minor and major SG specimens), was 0.749 (95% CI, 0.575–0; $I^2 = 52\%$); specificity, 0.984 (95% CI, 0.855–0.999; $I^2 = 0.0$); DOR, 188.33 (95% CI, 15.42–2299.96); FPR, 0.016 (95% CI, 0.001–0.145); PLR, 48.02 (95% CI, 4.6–501.25); NLR, 0.255 (95% CI, 0.14–0.46) (Fig. 2).

Pooled sensitivity across the 8 comparative studies [13, 14, 17, 19–23] using only anti-pS129-α-syn Abs was 0.66 (95% CI, 0.476–0.800; $I^2 = 70.6\%$); specificity, 0.974 (95% CI, 0.840–0.996; $I^2 = 0.0$); DOR, 71.4 (95% CI, 8.81–578.76); FPR, 0.026 (95% CI, 0.004–0.160); PLR, 24.96 (95% CI, 3.67–169.50); NLR, 0.349 (95% CI, 0.211–0.578) (Fig. 3).

Pooled sensitivity across the 5 comparative studies [13, 14, 17, 19, 21] using anti-pS129-α-syn Abs and major SG specimens was 0.761 (95% CI, 0.608–0.993; $I^2 = 55.6\%$); specificity, 0.993 (95% CI, 0.197–1.000; $I^2 = 0.0$); DOR, 460.08 (95% CI, 0.75–281555.50); FPR, 0.007 (95% CI, 0–0.803); PLR, 110.67 (95% CI, 0.196–62405.980); NLR, 0.241 (95% CI, 0.139–0.420) (Fig. 4).

Thus, the highest sensitivity (76.3%) and specificity (99.3%) were observed with major SG specimens and IHC for pS129-α-syn. Nevertheless, even with a lack of detailed description of techniques, biopsy sensitivity and specificity in terms of PD marker detection were 75% and 98.4%, respectively.

Discussion

The systematic review that included 16 studies with 260 patients who underwent SG biopsy and IHC examination of α-syn inclusions (a marker for PD) demonstrated relatively high significance of the method in idiopathic PD diagnosis. The majority of the studies analyzed and compared findings with those of controls. In over 80% of the studies, ICH involved Abs against modified forms of α-syn, whereas 50% of the studies employed the most effective method: double detection. Most studies used only qualitative data analysis. Semiquantitative analysis was less common, whereas quantitative analysis was described only in 2 recent studies. The majority of studies employed standard and effective histological and IHC techniques, which are straightforward to replicate in anatomical pathology laboratories and have no constraints on their extensive utilization. Nevertheless, at the outset of the method's investigation, the absence of quantitative techniques for analyzing the extent of α-syn inclusion spread may impede further research and the integration of the method into clinical practice.

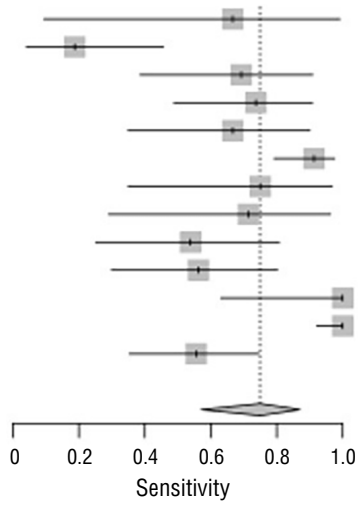
Regardless of qualitative or quantitative data analysis, the IHC effectiveness in PD verification using Abs against modified α-syn was relatively high: true-positive results exceeded 70% in half of all studies with or without controls and in 46% of the comparative studies. In one fifth of all studies, the positive results accounted for 100%.

In vivo biopsy safety was satisfactory: there were approximately 15% of patients with mild AEs across all the studies. AEs were more common in the studies using FNAB than those with IB.

The meta-analysis revealed that the pooled sensitivity and specificity of the method for detecting the PD marker in SG specimens (regardless of the anti-α-syn Abs and SG size) were 76.6% and 98%, respectively. We observed a decrease in sensitivity (66%) and specificity (97%) when analyzing the studies

A

Study	TP	Total (TP + FN)	Sensitivity	95% CI
Cersósimo M.G. et al. (2011)	3		0.67	[0.09; 0.99]
Folgoas E. et al. (2013)	16		0.19	[0.04; 0.46]
Gao L. et al. (2015)	13		0.69	[0.39; 0.91]
Adler C.H. et al. (2016)	19		0.74	[0.49; 0.91]
Vilas D. et al. (2016)	12		0.67	[0.35; 0.90]
Beach T.G. et al. (2016)	46		0.91	[0.79; 0.98]
Khudoerkov R.M. et al. (2016)	8		0.75	[0.35; 0.97]
Carletti R. et al. (2017)	7		0.71	[0.29; 0.96]
Iranzo A. et al. (2018)	13		0.54	[0.25; 0.81]
Shin J. et al. (2019)	16		0.56	[0.30; 0.80]
Ma L.-Y. et al. (2019)	8		1.00	[0.63; 1.00]
Fernández-Espejo E. et al. (2021)	45		1.00	[0.92; 1.00]
Mangone G. et al. (2022)	27		0.56	[0.35; 0.75]
Random-effects model			0.75	[0.57; 0.87]



B

Study	TN	Total (TN + FP)	Specificity	95% CI
Cersósimo M.G. et al. (2011)	3	3	1.00	[0.29; 1.00]
Folgoas E. et al. (2013)	7	9	0.78	[0.40; 0.97]
Gao L. et al. (2015)	13	13	1.00	[0.75; 1.00]
Adler C.H. et al. (2016)	7	9	0.78	[0.40; 0.97]
Vilas D. et al. (2016)	26	26	1.00	[0.87; 1.00]
Beach T.G. et al. (2016)	79	79	1.00	[0.95; 1.00]
Khudoerkov R.M. et al. (2016)	1	2	0.50	[0.01; 0.99]
Carletti R. et al. (2017)	7	7	1.00	[0.59; 1.00]
Iranzo A. et al. (2018)	32	33	0.97	[0.84; 1.00]
Shin J. et al. (2019)	14	14	1.00	[0.77; 1.00]
Ma L.-Y. et al. (2019)	7	7	1.00	[0.59; 1.00]
Fernández-Espejo E. et al. (2021)	30	30	1.00	[0.88; 1.00]
Mangone G. et al. (2022)	11	18	0.61	[0.36; 0.83]
Random-effects model			0.98	[0.87; 1.00]

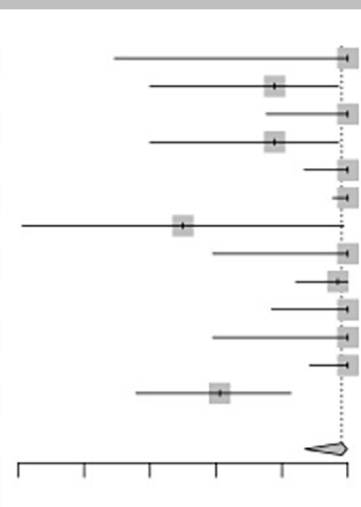
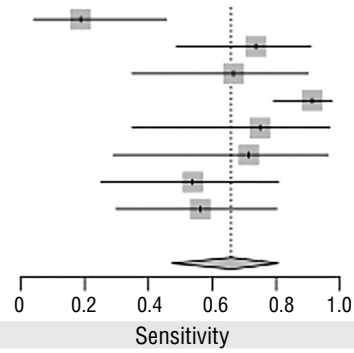


Fig. 2. Pooled sensitivity (*A*) and specificity (*B*) across all the comparative studies for methods of detecting α-syn in SGs of PD patients and controls, regardless of anti-α-synuclein Abs.
Here and in Fig. 3 and 4: TP, true-positive result; TN, true-negative result; FP, false-positive result; FN, false-negative result.

that used anti-pS129-α-syn Abs and did not specify the SG size. The studies that used anti-pS129-α-syn Abs and major SG specimens demonstrated greater sensitivity (76.3%) and specificity (99.3%) compared with the rest. This can be linked to the higher likelihood of detecting aggregates in specimens with more abundant monoaminergic innervation, as well as to significantly higher prevalence of α-syn forms with post-translational modifications among patients with neurodegenerative disorders compared with healthy individuals [31]. The latter conclusion is evidenced not only by the literature data but also by the pooled PLR of 110.6, indicating that likelihood of detecting modified α-syn using biopsy and IHC is 100-fold higher in PD patients than in healthy individuals. The fact that PLR was significantly higher than the generally accepted value of 10 allows us to assume the high diagnostic ability of the method in terms of detecting the studied PD marker. The meta-analysis revealed that the DOR of anti-pS129-α-syn Ab detection in major SG specimens was high (460.08), which suggests the potentially high effectiveness in differentiating participants by the presence or absence of PD.

A

Study	TP	Total (TP + FN)	Sensitivity	95% CI
Folgoas E. et al. (2013)	3	16	0.19	[0.04; 0.46]
Adler C.H. et al. (2016)	14	19	0.74	[0.49; 0.91]
Vilas D. et al. (2016)	8	12	0.67	[0.35; 0.90]
Beach T.G. et al. (2016)	42	46	0.91	[0.79; 0.98]
Khudoerkov R.M. et al. (2016)	6	8	0.75	[0.35; 0.97]
Carletti R. et al. (2017)	5	7	0.71	[0.29; 0.96]
Iranzo A. et al. (2018)	7	13	0.54	[0.25; 0.81]
Shin J. et al. (2019)	9	16	0.56	[0.30; 0.80]
Random-effects model			0.66	[0.47; 0.81]



B

Study	TN	Total (TN + FP)	Specificity	95% CI
Folgoas E. et al. (2013)	7	9	0.78	[0.40; 0.97]
Adler C.H. et al. (2016)	7	9	0.78	[0.40; 0.97]
Vilas D. et al. (2016)	26	26	1.00	[0.87; 1.00]
Beach T.G. et al. (2016)	79	79	1.00	[0.95; 1.00]
Khudoerkov R.M. et al. (2016)	1	2	0.50	[0.01; 0.99]
Carletti R. et al. (2017)	7	7	1.00	[0.59; 1.00]
Iranzo A. et al. (2018)	32	33	0.97	[0.84; 1.00]
Shin J. et al. (2019)	14	14	1.00	[0.77; 1.00]
Random-effects model			0.97	[0.83; 1.00]

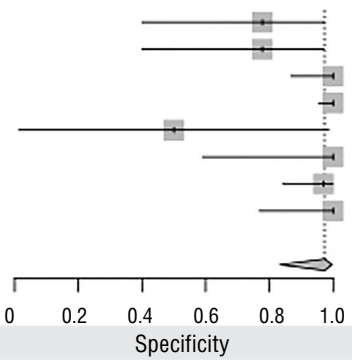


Fig. 3. Pooled sensitivity (*A*) and specificity (*B*) across the comparative studies for methods of detecting α-syn in SGs of PD patients and controls using anti-pS129-α-syn Abs.

It should be noted that there was no significant fluctuation in the pooled specificity values (from 0.974 to 0.993) owing to the sequential exclusion of the studies, depending on the Ab and specimen types.

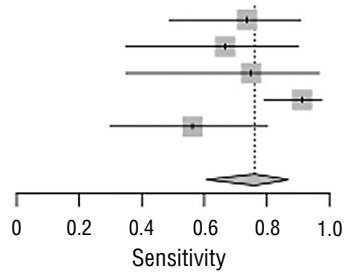
Interpreting sensitivity data with regard to clinical practice, we can conclude that 76% of patients who underwent IHC for pS129-α-syn inclusions in major SG are highly likely to get confirmed diagnosis of PD, previously established by the Movement Disorder Society diagnostic criteria. Based on the specificity data, the likelihood of false-positive results in healthy individuals is only 0.7%. However, we cannot rule out that participants with false-positive results are at risk of developing PD, and detection of modified α-syn inclusions may indicate the onset of neurodegeneration, which apparently starts in the nervous tissue decades before the typical clinical manifestations [32].

Our study is the first Russian meta-analysis, assessing the diagnostic significance of IHC in α-syn detection. The foreign meta-analysis of the English-language articles did not include Russian studies and some early publications with the findings of postmortem IHC examination of SG specimens sampled by IB [9]. Overall, the pooled sensitivity of the 5 comparative studies in our study and the 3 studies in another meta-analysis were comparable in terms of the pooled specificity: 0.99 in our meta-analysis and 0.96 in the 2019 meta-analysis [9]. The difference between the pooled sensitivity values in this study compared with those in the previous meta-analysis was 10.3% (76.3% vs 66%, respectively). The difference is probably due to the slightly larger number of studies in our analysis.

Despite the advantages of this meta-analysis and its value for critical evaluation of the diagnostic IHC, our study has limitations. First, the original studies included in the analysis had small sample sizes and some heterogeneity, which could have to some extent impacted our findings. Second, some publications did not meet the inclusion criteria due to the lack of full-text access, therefore some clinical studies and their results that could impact the sensitivity and specificity were not assessed.

A

Study	TP	Total (TP + FN)	Sensitivity	CI
Adler C.H. et al. (2016)	14		0.74	[0.49; 0.91]
Vilas D. et al. (2016)	8		0.67	[0.35; 0.90]
Khudoerkov R.M. et al. (2016)	6		0.75	[0.35; 0.97]
Beach T.G. et al. (2016)	42		0.91	[0.79; 0.98]
Shin J. et al. (2019)	9		0.56	[0.30; 0.80]
Random-effects model			0.76	[0.61; 0.87]



B

Study	TN	Total (TN + FP)	Specificity	CI
Adler C.H. et al. (2016)	7	9	0.78	[0.40; 0.97]
Vilas D. et al. (2016)	26	26	1.00	[0.87; 1.00]
Khudoerkov R.M. et al. (2016)	1	2	0.50	[0.01; 0.99]
Beach T.G. et al. (2016)	79	79	1.00	[0.95; 1.00]
Shin J. et al. (2019)	14	14	1.00	[0.77; 1.00]
Random-effects model			0.99	[0.99; 0.99]

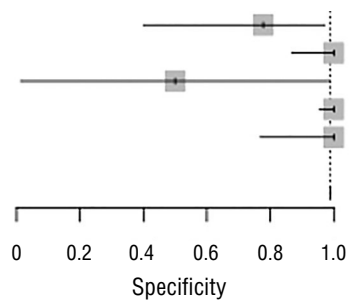


Fig. 4. Pooled sensitivity (*A*) and specificity (*B*) across the comparative studies for methods of detecting α-syn in SGs of PD patients and controls using anti-pS129-α-syn Abs and major SG specimens.

Conclusion

The meta-analysis results demonstrate a possibility of developing a diagnostic method of modified α-syn detection in major SG specimens and its implementation into clinical practice. The sensitivity and specificity were relatively high; however, comparative analysis with other methods of PD diagnosis is required. Further studies with quantitative data analysis are needed to gain greater insight into the method's role in verifying Parkinson's disease and informing of neurodegeneration severity and disease prognosis.

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Information about the authors

Kristina K. Khacheva – neurologist, laboratory assistant, Laboratory of neuromorphology, Brain Institute, Research Center of Neurology, Moscow, Russia, https://orcid.org/0000-0001-9441-4797
Alexey V. Karabanov – Cand. Sci. (Med.), neurologist, Consulting and diagnostic department, Research Center of Neurology, Moscow, Russia, https://orcid.org/0000-0002-2174-2412
Sergey N. Illarioshkin – D. Sci. (Med.), prof., RAS Full Member, Director, Brain Institute, Deputy director, Research Center of Neurology, Moscow, Russia, https://orcid.org/0000-0002-2704-6282

Authors' contribution. *Khacheva K.K.* – selection of publications for the meta-analysis, participation in the statistical processing, analysis of the results, writing the text of the publication; *Karabanov A.V.* – selection of publications for the meta-analysis, participation in the analysis of the results; *Illarioshkin S.N.* – participation in the analysis of the results, editing the text of the publication. All authors took part in forming the idea of the study and developing the methodology for conducting the meta-analysis.

Информация об авторах

Хачева Кристина Константиновна – врач-невролог, лаборант-исследователь лаб. нейроморфологии Института мозга Научного центра неврологии, Москва, Россия, https://orcid.org/0000-0001-9441-4797
Карабанов Алексей Вячеславович – канд. мед. наук, врач-невролог консультативно-диагностического отделения Научного центра неврологии, Москва, Россия, https://orcid.org/0000-0002-2174-2412
Иллариошкин Сергей Николаевич – д-р мед. наук, профессор, академик РАН, зам. директора по научной работе, директор Института мозга Научного центра неврологии, Москва, Россия, https://orcid.org/0000-0002-2704-6282

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Cellular and Molecular Mechanisms Underlying Transcranial Magnetic Stimulation: Experimental Data for Evaluating Changes in Nervous Tissue

Anna P. Krasilnikova[1], Anna V. Egorova[2, 3], Dmitry N. Voronkov[2], Alexandra G. Poydasheva[2], Valeria V. Glinkina[3], Vladimir S. Sukhorukov[2, 3]

[1]Lomonosov Moscow State University, Moscow, Russia;
[2]Research Center of Neurology, Moscow, Russia;
[3]Pirogov Russian National Research Medical University, Moscow, Russia

Abstract

Transcranial magnetic stimulation (TMS) is a non-invasive method for targeted modulation of the electrical activity of brain neurons with a magnetic field. Although TMS efficacy was demonstrated in the treatment of several neurological and mental disorders, changes in nervous tissue at the cellular and molecular levels with different duration and intensity of stimulation have been relatively understudied by cellular neurobiology methods. Aim. The aim of this review was to evaluate and summarize new experimental data on the fundamental mechanisms underlying the action of TMS and its potential in modulating structural and functional changes in nervous tissue. This article summarizes recent data on the effects of different TMS protocols on the mechanisms underlying synaptic plasticity, neurogenesis, and neuronal differentiation. Separate sections summarize the neuroprotective effects of this method and glial microenvironment response. Studies to investigate the mechanisms of TMS will contribute to the development of more effective and reliable treatment protocols.

Keywords: *transcranial magnetic stimulation; neuroplasticity; glia; neurogenesis; neuroprotection; synaptogenesis*

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For correspondence: 80 Volokolamskoye shosse, Moscow, 125367, Russia. Research Center of Neurology.
E-mail: av_egorova@bk.ru. Egorova A.V.

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Клеточные и молекулярные механизмы транскраниальной магнитной стимуляции: экспериментальные данные в оценке изменений нервной ткани

А.П. Красильникова[1], А.В. Егорова[2, 3] , Д.Н. Воронков[2], А.Г. Пойдашева[2], В.В. Глинкина[3], В.С. Сухоруков[2, 3]

[1]Московский государственный университет имени М.В. Ломоносова, Москва, Россия;
[2]Научный центр неврологии, Москва, Россия;
[3]Российский научно-исследовательский медицинский университет имени Н.И. Пирогова, Москва, Россия

Аннотация

Транскраниальная магнитная стимуляция (ТМС) – неинвазивный метод направленного воздействия на электрическую активность нейронов головного мозга магнитным полем. Несмотря на доказанную эффективность в лечении ряда неврологических и психических заболеваний, изменения в нервной ткани на клеточном и молекулярном уровнях при разной длительности и интенсивности стимуляции мало изучены методами клеточной нейробиологии. Целью работы явился анализ и обобщение новых экспериментальных данных о фундаментальных механизмах действия ТМС и потенциальных возможностях данного метода в модуляции структурно-функциональных изменений в нервной ткани. В работе систематизированы современные сведения о влиянии разных протоколов ТМС на механизмы синаптической пластичности, нейрогенез и дифференцировку нейронов. Отдельные разделы посвящены нейропротективным эффектам данного метода, а также ответной реакции глиального микроокружения. Исследования механизмов ТМС будут способствовать разработке более результативных и надёжных протоколов лечения.

Ключевые слова: *транскраниальная магнитная стимуляция; нейропластичность; глия; нейрогенез; нейропротекция; синаптогенез*

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Адрес для корреспонденции: Россия, Москва, Волоколамское шоссе, д. 80. Научный центр неврологии.
E-mail: av_egorova@bk.ru. Егорова А.В.

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Introduction

Transcranial magnetic stimulation (TMS) is a non-invasive method for targeting the electrical activity of neurons. It is used to stimulate nerve cells with short magnetic pulses that cause depolarization of the pre- and postsynaptic membrane. In the brain, a magnetic field induces an electric current that affects the electrophysiological parameters of neurons in the stimulated area [1–3].

TMS is widely used in current clinical practice for diagnosis, treatment, and rehabilitation of patients with various neurological and mental disorders. According to the European guidelines [4], this method was shown to be effective in the management of treatment-resistant depression [5–7], neuropathic pain [8–10] (level of evidence A) and in rehabilitation of patients with post-stroke motor deficit [11, 12] (level of evidence B). Statistically significant improvement was observed in Parkinson's disease [13, 14], spasticity in multiple sclerosis [15], migraine [16], etc.

In research practice, TMS is used to assess the excitability of the motor cortex, changes in cognitive processes over time, and functional brain mapping [3].

The method is usually well tolerated by patients. Compliance with safety recommendations minimizes the occurrence of such serious adverse effects as epileptic seizures (incidence rate less than 1 per 60,000 sessions) [17, 18]. Other side effects, such as pain at the stimulation site, are more

common but in most cases they do not affect the tolerability of the procedure [19].

Studying TMS effects on brain structures is challenging because the type of the effect is difficult to be assessed in non-motor areas of the cortex. Therefore, it is difficult to predict and interpret the results obtained by activating a set of neural networks. Simultaneous electroencephalography [20], functional magnetic resonance imaging, cognitive testing, and other methods [21] can only partially address detection issues.

While clinical effects of TMS are recognized, changes in neural tissue at the cellular and molecular levels with different duration and intensity of stimulation have been poorly studied by cellular neurobiology. Experiments in laboratory animals are complicated due to a mismatch between the size of the coil and a stimulated area of the brain. Targeted exposure and correlating experimental data with clinical results are difficult.

Fundamental studies to evaluate neural morphology, functional activity, and cellular environment in response to a magnetic field with different parameters would significantly improve the efficacy of this method.

Aim. The aim of this review was to evaluate and summarize new experimental data on the fundamental mechanisms underlying the action of TMS and its potential in modulating structural and functional changes in nervous tissue.

This review included experimental studies mainly from the last 5–7 years that assessed structural and functional TMS-induced changes in the cellular elements of the nervous tissue using neuromorphology and neuroimaging methods. The search was carried out in the PubMed and Google Scholar databases.

General aspects of TMS

Most studies investigating the cellular mechanisms of TMS in laboratory animals involved stimulation of a hemisphere or the whole brain of rats and mice. Due to their small size, focal stimulation in rodents is difficult; however, it can be achieved by using mini-coils of different design (including ferromagnetic cores) or shielding materials [22, 23]. Early studies showed that local stimulation was achievable in rats using clinically used figure-eight coils. Such coils for rats allowed generating unilateral motor evoked potentials of a single limb, thus indicating the possibility of fairly local effects without significant changes in coil design [22]. Another approach to achieve a local effect is to reduce magnetic field intensity [24], which, however, is criticized due to difficulties in translating experimental results to humans.

Differences in brain size, magnetic induction intensity, and electrical field interaction with nerve tissue make translation of preclinical results difficult, although computer modeling can facilitate the selection of similar stimulation conditions [25] and analysis of electric fields generated in cell cultures [26]. Additional limitations of TMS in animal studies include the use of anesthesia in some cases.

However, the advantages of studying the effects of TMS in experimental animal models are also obvious: controlled experimental conditions, homogeneity of the study sample, use of genetic models of diseases, use of the entire arsenal of modern neuroimaging methods, including *in vivo* microscopy, and neuromorphological studies to assess off-line effect (Table 1).

TMS can be classified into single-pulse, paired-pulse, and repetitive TMS (rTMS). In the latter case, a series of pulses with different frequency and intensity is generated. rTMS can be roughly classified into low-frequency stimulation (0.2–1.0 Hz), which reduces neuronal excitability, and high-frequency stimulation (5 Hz or more), which has an excitatory effect [2].

Low-frequency rTMS most often uses continuous delivery of single pulses, while high-frequency rTMS typically uses a train of stimuli lasting 2–10 s separated by pauses of 20–50 s.

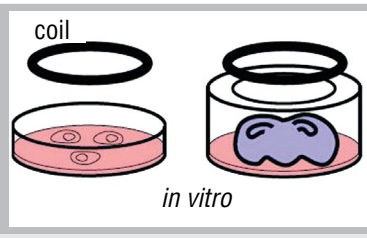
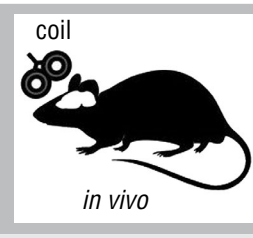
In addition to these conventional rTMS types, there are several other approaches, one of which uses θ-pattern, i.e. intermittent theta-burst stimulation (iTBS) or continuous theta-burst stimulation (cTBS) [3]. iTBS was shown to increase cortical excitability within 1 h of exposure while cTBS decreased it [27, 28].

Two groups of effects can be seen with TMS: online (during stimulation) and offline (after its completion).

A burst of action potentials is the most common online effect of single-pulse TMS at the neuronal level. The alternating magnetic field from the stimulator coil generates an induced electric field in the brain followed by an electrical current [29], with some neurons exhibiting combined activity. In this case, after the initial excitation, a long phase is observed, which combines periods of inhibition and excitation [30]. This phenomenon is likely to be caused by delayed activation of neighboring inhibitory interneurons. Not all neurons, even in the center of stimulation, respond to TMS. This heterogeneity in susceptibility to magnetic pulses may be due to differences in the local orientation of nerve cells relative to the TMS-induced electric field. The effect at the organism level also varies depending on coil orientation, with neural populations being recruited differently [31, 32].

According to modern concepts, the effects of TMS are most often associated with a neuroprotective effect, stimulation of neuro- and synaptogenesis, and optimization of synaptic transmission processes in the structures of the central nervous system [33, 34]. Pattern stimulation protocols and rTMS are used to induce an offline effect [35, 36].

Table 1. Summary of experimental methods to assess TMS effects

				
Object, assessed effects, level of organization	Cell cultures; online, offline effects; cell, intercellular interactions	Acute slices, online; cell, individual brain structures	Animal, online, offline; structures and systems	Mathematical model, online; structures and systems
Key study methods	Electrophysiology, fluorescence imaging, immunomorphology, biochemistry, molecular and biochemical methods	Electrophysiology, fluorescence imaging of fast processes	Electrophysiology, behavior (motor and cognitive tests), *in vivo* microscopy, immunomorphology, molecular and biochemical methods	
Excitation and synaptic transmission	+	+	+/–	Simulation of conditions and analysis of magnetic and electric fields in an object during stimulation
Proliferation, differentiation, and migration	+	–	+	
Intercellular, glioneuronal interactions	+	+/–	+	
Synaptogenesis	+	–	+	
Development of new clinical stimulation protocols	–	–	+/–	+

In response to rTMS, neuronal excitability changed due to a shift in the ionic balance around the population of stimulated cells. Depolarization dominates in the mechanism of excitability modulation, which resembles the induction of synaptic plasticity. However, hyperpolarization also plays an important role by influencing the membrane potential [37, 38].

Effects of TMS on synaptogenesis and synaptic transmission mechanisms

The functional effects caused by rTMS continue for a certain time after stimulation [39]. In addition to its effects on the metabolic cell profile and synaptic transmission, rTMS

causes changes in synaptic architecture. The most common theory suggests that this phenomenon is similar to synaptic plasticity mechanisms, such as long-term depression or potentiation, which are induced by stimulation of neuronal activity at different frequencies [40, 41]. According to modern concepts, the molecular mechanisms underlying structural and functional rearrangements of neural networks under the influence of TMS are associated with NMDA receptors on the postsynaptic membrane. For example, rTMS induced phenomena similar to long-term potentiation, thus triggering rearrangement of the actin cytoskeleton, which finally led to structural dendrite remodeling [42]. During long-term potentiation, dendritic spines first rapidly enlarge and deform due to increased actin polymerization and branching, and at following stages, proteins responsible for the functioning of postsynaptic densities and receptor clustering are attracted to the synapse area [43].

The effects of TMS on synaptogenesis and synaptic transmission processes were best studied in the motor areas of the cerebral cortex and hippocampus.

A.D. Tang et al. used two-photon imaging to track the plasticity of dendritic spines in the fifth layer of the motor cortex in mice of different ages. The study showed that a single train of subthreshold iTBS on the motor cortex increased the rate of dendritic spine loss 21 h after the session regardless of mice age and resulted in a significant decrease in the density of these structures 45 h after the session [44].

Meanwhile, a recent study showed that 5-day high-frequency rTMS (15 Hz) treatment increased total spine density in M1 L2/3 apical and basal dendrites 24 h post-stimulation in juvenile mice [45].

rTMS of hippocampal cell cultures was reported to induce clustering of postsynaptic AMPA receptors [42]. Data by M. Lenz et al. showed that high-frequency rTMS (10 Hz) *in vitro* affected synaptic transmission of predominantly excitatory synapses located on the proximal dendrites of cultured CA1 pyramidal neurons. AMPA receptor stimulation and retrograde membrane depolarization activated voltage-gated sodium and calcium channels and removed a reversible magnesium block from NMDA receptors [46]. This led to a local increase in calcium levels, rapid dendrite depolarization, generation of so-called "proximal area of dendritic plasticity" and a calcium-dependent increase in AMPA levels on the postsynaptic membrane of the dendritic spine. Moreover, selective pharmacological inhibition of NMDA receptors or α-1 subunit of calcium channels (L-VGCC) inhibited the rTMS effect on the proximal dendrites [47].

Dysfunction of neural networks may be explained by an imbalance of excitation and inhibition, so TMS effects on inhibitory synapses of neuronal circuits should be also considered. A study by M. Lenz et al. showed that 10 Hz magnetic stimulation affected Ca^{2+}/calcineurin-dependent oligomerization of gephyrin [48], a postsynaptic scaffold protein that mediates stabilization and clustering of ionotropic glycine and γ-aminobutyric acid (GABA-A) receptors. The main cluster of GABA-A receptors is located on the soma and axonal hillocks of hippocampal neurons [49]. Long-term potentiation of excitatory synapses (described above) was associated with gephyrin-mediated Ca^{2+}/calcineurin-dependent restructurization of inhibitory synapses. These structural and functional changes require activation of voltage-gated L-type sodium and calcium channels and NMDA receptors, and they were not observed when calcineurin protein phosphatases were pharmacologically blocked [50]. Accordingly, 10 Hz stimulation was associated with destabilization of gephyrin, GABA-A, and glycine receptor clusters and a decrease in the activity of inhibitory synapses.

A. Thomson et al. illustrated the excitatory effect of iTBS using SH-SY5Y cells (a human neuroblastoma cell line) pre-incubated with Fluo-4 AM, a fluorescent calcium indicator, as a synaptic plasticity model. A protocol similar to iTBS was associated with increased fluorescent response to the addition of KCl (depolarization-induced neuronal activation), while a protocol similar to cTBS was associated with decreased fluorescent response compared with control [51].

Phosphorylation of ribosomal S6 in neurons is known to be a marker of NMDA-dependent signaling pathway activation and induce synaptic and cellular changes that underlie plasticity. High-frequency TMS (400 Hz) was associated with activation of mTORC1 signaling pathway, which phosphorylates threonine at position 389 of S6 protein, thus activating rpS6 kinase. There was a more than 3-fold increase in rpS6 phosphorylation 15 min, 2 h, and 4 h after completion of high-frequency TMS. These effects were eliminated by treatment with rapamycin, which blocks the activation of this signaling pathway [52].

In a study with high-frequency (400 Hz) TMS in mice, there was an increase in the content of phosphorylated ribosomal protein S6 in the islands of Calleja and the paraventricular nucleus of the hypothalamus, ventromedial-lateral posterior nuclei of the thalamus, piriform cortex, and central nucleus of the amygdala [53]. A group of rpS6 phospho-mutant mice did not show any long-term potentiation and excitatory post-synaptic currents after high-frequency TMS (100 Hz) [54].

In hippocampal cell cultures, low-intensity TMS (1.14 T, 1 Hz) caused dendritic sprouting and an increase in synaptic contact density, while high-intensity TMS (1.55 T, 1 Hz) had a destructive effect, leading to a decrease in the number of processes and synapses. The authors showed that low-intensity low-frequency TMS (1.14 T, 1 Hz) could induce dendritic and axonal growth in cultured hippocampal neurons by activating brain-derived neurotrophic factor (BDNF)/extracel-

lular signal-regulated kinase (ERK) signaling pathway, which resulted in increased expression of postsynaptic density protein (PSD95) and synaptophysin [55], as well as postsynaptic membrane thickening [56].

According to other data, a protocol similar to iTBS (2-second trains of stimuli every 10 s, total exposure time 180 s) stimulated PSD95 and synaptophysin transcription, while low-frequency TMS did not have any similar effect [57].

Low-intensity TMS is associated with remodelling of abnormal neural connections into a topographically more appropriate position. Ephrin-A2/A5 double knockout mice lack key signals for axonogenesis and, therefore, have impaired topography of the visual pathways. Two-week low-intensity rTMS (10 mT; 10 min/day) reduced the number of abnormal projections in subcortical [58] and cortical visual circuits [59].

The metabolic profile of neurons pre-treated with TMS showed depleted pools of aspartate, phenylalanine and isoleucine, which was explained by the authors by the need to replenish the tricarboxylic acid cycle. Low-frequency TMS was associated with an increase in GABA synthesis and spontaneous release (which may be associated with decreased levels of pyroglutamate and alanine). The content of serine and glycine also decreased significantly after 1 Hz and 10 Hz stimulation, which is likely to be due to increased synthesis of proteins such as BDNF, c-fos, and various neurotransmitter receptors [60].

The cellular and molecular changes that are associated with synaptic plasticity and develop after TMS were illustrated by very few studies in animals and cell cultures with inconsistent results (Table 2). The most significant improvement in synaptic plasticity was found when high-frequency TMS (10 Hz) was used in cell cultures; however, there is no commonly accepted position regarding the intensity of the effect. Low-intensity TMS using various protocols led to positive effects in neuronal cultures but did not improve synaptogenesis at the organism level. Additional studies are needed to clarify the effects of TMS protocols, especially regarding the intensity of magnetic stimulation. An analysis of recent literature showed that fundamental experimental studies overall confirmed that some TMS protocols induced processes similar to long-term depression, while others induced long-term potentiation. However, delayed effects of TMS are often variable and depend not only on exposure parameters but also on previous neuronal activity and several other factors. The long-term effects of TMS may be mediated by a combination of different types of plasticity, including metaplasticity [61].

Table 2. Effects of TMS on synaptogenesis and synaptic transmission mechanisms

Effects of TMS	TMS type	Frequency, Hz	Effect	Reference
Positive	Low intensity	15	Density of dendritic spines on pyramidal neurons increased	[45]
		1	Dendritic sprouting and synaptic contact density increased through activation of BDNF/ERK pathway	[55, 56]
		10	Synaptic potentiation of predominantly excitatory synapses on proximal dendrites of cultured CA1 pyramidal neurons induced	[46]
		10	Structural and functional plasticity of inhibitory synapses induced	[48]
		400	NMDA-dependent pathways upregulated via mTORC1 pathway	[52]
		400	NMDA-dependent pathways upregulated through S6 increased	[53]
		6,67/10	Neuron connections remodelled	[58, 59]
Negative	Low intensity	50	Density of dendritic spines decreased after 45 h; loss rate increased after 21 h	[44]
	High intensity	1	Number of processes and synapses decreased	[56]

Neuroprotective and regenerative effects of TMS

Studies in experimental neurological disease models showed anti-apoptotic and restorative effects of low-intensity TMS, which were mediated by profound changes in regulatory cascades in neurons. In one study, rTMS treatment was applied at a frequency of 10 Hz, 10 min per day during 14 days to mice with spinal cord transection at the T9–T11 level; proteomic analyses showed a decrease in the levels of several pro-apoptotic proteins, such as annexin A2, thus contributing to neuron survival and remyelination. This study also demonstrated that TMS with these parameters was associated with increased proliferation of progenitor nerve cells of the spinal cord and increased levels of NEUM, CDC42, and RHOG proteins, which are known to cause increased axon growth and branching [62].

Another study showed that in middle cerebral artery occlusion TMS reduced neuronal death in the blood supply area by affecting apoptosis regulator proteins, enhancing anti-apoptotic Bcl-2 expression, and inhibiting pro-apoptotic Bax expression [63]. A study in a genetic Alzheimer's disease model showed that high-frequency TMS (25 Hz) reduced neuronal loss and apoptosis of hippocampal cells due to activation of PI3K/Akt/GLT-1 pathway, which is associated with decreased excitotoxicity [64].

However, TMS can also have detrimental effects on cells. Experiments on primary neuron cultures showed that 10 and 100 Hz modes with continuous stimulation were associated with an increase in the number of apoptotic cells [65].

In a study in a culture of primary hippocampal neurons, rTMS (40% and 60% of the maximum power of the stimulator) increased the expression of catalase and aconitase (i.e. iron-containing proteins that are involved in antioxidant protection) and increased neuron survival. It is interesting that high-intensity TMS accelerated their damage [66].

Therefore, different experimental models demonstrated that several TMS modes suppressed molecular mechanisms that underlie neuronal damage and death such as apoptosis, excitotoxicity, and oxidative stress. Continuous and high-intensity TMS exacerbated cell damage (Table 3).

Effects of TMS on neurogenesis and neuron differentiation

E. Ueyama et al. assessed BrdU incorporation into proliferating cells and showed that 14-day 25 Hz rTMS enhanced neurogenesis in the hippocampus of intact mice [67]. Studies in models of spinal cord damage showed that neural stem cells resting near the central canal of the spinal cord differentiated into astrocytes [68, 69] and oligodendroglia under the influence of TMS [62]. TMS effects on the proliferation, differentiation, and migration of neuronal precursors in neurogenic niches was best studied *in vivo* in stroke models in order to justify its use in patient rehabilitation.

In an ischemic brain injury model, 10 Hz rTMS promoted the proliferation of neuronal precursors in the subgranular zone of the hippocampus of experimental rodents. In TMS-treated animals, expression of BDNF, TrkB, p-AKT, and anti-apoptotic Bcl-2 was increased while expression of pro-apoptotic Bax was significantly decreased [63]. BDNF plays a critical role in promoting neuronal survival by specifically binding to tropomyosin receptor kinase B (TrkB). This binding results in auto-phosphorylation and dimeriza-

Table 3. Effects of TMS on mechanisms underlying neuroprotection and regeneration

Effects of TMS	TMS type	Frequency, Hz	Effect	Reference
Positive	Low intensity	10	Levels of several pro-apoptotic proteins decreased, those of proteins affecting axonogenesis and antioxidant enzymes increased	[62]
		10	Expression of anti-apoptotic Bcl-2 increased and expression of pro-apoptotic Bax suppressed	[63]
		25	Neuronal loss and apoptosis of hippocampal cells reduced	[64]
		Not specified	Expression of aconitase and catalase, which are involved in antioxidant defense, increased	[66]
Negative	High intensity	10/100	Number of apoptotic cells increased	[65]

tion of the TrkB receptor, thus triggering the activation of phosphatidylinositol 3-kinase PI3K. The PI3K/Akt signaling pathway is the main TrkB-mediated survival pathway that protects against apoptosis [70]. In a similar experiment with a similar frequency of stimulation, a significant increase in the expression of miR-25 (i.e. microRNAs that are involved in the differentiation and proliferation of neural stem cells) was shown in the subventricular zone [71]. High-frequency rTMS (20 Hz) also stimulated BDNF and pERK1/2 expression, which confirmed the influence of the BDNF/ERK signaling pathway on increased proliferation of neural stem cells in the hippocampus [72, 73]. The authors highlighted the similarity of the changes with the effects of antidepressants and electroconvulsive therapy.

Therefore, one of the mechanisms underlying the effects of TMS includes the enhancement of neurogenesis and repair processes due to stimulation of BDNF production, which promotes the survival of stem cells and neuronal differentiation, as well as the formation of new synapses. The neuroprotective effect of BDNF was shown in animal models of Alzheimer's disease [74, 75].

However, besides BDNF effects, other mechanisms were investigated. For example, N. Liu et al. found that the proliferation of neural stem cells *in vitro* after high-frequency rTMS was associated with a dose-dependent increase in expression of microRNAs of miR-106b~25 cluster (miR-106b, miR-93, miR-25), which are involved in cell cycle regulation [76].

In addition to enhancing neurogenesis in neurogenic niches, TMS was shown to have an effect on the migration of neurons to the damage area. For example, rTMS (10 Hz every 24 hours for 5 days) was associated with an increase in the levels DCX-positive neuronal precursors in the cortex in a hemorrhagic stroke model. In an *in vitro* experiment with neurospheres, the same authors showed an increase in the percentage of Sox2 and $Ki67^+$ cells, which suggested increased proliferation of neural stem cells associated with TMS (10 Hz every 24 hours for 72 hours) [77].

The study showed an increase in proliferation and a role of chemokine receptors in 10 Hz rTMS effects on the migration of neural stem cells from the subventricular area to the perifocal area of ischemic infarction. TMS was also associated with improved behavioral parameters of rats in this experiment [78].

Similar conclusions were made after staining with Nestin/SOX2 and Nestin/beta3-tubulin: rTMS increased the pool of neuronal progenitors in the peri-infarction area of the cerebral cortex in post-stroke setting. The number of immature neurons in the peri-infarction area was higher in animals exposed to rTMS; the authors concluded that cells migrated to the peri-infarction area due to the direction of $\beta3\text{-tubulin}^+$ processes [79].

Although most studies showed that TMS was associated with a improvement in neurological deficit in stroke models, it is still unclear whether TMS promotes the integration of newly formed nerve cells in the perifocal area of the infarction or recovery occurs due to other TMS-stimulated mechanisms such as the prevention of neuronal death, or the reorganization or restoration of neuronal connections.

There is little data on TMS effects on human neuron differentiation, although they are of particular interest in the context of the development of cell therapy methods. For example, a study in human neurons derived from induced pluripotent stem cells *in vitro* showed effects of different TMS protocols on neuron differentiation and maturation: high-frequency TMS promoted the differentiation of neuronal precursors into glutamatergic neurons, while iTBS enhanced synaptogenesis, suggesting its effect on neuron maturation [57].

The influence of TMS on differentiation of transplanted neural stem cells remains almost not studied. J.J. Peng et al. showed that animals with transplanted human neural stem cells who received TMS (10 Hz) demonstrated better functional recovery after ischemic infarction compared with animals with no TMS exposure; this was associated by the authors with the activation of the BDNF/TrkB signaling pathway that we discussed previously [80].

In these studies, the effect of TMS on neurogenesis both in the dentate gyrus of the hippocampus and the subventricular area was repeatedly demonstrated using immunohistochemical markers of neuronal precursor proliferation and neuronal differentiation. The vast majority of studies used high-frequency TMS protocols, most commonly 10 and 20 Hz. The stimulating effect of high-frequency TMS on the migration of progenitor cells to peri-infarction areas was consistently demonstrated. We can assume that TMS influences neurogenesis mainly through activation of the BDNF/TrkB pathway and effects on transcripts of genes that regulate the cell cycle.

Effects of TMS on glial cells

Although several studies did not reveal any direct effects of rTMS on glial cell cultures, changes in all types of neuroglia were repeatedly shown when pathological conditions were simulated. There is growing evidence that glial cells may actively participate in the neuroprotective effect of TMS [81].

Besides the direct response of gliocytes to TMS, which remains controversial, glia changes in mixed cultures or tissue can be also explained by increasing electrical activity of neurons, which cause a response in glial cells.

Closely interacting with neurons, astrocytes participate in the regulation of synaptogenesis. Addition of astrocyte-conditioned medium or their co-culturing with nerve cells in-

creased the number of functional excitatory synapses formed in the culture, while removal of astrocytes had the opposite effect [82]. Thrombospondins (TSPs) are factors that are secreted by astrocytic glia and associated with the regulation of synaptogenesis [83]. For example, TSP1/β-integrin signaling pathway controls the excitation/inhibition ratio in the spinal cord by upregulating glycinergic receptors and downregulating surface expression of AMPA receptors. Astrocyte-mediated TSP1/α2δ-1 signaling in the striatum was shown to modulate the activity of excitatory synapses [84].

Astroglia also controls the number of synapses through phagocytosis. Synaptic elimination is mediated by the transmembrane protein Megf10, which is expressed by astrocytes [85]. Astrocytes were shown to phagocytize synapses via the Megf10 and Mertk pathways in both developing and adult brains [86]. J. Lee et al. also confirmed that astrocytic Megf10 mediated the elimination of excitatory synapses in the CA1 region of the adult hippocampus [87].

Clustering of AMPA receptors at postsynaptic terminals of excitatory synapses, which may be astrocyte-dependent, is one of the mechanisms underlying synaptic plasticity, which is also seen with TMS. One of the mechanisms regulating the clustering process is mediated by ephrin A3 of astrocytic processes and its receptor EPHA4, which is expressed by dendritic spines [88, 89]. To support the connection between neuroplasticity and astrocyte response, we can mention a study by N. Monai et al. They showed that direct current-stimulated astrocyte response affected long-term potentiation of neurotransmission, was associated with fluctuations in Ca^{2+} levels, and depended on adrenergic receptors [90].

TMS (1 Hz for 10 min) increased STIM1 and ORAI3 protein expression in astrocytes; STIM1 protein acts as a sensor for Ca^{2+} stores depletion in the endoplasmic reticulum, while ORAI3 is a Ca^{2+} influx channel. This study demonstrated decreased expression of several inflammatory response genes in astrocytes associated with frequencies of 1 and 10 Hz [91].

A recent study in a mixed culture exposed to high-frequency TMS showed that astrocytes released a neurotrophic factor that induced the neuronal expression of ERK1/2 gene, associated with synaptic plasticity and neuronal activation, and immediate-early *c-fos* gene, thus confirming the bidirectional interaction of astroglia and neurons after stimulation [92].

High-frequency TMS and a very low-intensity magnetic field (0.5 mT) induced a transient increase in the expression of the astrocytic marker GFAP *in vivo* in mice after ischemic injury and reperfusion, which may indirectly indicate the recruitment of astrocytes to the damaged area (continuous 50 Hz exposure for 7 days) [93]. Similar data were obtained in a murine model of spinal cord injury. 1 Hz magnetic stimulation with 5-min sessions on 14 consecutive days induced GFAP expression by astrocytes and ERK1/2-dependent migration into the lesion areas [94].

A number of articles highlighted the role of microglia in the response of nervous tissue to TMS. In microglia-depleted tissue cultures, CA1 pyramidal neurons did not show any local depolarization of the postsynaptic membrane associated with 10 Hz TMS. Depletion of microglia *in vivo* had no significant effect on baseline synaptic transmission. In experiments with TMS, control mice with intact microglia showed spontaneous depolarizations of post-synaptic membranes (mEPSCs) in excitatory synapses in the medial prefrontal cortex vs. no such potentials in mice with depleted microglia [95].

S. Chen et al. showed that high-frequency TMS (20 Hz) was associated with an improvement in the cognitive functions of mice on day 28 after temporary occlusion of the middle cerebral artery. The volume of white matter lesions reduced, levels of pro-inflammatory cytokines decreased, and microglia switched to the M2 phenotype [96].

Oligodendrocyte proliferation was evaluated in several studies. Their results were inconsistent. G. Liu et al. reported stimulation of oligodendrocyte proliferative capacity [57] and induction of progenitor cell differentiation into oligodendrocytes in a study with high-frequency rTMS. A study by C.L. Cullen et al. did not confirm these effects [97]. Effects of iTBS and cTBS on oligodendrocytes were evaluated in Plp-CreER:Tau-mGFP and Pdgfra-CreERT2 transgenic mice. iTBS was shown to increase the number of newly formed oligodendrocytes [98].

Information about the effects of TMS on glia is currently insufficient, and this aspect requires further investigation. The neuroprotective effect of glial cells on ischemic and damaged tissues was shown indirectly. TMS modulates glia to create anti-inflammatory environment by switching microglia and astrocytes to a pro-inflammatory phenotype. A special role is played by TMS-induced release of glial cell neurotrophic factor from astrocytes, which leads to an increase in ERK1/2 expression in neurons. ERK1/2 activation is required for the BDNF cascade, which results in increased dendritic density and proliferation of neuronal progenitors. However, studies to investigate the effects of rTMS on glial cells are extremely scarce, so additional research is needed in this topic.

Conclusion

The effects of TMS discussed in the review, which are associated with the regeneration and restoration of nervous system functions, cell differentiation, and stimulation of synaptic plasticity, can substantiate the use of this method in cell therapy of neuropsychiatric disorders. However, many questions remain unresolved. The effect of TMS on

the differentiation and maturation of neuronal precursors is little studied. Isolated effects of TMS on glial cells remain a controversial issue.

Many studies in cell cultures were conducted using frequencies that are not relevant for clinical practice. Special attention should be paid to standardizing the intensity of stimulation, since in affects glial and neuronal responses. It should be remembered that results obtained in cell cultures do not always correlate with the response at the organism level.

Further studies to evaluate the mechanisms of TMS would contribute to the development of more effective treatment protocols with this method.

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Information about the authors

Anna P. Krasilnikova – student, Lomonosov Moscow State University, Moscow, Russia, https://orcid.org/0009-0006-2258-6155
Anna V. Egorova – Cand. Sci. (Med.), Assistant professor, researcher, Laboratory of neuromorphology, Brain Institute, Research Center of Neurology; Assistant professor, Department of histology, embryology and cytology, Pirogov Russian National Research Medical University, Moscow, Russia, https://orcid.org/0000-0001-7112-2556
Dmitry N. Voronkov – Cand. Sci. (Med.), senior researcher, Laboratory of neuromorphology, Brain Institute, Research Center of Neurology, Moscow, Russia, https://orcid.org/0000-0001-5222-5322
Alexandra G. Poydasheva – Cand. Sci. (Med.), researcher, Non-invasive neuromodulation group, Institute of Neurorehabilitation and Rehabilitation Technologies, Research Center of Neurology, Moscow, Russia, https://orcid.org/0000-0003-1841-1177
Valeria V. Glinkina – Dr. Sci. (Med.), Professor, Head, Department of histology, embryology and cytology, Pirogov Russian National Research Medical University, Moscow, Russia, https://orcid.org/0000-0001-8708-6940
Vladimir S. Sukhorukov – Dr. Sci. (Med.), Professor, Head, Laboratory of neuromorphology, Brain Institute, Research Center of Neurology; Professor, Department of histology, embryology and cytology, Pirogov Russian National Research Medical University, Moscow, Russia, https://orcid.org/0000-0002-0552-6939

Authors' contribution. *Krasilnikova A.P.* – analysis and review of publications on the theme of the article, article writing; *Egorova A.V.* – definition of the general concept of the article, working with the text, editing the article; *Voronkov D.N.* – definition of the general concept of the article, guidance, working with the text; *Poydasheva A.G.* – working with the text, editing the article; *Glinkina V.V., Sukhorukov V.S.* – working with the text, editing the article, guidance and coordination of work.

Информация об авторах

Красильникова Анна Павловна – студент МГУ им. М.В. Ломоносова, Москва, Россия, https://orcid.org/0009-0006-2258-6155
Егорова Анна Валериевна – канд. мед. наук, доцент, н. с. лаб. нейроморфологии Института мозга Научного центра неврологии, Москва, Россия; доцент каф. гистологии, эмбриологии и цитологии РНИМУ им. Н.И. Пирогова, Москва, Россия, https://orcid.org/0000-0001-7112-2556
Воронков Дмитрий Николаевич – канд. мед. наук, с. н. с. лаб. нейроморфологии Института мозга Научного центра неврологии, Москва, Россия, https://orcid.org/0000-0001-5222-5322
Пойдашева Александра Георгиевна – канд. мед. наук, н. с. группы неинвазивной нейромодуляции Института нейрореабилитации и восстановительных технологий Научного центра неврологии, Москва, Россия, https://orcid.org/0000-0003-1841-1177
Глинкина Валерия Владимировна – д-р мед. наук, профессор, зав. каф. гистологии, эмбриологии и цитологии РНИМУ им. Н.И. Пирогова, Москва, Россия, https://orcid.org/0000-0001-8708-6940
Сухоруков Владимир Сергеевич – д-р мед. наук, профессор, зав. лаб. нейроморфологии Института мозга Научного центра неврологии, Москва, Россия; профессор каф. гистологии, эмбриологии и цитологии РНИМУ им. Н.И. Пирогова, Москва, Россия, https://orcid.org/0000-0002-0552-6939

Вклад авторов: *Красильникова А.П.* – анализ и обзор публикаций по теме статьи, написание текста статьи; *Егорова А.В.* – определение общей концепции статьи, работа с текстом, редактирование статьи; *Воронков Д.Н.* – определение общей концепции статьи, руководство, работа с текстом; *Пойдашева А.Г.* – работа с текстом, редактирование статьи; *Глинкина В.В.*, *Сухоруков В.С.* – работа с текстом, редактирование статьи, руководство и координация работы.

Complication of COVID-19: Mild Encephalopathy Syndrome with Reversible Splenial Lesion

Tatiana V. Matveeva[1], Rustem T. Gayfutdinov[1], Dinara S. Kamalova[1], Gulnaz A. Fasakhova[2]

[1]Kazan State Medical University, Kazan, Russia;
[2]Central City Clinical Hospital No. 18, Kazan, Russia

Abstract

A syndrome of mild encephalopathy with reversible splenial lesion (MERS) was described in a post-COVID-19 male patient. The clinical manifestations included neuropsychiatric and visual abnormalities; when focusing separately on an object (one eye closed), the left eye perceived it as normal, but the right eye perceived it as multiple images moving diagonally into the distance. T2, FLAIR, and ADC magnetic resonance imaging (MRI) showed a splenial lesion that resolved rapidly without using corticosteroids. The patient was diagnosed with cerebral polyopia because he saw images arranged in ordered rows after focusing on an object. Differential diagnoses included astigmatism, palinopsia, and polyopic visual hallucinations. Monocular polyopia is explained by anomia associated with the patient's partial split-brain syndrome (the splenial lesion, neuropsychiatric abnormalities); involvement of the pathways from the frontal eye fields to the brainstem structures responsible for initiating extraocular eye movements. The association of neurological complications with prior COVID-19, rapid resolution of symptoms, and MRI lesions without initiating immunosuppressive therapy suggested endotheliopathy as the cause of COVID-19 complications.

Keywords: *splenium of the corpus callosum; MERS; polyopia; split-brain syndrome; anomia*

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For correspondence: 2 Mavlyutina str., Kazan, 420101, Russia. Kazan State Medical University. E-mail: gaifutdinov69@mail.ru. Gaifutdinov R.T.

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Осложнение коронавирусной инфекции: синдром умеренной энцефалопатии с обратимым поражением валика мозолистого тела

Т.В. Матвеева[1], Р.Т. Гайфутдинов[1], Д.Ш. Камалова[1], Г.А. Фасахова[2]

[1]Казанский государственный медицинский университет, Казань, Россия;
[2]Центральная городская клиническая больница № 18, Казань, Россия

Аннотация

Описан синдром умеренной энцефалопатии с обратимым поражением валика мозолистого тела у мужчины, перенёсшего COVID-19. Клиническими проявлениями заболевания были нейропсихические отклонения и нарушение зрения – при раздельной фокусировке взора на объекте (один глаз закрыт) левый глаз воспринимал его как обычно, правый – как множество уходящих вдаль по диагонали изображений. На магнитно-резонансной томограмме (МРТ) в режимах T2, FLAIR, ADC зафиксировано быстро регрессирующее без назначения глюкокортикоидов образование в валике мозолистого тела. Видение пациентом изображений в виде упорядоченных рядов после фиксации взора на объекте позволило диагностировать у него церебральную полиопию. Дифференциальный диагноз проводился с астигматизмом, палинопсией, зрительными полиопическими галлюцинациями. Моноокулярная полиопия объяснена аномией, сопряжённой с имеющимся у пациента синдромом частично «расщеплённого мозга» (очаг в валике мозолистого тела, нейропсихические отклонения); заинтересованностью путей от лобных полей глаза к структурам ствола, ответственных за инициацию экстраокулярных движений глаз. Ассоциация неврологических осложнений с перенесённым COVID-19, быстрый регресс симптомов заболевания и изменений на МРТ без назначения иммуносупрессивной терапии позволило в качестве генеза осложнения COVID-19 предположить эндотелиопатию.

Ключевые слова: *валик мозолистого тела; синдром MERS; полиопия; синдром «расщеплённого мозга»; аномия*

Этическое утверждение. Исследование проводилось при добровольном информированном согласии пациента, в том числе на публикацию клинического случая.

Источник финансирования. Авторы заявляют об отсутствии внешних источников финансирования при написании данной статьи.

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

Адрес для корреспонденции: 420101, Россия, Казань, ул. Мавлютова, д. 2. ЦГКБ № 18. E-mail: gaifutdinov69@mail.ru. Гайфутдинов Р.Т.

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Introduction

Neurological complications of the novel coronavirus infection (COVID-19) are common, ranging from headache to clinical symptoms and signs of encephalitis, meningitis, encephalomyelitis, acute stroke, Guillain–Barré syndrome, neuropathies, etc. [1–5]. This suggests that their origin is complex and unclear. The possibility of direct cell infection by coronaviruses is under discussion. Angiotensin-converting enzyme 2 and membrane-bound serine protease matriptase-2 are thought to be viral receptors and entry points for some coronaviruses [6, 7]. SARS-CoV-2 enters the brain by the neurogenic route via the axons of olfactory cells and then spreads transsynaptically to various brain structures [8]. The hematogenous spread of the virus is mediated by infected monocytes and macrophages, which contributes to the damage of brain vessel endothelium [9], affecting the function of the neurogliovascular unit (neuron–astrocyte–vessel). This includes an increased permeability of the blood–brain barrier, leading to extravasation of plasma components into the vascular wall and perivascular space, inflammation, loss of autoregulatory function of the

brain, smooth muscle involvement, and ultimately occlusion of the vascular lumen at the final stage of the disease [10].

Angiotensin-converting enzyme 2 receptors are found in skeletal muscles, arterial and venous endothelial cells, arterial smooth muscle cells of many organs and the brain [3, 11, 12]. The somatic manifestations and other complications of COVID-19 can be explained by the effects of the viral toxin as well as by inducing effect of the pathogen, leading to a systemic inflammatory response syndrome. A cytokine storm is one of its components. High levels of proinflammatory cytokines (interleukin-1β, -2, and interleukin-2, -4, -10, -18 receptors, interferon-γ, C-reactive protein, tumor necrosis factor-α, granulocyte colony growth factor, etc.) have been reported in severe COVID-19 [2]. However, a retrospective cohort study conducted by M.L. Ciampa et al. in patients hospitalized with COVID-19 did not confirm these data [13]. The difficulty in assessing the role of cytokines in the development of COVID-19 is that cytokines have different functions due to their biological activity. In COVID-19, most conclusions are based on a quantitative assessment of cytokines without considering their functional purpose as substances that activate (stimulate) or suppress (inhibit) the immune response. Considering these factors, the role of cytokines in the development of COVID-19 needs to be clarified.

SARS-CoV-2, like dengue virus, is thought to infect the endothelium directly, resulting in extensive vascular involvement that occurs 3–6 days after disease onset [14, 15]. Endotheliopathy in COVID-19 increases the release of multimeric von Willebrand Factor (VWF) and platelet adhesion, and decreases levels of anticoagulant proteins on the endothelial surface. These processes, together with coagulopathy and infection-induced platelet hyperactivity, trigger blood clot formation and thrombotic microangiopathy [16]. One of the mechanisms that lead to the central nervous system damage in COVID-19 is local hemostasis defects due to an imbalance between high levels of highly active VWF multimers and low levels of ADAMTS13 (a disintegrin and metalloproteinase with thrombospondin motifs 13), which cleaves newly released highly active VWF multimers. In patients with sepsis and a significant imbalance between VWF and ADAMTS13, systemic thrombotic microangiopathy is observed. The imbalance of systemic coagulation factors in COVID-19 patients is mild and may manifest locally. However, it may be one of the reasons for delayed strokes or structural brain lesions [16]. The SARS-CoV-2 induces autoimmune responses by several mechanisms such as molecular mimicry between viral proteins and host antigens, formation of viral superantigens, activation of macrophage and monocyte defense mechanisms, and polyclonal activation of B cells [17].

Viral neuroinvasion can contribute to the exacerbation and progression of acquired, inherited, demyelinating, metabolic, neurodegenerative, and neuromuscular diseases [2, 18–20]. It is still unclear how the underlying mechanism for a particular form of the disease can be identified.

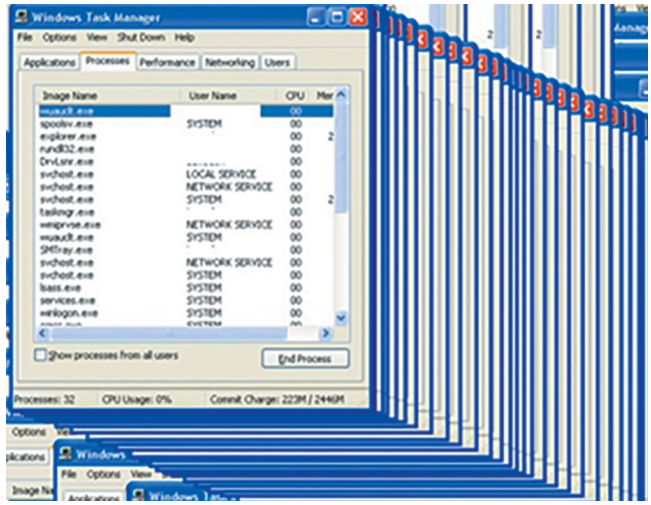


Fig. 1. This is how patient A. saw when he fixes his gaze on something.

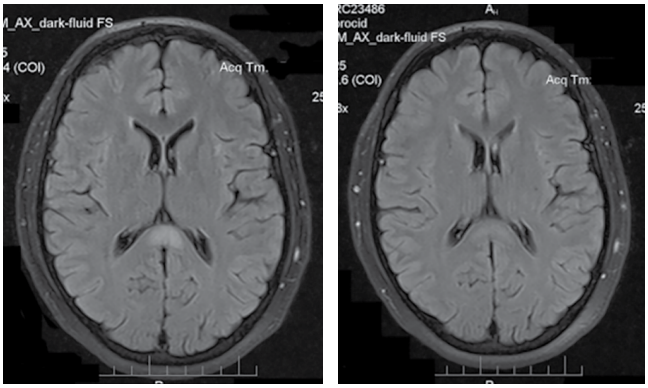
A case report

Patient A., 31 years old, was admitted with complaints of visual impairment. When looking with both eyes, he saw an object as "a set of identical objects arranged in a row" (Figure 1). When the patient covered his left eye, the right eye's perception remained distorted. With the right eye closed, the left eye's perception was normal. The same visual disturbances occurred when refocusing on a new object. The object and background were clearly seen. Duration of visual impairment was approximately 1 minute.

The patient noted gait instability, impaired memory for recent events (did not remember if he took the drug the day before or at the day of examination). The patient felt constantly anxious, detached from his surroundings, fearful of the future. It seemed as if "everything was happening not to" him. This experience was accompanied by general weakness, sweating, and a blood pressure increase to 160/90 mm Hg.

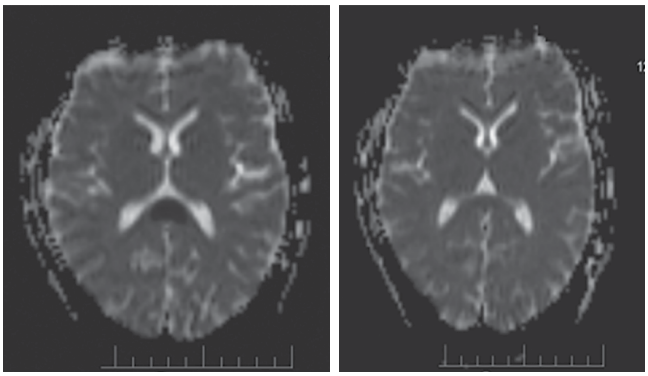
Visual impairment occurred on day 21 after moderate COVID-19. After being discharged from COVID-19 department, he could not return to work due to his inability to concentrate and perform routine tasks. He became more anxious. Prior to this case, he rarely had acute respiratory viral infections.

The neurological examination on admission to the Neurology department did not reveal any visual fields deficit in confrontation test. No sensory or motor disorders were observed. Deep tendon reflexes were active and symmetrical. Abdominal reflexes were absent, plantar reflexes were reduced. No abnormal signs were detected. Coordination tests revealed slight bilateral dysmetria. Bilateral dysdiadochokinesis was inconclusive. On Romberg test, the patient swayed slightly. Gait was unsteady, tandem walking was normal. A Montreal Cognitive Assessment Scale score was 27. The patient



A *B*

Fig. 2. Axial brain FLAIR MRI of Patient A.
A: day 1 of hospitalization; a single symmetric hyperintense splenial lesion with relatively smooth and clear contours;
B: day 13 of hospitalization; lesion almost completely resolved.



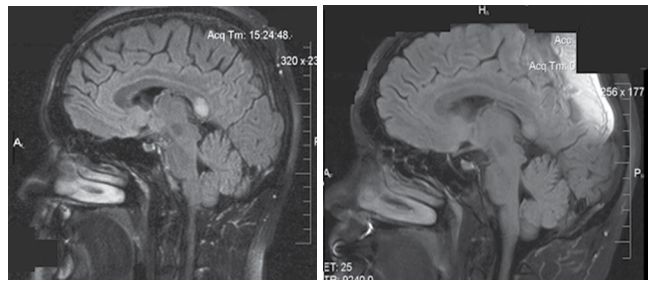
A *B*

Fig. 3. ADC Map MRI of Patient A.
A: day 1 of hospitalization, low signal intensity in the splenium projection; *B*: day 13 of hospitalization; average signal intensity in the splenium projection.

was unable to accurately redraw the cube and reproduce two words by heart (but was able to do so when prompted by category). In a ten-word test, the patient could recall only 4 of 10 words after 1 repetition. After the subsequent repetitions, he could reproduce 7, then 9, 9, and all 10 words. The volume of a correction task was 583 characters. A concentration score was 4.85. High attention span, decreased work capacity, and significant emotional instability were observed.

Laboratory tests showed ALT of 53 U/L (reference: < 37 U/L), AST of 29 U/L (reference: < 29 U/L), ferritin of 272 ng/mL (reference: 2–250 ng/mL). Coagulation test was unchanged. Other parameters were normal. The ophthalmologist's conclusion was simple astigmatism of the left eye, bilateral retinal angiopathy.

Magnetic resonance imaging (MRI) of the brain showed a hydrophilic splenial lesion measuring 12 × 18 × 15 mm without perifocal changes or contrast enhancement, consistent with cytotoxic lesions of the corpus callosum (CLOCCs; Figures 2–4).



A *B*

Fig. 4. Sagittal brain FLAIR MRI of patient A.
A: day 1 of hospitalization; hyperintense splenial lesion; *B*: day 13 of hospitalization, decreased intensity of the lesion.

The patient received metabolic therapy. On day 4 of hospitalization, visual disturbances resolved. The patient was discharged in satisfactory condition.

Discussion

In our case, the visual impairment could not be explained by the ophthalmologist's diagnosis of astigmatism, with its main symptoms being blurred and double vision. The patient clearly saw multiple images of objects arranged in a row.

This visual impairment was classified as cerebral polyopia because of its dependence on gaze fixation and its specific character. Polyopia is seeing two or more images arranged in ordered rows, columns, or diagonals after fixation on an object [21].

Cerebral polyopia differs from ocular polyopia, which is associated with the formation of areas in the optical media of the eye (cornea, lens) that refract light rays unevenly, resulting in the projection of multiple object images onto the retina. In our case, one of the objects could be perceived clearly, while the other could appear blurred. The visual defect was not associated with fixation, persisted when one eye was closed, and did not disappear when the patient was attempting to look at an object using a needle-hole occluder.

The patient's visual impairment also differed from palinopsia. Palinopsia is a visual perseveration. Patients continue to perceive or see the image again for a brief time even after the visual stimulus stops. The virtual image is perceived as real in the environment and does not disappear when the eyes are closed. Illusion occurs on the side of the defective visual field. Palinopsia occurs when the process is located in the temporo-occipital, parietal-occipital regions of the brain and, less commonly, in the posterior regions of the left hemisphere [22].

Cerebral polyopia is observed in cases of damage to the occipital or parieto-occipital regions of the brain [23] or to the parietal region [24]. The neurophysiological mechanism of polyopia is associated with increased excitability of neurons in the visual cortex [25]; with recoding of visual receptive

fields in the primary visual cortex with bilateral lesions in the occipital lobe [26]; with disruption of connections between the posterior parietal cortex, where visual-spatial analysis is performed, and the cortical gaze center, subcortical structures, and the stem gaze center [24]. Due to the lack of neuroimaging data confirming brainstem and hemisphere damage, preservation of visual fields, and monocular character of the defect, visual impairment is unlikely to be explained by the above mechanisms.

The dependence of the visual impairment on fixation and the monocular polyopia associated with an existing object excludes the possibility of visual polyopic hallucinations with the virtual image seen [22].

Foveal fixation and stereopsis (the sense of spatial expansion and relief of real objects) are performed by each eye separately. Perception of an object by a person with normal binocular vision requires precise alignment of the visual axis to re-fixate the object in each eye in the same dimension, which is achieved by extraocular (vergent) movements of the eyeballs (saccades) [27]. Visual information enters the primary visual field, the striate cortex or visual area V1, whose axons form the dorsal and ventral visual pathways. The dorsal pathway provides the answer to the question "Where?", terminates in neurons located in the posterior parietal region, and is associated with the spatial orientation of objects. The ventral pathway provides the answer to the question "What?", is associated with the identification of objects, and is adapted to the structures of the extrastriate visual cortex (V2, V3, V4, V5 fields). From the parietal region, the information reaches the frontal eye fields, which generate a targeted motor effect of the eyeballs in the form of saccades. At the same time, the role of the frontal eye fields is reduced to selecting the appropriate saccadic amplitude and transmitting the information through a number of intermediate structures, including the reticular and paramedian structures of the brainstem, to the muscles that provide extraocular eye movements [24, 27]. In our case, the patient clearly saw and recognized the object, and this suggested the spared ventral visual pathway. This allowed concluding that the patient's image perception was associated with a defect in foveal fixation and stereopsis of the object. Given the location of the process, it can be assumed that pathways from the frontal fields of the eye to the brainstem were affected. However, we cannot explain monopolyopia by a disruption of the considered connections.

In our case, monopolyopia was accompanied with a splenial lesion, which suggested the possibility of a partially split-brain syndrome associated with damage to the callous structures. This was confirmed by the experiment of Gazzaniga, 1999 [28]. When an object was presented to the left hemisphere, a split-brain patient gave the correct answer with an emotional response. When the same object was presented to the right hemisphere, the patient responded that she saw nothing. In cases of combined damage to the visual pathways and centers, partial transection of the posterior corpus callosum resulted in visual anomia [28]. This is the most likely defect in our patient.

Lesions in the isthmus/splenium and corpus callosum are associated with confusion, altered mental status, hallucinations, psychosis, mutism, and cognitive impairment [29–31]. This location may explain impaired anterograde memory, anxiety, fear, emotional instability, impaired auditory short-term memory (with less words retained and reproduced for the first sequence of the Luria test); depersonalization and derealization.

In our case, instability of walking cannot be explained by cerebellar symptoms. It does not correlate with inconclusive cerebellar symptoms, normal tandem walking, and unsteady but not ataxic gait. Instability is one of the corpus callosum lesion symptoms, with some patients unable to move [28].

There are acute and chronic variants of the split-brain or disconnection syndrome. Acute symptoms of disconnection may develop gradually or rapidly, within 4–7 days, and may partially or completely resolve [32–34], as in our patient.

Prior COVID-19, as well as the corresponding described symptoms suggested the diagnosis of mild encephalopathy with reversible splenial lesion (MERS) associated with COVID-19; partially split-up brain syndrome in the form of transient mononuclear cerebral polyopia, visual anomia, and mild neuropsychiatric disorders.

Based on the above-mentioned mechanisms of nervous system damage in SARS-CoV-2 infection, the presence of CLOCCs, the absence of toxic infectious manifestations or hemostasis disorders on admission to the Neurology department, rapid regression of visual impairment without using steroids (the patient received only metabolic therapy) suggest the vascular nature of the disease and associates the resulting condition with local endotheliopathy, which is possible in COVID-19 [16, 35] and may be the cause of CLOCCs [29].

MERS is a new disease entity with limited experience in diagnosing and understanding clinical manifestations.

We detected and confirmed monocular cerebral polyopia and visual anomaly in the patient with the splenial lesion. According to our hypothesis, these syndromes are thought to develop due to the splenial lesion and partial damage to the callous pathways and descending visual connections that pass near the corpus callosum. These syndromes in patients with lesions of the corpus callosum have not been previously described, so this information may contribute to the understanding of the function of the posterior corpus callosum.

There are almost no reports of MERS in COVID-19, so this report may be interesting for clinicians and brain researchers.

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Information about the authors

Tatiana V. Matveeva – Dr. Sci. (Med.), Prof., Department of neurology, neurosurgery and medical genetics, Kazan State Medical University, Kazan, Russia, https://orcid.org/0000-0002-1889-0094
Rustem T. Gaifutdinov – Cand. Sci. (Med.), Associate Professor, Department of neurology, neurosurgery and medical genetics, Kazan State Medical University, Kazan, Russia, https://orcid.org/0000-0001-5591-7148
Dinara Sh. Kamalova – neurologist, Central City Clinical Hospital No. 18, Kazan, Russia, https://orcid.org/0000-0002-3123-9546
Gulnaz A. Fasakhova – Head, Neurology department, Central City Clinical Hospital No. 18, Kazan, Russia,
https://orcid.org/0009-0004-4843-6767

Authors' contribution. *Matveeva T.V.* – writing an article, selection of literature; *Gaifutdinov R.T.* – work with the patient, selection of literature, analysis of neuroimaging data, analysis and discussion of the results; *Kamalova D.S., Fasakhova G.A.* – clinical observation of the patient, organization of examinations, consultations, participation in the discussion of the results.

Информация об авторах

Матвеева Татьяна Всеволодовна – д-р мед. наук, профессор каф. неврологии, нейрохирургии и медицинской генетики Казанского государственного медицинского университета, Казань, Россия,
https://orcid.org/0000-0002-1889-0094
Гайфутдинов Рустем Талгатович – канд. мед. наук, доцент каф. неврологии, нейрохирургии и медицинской генетики Казанского государственного медицинского университета, Казань, Россия,
https://orcid.org/0000-0001-5591-7148
Камалова Динара Шамилевна – врач-невролог ЦГКБ № 18, Казань, Россия, https://orcid.org/0000-0002-3123-9546
Фасахова Гульназ Ануровна – зав. неврологическим отделением ЦГКБ № 18, Казань, Россия, https://orcid.org/0009-0004-4843-6767

Вклад авторов: *Матвеева Т.В.* – написание статьи, подбор литературы; *Гайфутдинов Р.Т.* – работа с пациентом, подбор литературы, анализ данных нейровизуализации, анализ и обсуждение результатов; *Камалова Д.Ш., Фасахова Г.А.* – клиническое наблюдение за больным, организация обследований, консультаций, участие в обсуждении результатов.

Amyotrophic Lateral Sclerosis and Myasthenia Gravis: Comorbidities and Differential Diagnosis

Ekaterina V. Pervushina[1], Mansur A. Kutlubaev[1], Rim V. Magzhanov[1], Maksim V. Brazhnikov[2], Svetlana M. Farrakhova[2]

[1]Bashkir State Medical University, Ufa, Russia;
[2]Republican Clinical Hospital named after G.G. Kuvatov, Ufa, Russia

Abstract

Amyotrophic lateral sclerosis (ALS) and myasthenia gravis (MG) are both characterized by primarily motor deficit, and their differential diagnosis may be sometimes challenging. We present a case report of a patient with late-onset ALS, which was initially misdiagnosed for anti-acetylcholine (anti-AChR) antibody-positive MG. In some cases, ALS has been thought to be triggered by MG. In the presented case report, elevated anti-AChR antibody titers (positive anti-AchR Ab) had no clinical significance and possibly indicated an immune response to structural changes in the postsynaptic membrane of the neuromuscular synapse in the ALS patient.

Keywords: *amyotrophic lateral sclerosis; myasthenia gravis; anti-acetylcholine receptor antibodies; motor neuron disease*

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For correspondence: 3 Lenina str., Ufa, 450008, Russia. Bashkir State Medical University. E-mail: mansur.kutlubaev@yahoo.com. Kutlubaev M.A.

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Боковой амиотрофический склероз и миастения гравис: коморбидность и дифференциальная диагностика

Е.В. Первушина[1], М.А. Кутлубаев[1], Р.В. Магжанов[1], М.В. Бражников[2], С.М. Фаррахова[2]

[1]Башкирский государственный медицинский университет, Уфа, Россия;
[2]Республиканская клиническая больница имени Г.Г. Куватова, Уфа, Россия,

Аннотация

Боковой амиотрофический склероз (БАС) и миастения характеризуются чисто двигательным неврологическим дефицитом, и в некоторых случаях их дифференциальная диагностика может вызывать трудности. Представлен случай позднего дебюта БАС, который изначально был ошибочно принят за миастению с положительными антителами к ацетилхолиновым рецепторам (АХР). В некоторых случаях миастения рассматривается как триггер БАС. В представленном случае положительный титр антител к АХР не имел клинического значения и, возможно, указывал на иммунную реакцию на структурные изменения в постсинаптической мембране нервно-мышечного синапса у пациента с БАС.

Ключевые слова: *боковой амиотрофический склероз; миастения; антитела к ацетилхолиновым рецепторам; болезнь двигательного нейрона*

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Адрес для корреспонденции: 450008, Россия, Уфа, ул. Ленина, д. 3. Башкирский государственный медицинский университет. E-mail: mansur.kutlubaev@yahoo.com. Кутлубаев М.А.

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Amyotrophic lateral sclerosis (ALS) and myasthenia gravis (MG) are two diseases with motor disorders at the core of their clinical picture. However, a comprehensive examination of the clinical manifestations generally allows for a correct diagnosis to be established already at the screening. ALS is characterized by progressive mixed asymmetric paresis without oculomotor muscle involvement. The disease is irreversibly progressive and unresponsive to treatment. Myasthenia gravis (MG) is predominantly manifested by muscle weakness and abnormal fatigability. MG is classified by the distribution of paresis affecting mainly oculomotor, mimic, and bulbar muscles, as well as the muscles of upper and lower limb girdles and proximal limbs. MG course is usually fluctuating with MG patients quickly responding to acetylcholinesterase inhibitors (AChEIs).

A number of factors may impede differential diagnosis of ALS and MG. In some ALS subtypes, the peripheral motor neurons are primarily affected. At the early stages, only the bulbar muscles are involved, and there are no clear signs of tongue atrophy or fasciculation. This clinical picture resembles that of MG. On the other hand, MG may mimic ALS. For instance, MG with antibodies against muscle-specific tyrosine kinase (MuSK-MG) is characterized by asymmetric paresis sparing oculomotor muscles, although with an early onset of respiratory disturbances. In this subtype of MG, progression of paresis is rapid leading to atrophy and the patients show no response to AChEI [1].

Differential diagnosis of MG and ALS is of paramount importance as these two diseases require different treatment approaches. In MG patients, immunomodulatory therapy may be beneficial, while in ALS patients, glucocorticoid therapy fails and the most promising treatment options are associated with various neuroprotective strategies. We present a case report of an anti-AchR Ab positive patient who initially was diagnosed with late-onset MG. A more comprehensive review of the clinical and instrumental data ultimately led to the ALS diagnosis.

A case report

Patient N. aged 81 years.

Complaints: difficulty swallowing (both solid foods and liquids), impaired chewing, choking, nasality, and decreased voice pitch, severe generalized muscle weakness, especially in the neck ("dropping head") and legs.

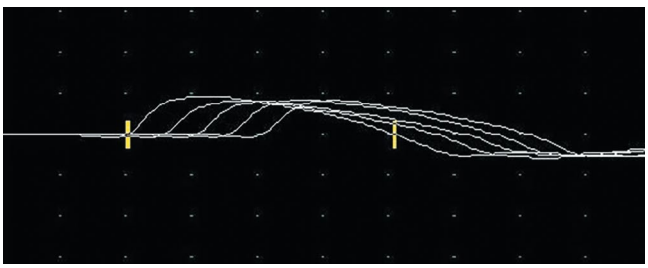
Medical history. Approximately one year ago, the patient first observed weakness in the right arm, which subsequently extended to the whole body. One year later, his close ones started noticing his slurred speech. One month prior to hospital admission, the patient experienced an acute decline in his condition, which manifested as acute dysphagia, including failure to swallow saliva, and weakness in the legs, which gradually increased over the course of the month.

Concomitant diseases. The patient had a history of basal cell skin carcinoma of the right anterior thorax which was operated in 2021; neither radiotherapy or chemotherapy was given; prostate adenoma (operated in 2021); stage III arterial hypertension > 10 years managed by triple antihypertensive therapy with combination of amlodipine, valsartan, and hypothiazid 5/160/12.5 mg. The patient had smoked for 40 years (one pack of cigarettes per day) and ceased smoking in 2004; worked at construction sites for a long time, and as a crane operator for the last 20 years before retirement at the age of 65. No family history of similar disorders, the patient's father died early in life because of the trauma, mother had symptoms resembling parkinsonism (limb tremor, bradykinesia, and muscle rigidity), we have no information about her diagnosis.

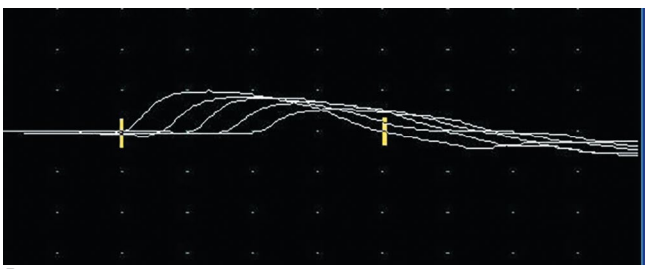
The patient was urgently admitted to the central district hospital. Diagnosis at admission: cerebrovascular disease. The complete blood count and metabolic panel test results fell within reference limits; a urinalysis revealed moderate leukocyturia; electrocardiography showed chronic atrial fibrillation, which was detected for the first time. Prior to AChEI

treatment initiation, the patient underwent electromyography (EMG) with low-frequency (3 Hz) repetitive stimulation of the following muscles: m. orbicularis oculi, m. nasalis, m. digastricus, m. deltoid, and m. abductor digiti minimi. The EMG results were suggestive of abnormalities in postsynaptic neuromuscular transmission (muscle action potential (MAP) decrement 16–32%, post-activation depression and post-activation exhaustion, see Figure 1). Consequently, the diagnosis was revised to myasthenia gravis. Upon insertion of a nasogastric tube, the patient started AChEI (pyridostigmine bromide 180 mg daily) and glucocorticosteroid treatment (prednisolone 70 mg daily). General weakness, particularly in the legs, progressively increased with the treatment. Additionally, dyspnea developed, which may have been caused by the side effects of the ongoing treatment, also including fluctuations in blood pressure, progressive cardiac rhythm disturbances, and glycemia. The patient stopped walking without assistance.

To verify the diagnosis and modify the treatment, the patient was transferred to the Neurology Department of G.G. Kuvatov Republican Clinical Hospital.



A



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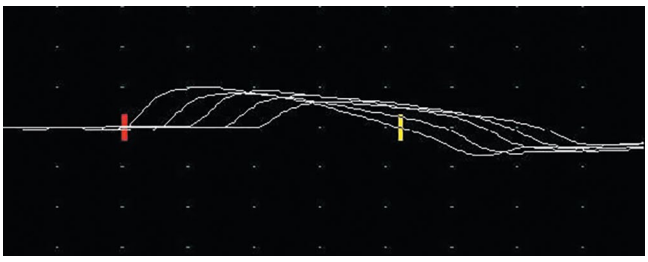

C

Fig. 1. Electromyogram of the patient N.
A – *m. deltoideus dextra,* primary amplitude decrement (1–5) 35%; *B* – post-activation depression; *C* – post-activation exhaustion.

At admission to the Neurology Department: height 180 cm, weight 67 kg (weight loss of 15 kg within 1.5 months), BMI 20.68 kg/m^2, respiration rate 19 breaths per min, chest expansion decreased. Lower legs are swollen. Blood oxygen saturation was 91%, with oxygen insufflation increased to 97%. Cardiac arrhythmia, tachycardia, heart rate 100 bpm. Nasogastric feeding. Other physical characteristics without visible changes.

Neurological examination: normal ranges of eye movement, no diplopia, the orbicularis oculi muscle strength grade 5; masticatory muscle hypotrophy with strength down to grade 3; the jaw jerk reflex not exaggerated, symmetric face, mimic muscle strength sufficient, hypophony, poor velar elevation, reduced swallowing reflex and palatal and pharyngeal reflexes, dysphagia, dysarthria, fibrillations on the tongue without hypotrophy, tongue movements and strength within normal range. The snout reflex and the nasolabial reflex were positive. The strength of the neck extensor muscles is at grade 1 and of the neck flexor muscles is at grade 2 (dropped head syndrome, DHS). The patient had a clinical picture of severe, predominantly flaccid, asymmetric tetraparesis. Active movements of the limbs are limited, predominantly on the right side: muscle strength in the proximal limbs decreased to grade 1 in the leg and grade 2 in the arm with plegia in the distal parts. Muscle strength in the left-side limbs decreased to grade 3, predominantly in the proximal parts. The muscle tone is decreased. Asymmetric diffuse upper and lower limb hypotrophy andingle fasciculations in the upper and lower limb muscles were observed. Tendon reflexes were symmetrically reduced, no pathological reflexes elicited. Moderate muscle fatigability was observed: a grade 0.5–1.0 muscle strength decrease during muscle strength re-assessments. Coordination in the left-side limbs within normal range. Gait assessment was impossible [due to the patient's immobility]. No sensory disturbances. The patient had urinary dysfunction associated with prostatic hyperplasia. After recommendation of the urologist, the patient was catheterized.

The patient was differentially diagnosed between motor neuron disease and neuromuscular junction disorder. A neostigmine test was performed twice with no apparent positive effect.

MAP decrement assessment by needle electromyography (EMG) in m. trapezius, *m. abducens digiti minimi dextr.* performed 12 h after AChEI withdrawal detected no neurophysiological signs of disturbance in neuromuscular transmission. No significant changes in M-response or decremental response were observed during post-tetanic depression and exhaustion. Needle EMG results showed a pattern of neurogenic changes in motor action potentials with greater average duration and amplitude in *m. rectus femoris, m. tibialis anterior, m. deltoideus*, and *m. interosseous* I (Fig. 2). Detection of single spontaneous fibrillation potentials and fasciculations confirms generalized damage to motor neurons in the spinal

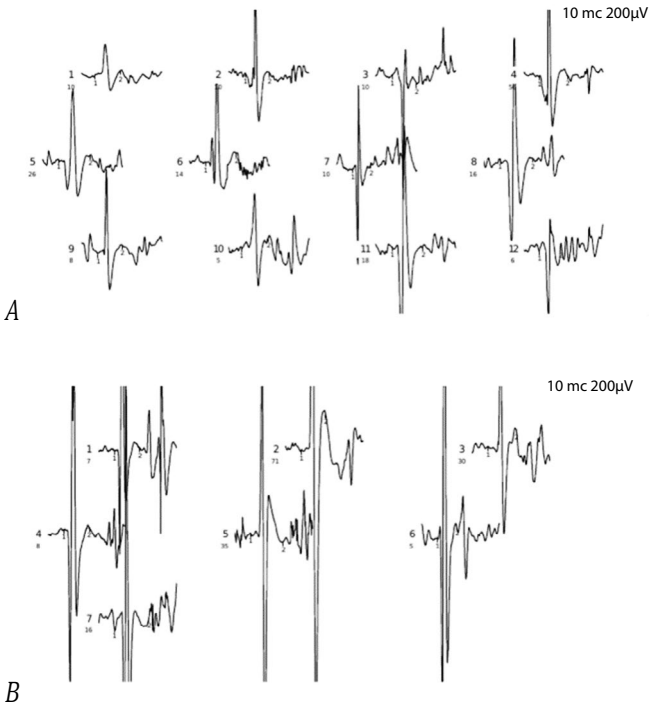

Fig. 2. Needle electromyogram of patient N.
A – m. deltoideus dextra, average duration 13.3 ms (8.9–16.3 ms, ref. values < 12 ms), average amplitude 1408 μV (459–2164 μV, ref. values < 550 μV), spontaneous activity (single fibrillation and fasciculation potentials); *B – m. interosseous I dextra*, average duration 14 ms (9.9–17.8 ms, ref. values < 10.3 ms), average amplitude 4863 μV (2125–8427 μV, ref. values < 750 μV), and moderate spontaneous activity (fasciculation potentials).

cord. The EMG assessment of the tongue muscles detected a single spontaneous fibrillation potentials and an increased average amplitude of motor action potentials without any changes in duration.

To rule out focal lesions in the brainstem area, a brain MRI was conducted, which revealed the signs of chronic cerebrovascular insufficiency (Fig. 3).

Complete work-up of the patientrevealed left kidney cyst, moderate right kidney pyelectasia, diverticula and pseudo-diverticula in the urine bladder, and diffuse pulmonary sclerosis.

The complete blood count results were within normal range; urinalysis detected transitory macrohematuria and leukocyturia caused by uroinfection due to insertion of urinary catheter; comprehensive metabolic panel results indicated hypoproteinemia (51.0 g/L against ref. values of 66–83 g/L) and hypoalbuminemia (31.6 g/L against ref. values of 35–52 g/L), elevated urine acid levels up to 440.9 μM/L (ref. values: 208.3–428.3 μM/L), and other parameters within a range of reference values. Prostate-specific antigen: 2.450 ng/mL (ref. value < 6.5 ng/mL). Elevated anti-AChR antibody titers: > 20 nM/L (ref. values < 0.5 nM/L).

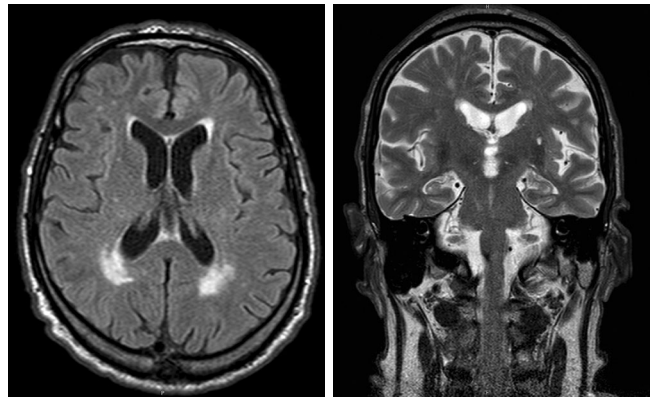

Fig. 3. Periventricular hyperintensity of vascular origin on T2 (*A*) and FLAIR (*B*) MRI scans of the patient's brain.

Based on progressive asymmetric paresis, the absence of any other symptoms since its onset in the upper right limb, electrophysiological signs of neuronal damage present on threelevels of central nervous system, and the absence of positive response to AChEI administration, the diagnosis was established as motor neuron disease (MND). Amyotrophic lateral sclerosis, spinal onset with asymmetric tetraparesis to the degree of plegia in the distal parts of the upper right limbs, neck muscles involvement, bulbar-pseudobulbar syndrome, and respiratory disturbances. Insignificant anti-AchR positivity. Concominant diagnosis: coronary artery disease associated with arrhythmia. Chronic atrial fibrillation and congestive heart failure (ACC/AHA Stage B, NYHA Class II). Stage III arterial hypertension. Stage I prostatic hyperplasia.

Following the gradual withdrawal of prednisolone and pyridostigmine bromide, the patient exhibited a slight improvement in blood oxygen saturation levels and respiratory function.

Discussion

At the district hospital (Level II of healthcare system), the initial diagnosis for patient N was myasthenia gravis, which was subsequently revised to amyotrophic lateral sclerosis (ALS). However, the presence of anti-AChR antibodies raised doubts about the absence of myasthenia gravis.

The potential association between ALS and myasthenia gravis is manifold. The most straightforward explanation is that they are capable of mimicking each other, as previously stated. Nevertheless, clinical cases with combination of these two diseases are particularly intriguing. In the majority of MG-ALS comorbidity cases documented in the literature, classical myasthenia gravis preceded the symptoms indicative of upper and lower motor neuron damage, which may be considered a transformation of MG into ALS. Few cases of

patients with MG followed by ALS have also been previously described. Epidemiological data analysis showed that MG patients, who further developed ALS, were of older age, had two times more frequently a bulbar onset, and a more severe course of disease [2].

The majority of authors consider MG and ALS coexistence to be far beyond coincidence. Based on western countries incidence rates the co-occurrence of both diseases is a really exceptional event ($7.5/10^9$), while S. de Pasqua et al. reported higher incidence rates based on their studies ($1.87/10^7$) [2]. In terms of pathogenesis, the immunological dysfunctions in patients with MG may trigger the ALS development, especially if there is genetic predisposition. Antibodies to lipoprotein–related receptor protein 4 (LRP4) can be found both in some MG subtypes and in ALS, as this protein plays an important role in the neuromuscular synapse and motor neuron functioning. This mechanism is uncommon and is unlikely in the present case, as the patient was anti-AChR antibody positive with elevated anti-AChR antibody titers. On the other hand, dysfunction of the regulatory T-cells (Tregs) typical for MG may be considered a trigger to motor neuron damage. Tregs are shown to suppress proinflammatory cytokine production, stimulate production of antiinflammatory cytokines and neurotrophic factors, as well as mediate microglia activation, etc. [3]. In MG, suppressive function of Tregs is compromised [4], whereas in ALS, Tregs are thought to suppress microglial activation and production of free radicals [5]. Experimental studies suggest that decreased blood levels of Tregs are associated with rapid disease progression [6].

Neuromuscular synapse dysfunction plays a role in the pathogenesis of ALS due to a "dying back" phenomenon, i. e. progressive degeneration of motor axons, muscle denervation and decreased neuromuscular transmission [7]. The pathogenetic similarities between ALS and MG are supported by electrophysiological studies. In their compound muscle action potential (CMAP) decrement study, D. Zhang et al. demonstrated a decrease in M-response amplitude in m. abductor pollicis brevis in ALS patients — the so-called neurogenic decrement [8]. Nowadays, neuromuscular junction dysfunction is considered to play a special role in the pathogenesis of ALS [9].

The patient's history of basal cell skin carcinoma does not exclude paraneoplastic ALS [10]. However, in the presented case, cancer was highly differentiated with low immunogenicity, which resulted in a low risk of paraneoplastic response. The tumor as a source of antigenic stimulation was removed two years prior to disease onset, and the patient did not respond to immunosuppressive therapy.

The patient with predominantly flaccid tetraparesis was diagnosed with late-onset MG and presumptive therapy (AChEI + glucocorticosteroids) at the district l hospital failed to yield any positive results. Moreover, administration of glucocorticosteroids had a transient negative effect that leveled off after their withdrawal. Results of follow-up examination ruled out MG and were suggestive of ALS. At the same time, the anti-AChR ab test was positive. A comprehensive analysis of clinical and electrophysiological data, and review of the patient's family medical history revealed no signs of neuromuscular synapse dysfunction, and the patient was diagnosed with ALS. The presence of autoantibodies was regarded clinically insignificant. In the presented case, positive anti-AChR ab test result may indicate an immune response to the AChR degeneration in the neuromuscular synapse [11, 12]. Several authors suggested that pathogenesis of certain ALS subtypes includes autoimmune traits [11], but failure of the glucocorticosteroid therapy is inconsistent with this hypothesis.

Thus, MG-ALS co-morbidity is a rare but explainable phenomenon in terms of pathogenesis [13–17]. A case report presented in this article highlights the importance of a comprehensive analysis of the clinical picture in the diagnosis of neuromuscular diseases. Even the presence of disease-specific autoimmune antibodies is not sufficient to diagnose MD. The diagnosis must be supported by other typical manifestations, such as oculomotor muscle involvement, pronounced muscle fatigability, response to AchRI therapy, cold pressure test, and muscle action potential decrement by low-frequency repetitive nerve stimulation.

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Information about the authors

Ekaterina V. Pervushina – Cand. Sci. (Med.), Assistant Professor, Department of neurology, Bashkir State Medical University, Ufa, Russia, https://orcid.org/0000-0002-9352-5783
Mansur A. Kutlubaev – Dr. Sci. (Med.), Head, Department of neurology, Bashkir State Medical University, Ufa, Russia, https://orcid.org/0000-0003-1001-2024
Rim V. Magzhanov – Dr. Sci. (Med.), Professor, Department of neurology, Bashkir State Medical University, Ufa, Russia, https://orcid.org/0000-0003-1246-4833
Maksim V. Brazhnikov – neurologist, Republican Clinical Hospital named after G.G. Kuvatov, Ufa, Russia, https://orcid.org/0009-0008-5959-388X
Svetlana M. Farrakhova – neurologist, Republican Clinical Hospital named after G.G. Kuvatov, Ufa, Russia, https://orcid.org/0009-0007-3890-264X

Authors' contribution. *Pervushina E.V., Farrakhova S.M.* – writing the article; *Kutlubaev M.A.* – study of the literature sources, writing the article, editing the article; *Magzhanov R.V.* – editing the article; *Brazhnikov M.V.* – study of the literature sources.

Информация об авторах

Первушина Екатерина Владимировна – канд. мед. наук, доцент каф. неврологии Башкирского государственного медицинского университета, Уфа, Россия, https://orcid.org/0000-0002-9352-5783
Кутлубаев Мансур Амирович – д-р мед. наук, доцент, зав. каф. неврологии Башкирского государственного медицинского университета, Уфа, Россия, https://orcid.org/0000-0003-1001-2024
Магжанов Рим Валеевич – д-р мед. наук, профессор каф. неврологии Башкирского государственного медицинского университета, Уфа, Россия, https://orcid.org/0000-0003-1246-4833
Бражников Максим Владимирович – врач-невролог Республиканской клинической больницы им. Г.Г. Куватова, Уфа, Россия, https://orcid.org/0009-0008-5959-388X
Фаррахова Светлана Мизхатовна – врач-невролог Республиканской клинической больницы им. Г.Г. Куватова, Уфа, Россия, https://orcid.org/0009-0007-3890-264X

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