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As a manuscript

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COMPARISON OF CLINICAL AND PATHOPHYSIOLOGICAL CHARACTERISTICS OF MYALGIC ENCEPHALOMYELITIS/CHRONIC FATIGUE SYNDROME AND CHRONIC FATIGUE ASSOCIATED WITH POST-COVID-19 SYNDROME

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CONTENT

INTRODUCTION

Relevance of the research topic

Coronavirus disease 2019 (COVID-19) has become a global problem for the medical community, not only because of its widespread prevalence but also because of the high incidence of long-term sequelae following the acute phase of a SARS-CoV-2 infection. On 6 October 2021, the World Health Organization (WHO) proposed the term "post COVID-19 condition" and developed a clinical case definition to describe the wide range of symptoms which could be observed after COVID-19 [50]. According to this definition, "post-COVID-19 condition occurs in individuals with a history of probable or confirmed SARS-CoV-2 infection, usually 3 months from the onset of COVID-19 with symptoms that last for at least 2 months and cannot be explained by an alternative diagnosis. Common symptoms include fatigue, shortness of breath, cognitive dysfunction but also others and generally have an impact on everyday functioning. Symptoms may be new onset following initial recovery from an acute COVID-19 episode or persist from the initial illness. Symptoms may also fluctuate or relapse over time". The proposed term "post COVID-19 condition" was included in the International Classification of Diseases, 10th revision (ICD-10), code U09.9. In English-language medical scientific literature and in the field of public health, several synonymous equivalents are used in this meaning [50]. In the Russian-language literature, the most common term is "post-COVID-19 syndrome" (PCS).

The prevalence of PCS was estimated by WHO to be 10-20% among COVID-19 survivors [290]. However, a 2023 meta-analysis of 194 studies including 735,006 individuals showed that 4 months after COVID-19, one or more symptoms persist in 45% of individuals [303]. Twelve months after acute illness, the prevalence of PCS remains high (18.3% among COVID-19 survivors in the USA and 5.2% in the UK) [192, 259].

By the middle of 2021, the main symptom complexes characteristic of PCS had already led to the recognition of the similarity between PCS and myalgic

encephalomyelitis/chronic fatigue syndrome (ME/CFS) [179, 196, 238], and the need to raise awareness of ME/CFS among general practitioners [176]. According to modern concepts, reflected, in particular, in expert consensus documents and clinical guidelines on this syndrome published in 2021, ME/CFS is a separate nosological form, a chronic multisystem disease [128, 216, 224]. During 1990-2015 several sets of diagnostic criteria which allow differentiating ME/CFS from other diseases with similar symptoms were developed [174]. A certain challenge is the lack of universally recognised biomarkers of ME/CFS, therefore all proposed diagnostic criteria are clinical. WHO included ME/CFS in the ICD in 1969. ICD-8 and ICD-9 used the term "benign myalgic encephalomyelitis" (code 323), and ICD-10 and ICD-11 use the term "post-viral fatigue syndrome" to describe this nosological form (codes G93.3 and 8E49, respectively) because, according to current data, up to 80% of cases of ME/CFS manifest after an acute illness with a clinical picture resembling a viral infection [214]. In the Russian medical literature, the cluster of symptoms corresponding to ME/CFS was first described by A.G. Chuchalin and D.G. Soldatov in 1989, who proposed the term "post-viral asthenia syndrome" [46]. The authors associated the development of this cluster of symptoms with viral infections, indicating two possible mechanisms: metabolic changes during the acute period of infection or, as more probable, viral persistence. Such conclusions were made based on the analysis of literature data on the detection of high titers of antibodies to viral antigens in the sera of patients, and in some cases – detection of viral DNA/RNA in organs and tissues confirming the persistence of herpes- and enteroviruses.

Despite the fact that the history of the ME/CFS is inextricably linked to outbreaks of viral infections, the increasing prevalence of sporadic cases since the late 1990s has led to the conclusion that there is a possibility of a non-infectious etiology and more diverse mechanisms of pathogenesis of this disease, among which dysfunction of the body's regulatory systems (nervous, endocrine, and immune) have increasingly attracted the attention of researchers. Thus, in 1999, A.S. Zaichik and L.P. Churilov [10] for the first time suggested that ME/CFS may represent a limited chronic autoimmune hypothalamitis. In 2001 a group of authors concluded after outlining the main hypotheses

of the pathogenesis of ME/CFS: "the pathogenesis of this disease is based on manifestations of dysregulation in the integrative systems of the organism: nervous, endocrine, and immune ones" [38]. Signs of abnormalities in the function of these systems have been identified in ME/CFS in numerous original works during last twenty years [214]. The presence of evident microcirculatory alterations in the acute period of COVID-19 led to the assumption of their important role in the pathogenesis of PACS and ME/CFS, and to the emergence of studies on the clinical pathophysiology of microcirculatory disorders in these diseases [327]. At the same time, original studies on the pathogenesis of PCS carried out in the Russian adult population are few, which resulted in insufficient awareness of Russian physicians about this disease, its pathogenesis, and treatment approaches.

Insufficient awareness of medical specialists about the pathogenesis, principles of diagnostics and therapy of PCS and ME/CFS negatively affects the quality of medical care received by such patients. This issue became especially relevant during the COVID-19 pandemic when the number of patients who present to physicians of different specialities with symptoms characteristic for ME/CFS, occurring after COVID-19 and persisting for several months – increased sharply [143]. The prevalence of ME/CFS in the pre-pandemic period was quite high (estimated as 0.89% in the general population) and similar in all regions of the world, according to the epidemiological study conducted in 13 countries [288]. The first post-pandemic studies showed that the prevalence of ME/CFS in the population increased 5-fold due to the PCS cases fulfilling the diagnostic criteria for ME/CFS (which may account for 43-58% of all PCS cases) [54, 173, 193, 218, 220]. Despite the fact that ME/CFS does not affect the life expectancy of patients, it makes a significant contribution to the global burden of diseases, in particular, in the USA it exceeds HIV infection and multiple sclerosis by 2 times in terms of Disability-adjusted life years (DALY) [113].

The above facts determine the scientific significance and practical relevance of studying and comparing the pathogenesis of ME/CFS and PCS, which are increasingly common in general medical practice. Another issue important for the development of

treatment approaches to these syndromes is to clarify whether chronic fatigue associated with PCS is clinically identical to ME/CFS, which is known to doctors from the 20th century, or represents a qualitatively different condition.

Current state of research in the field

In the English-language literature there is a large body of research regarding the etiology, pathogenesis and potential biomarkers of ME/CFS. The achievements of the last twenty years in this field are outlined in several review articles [79, 179, 206, 214]. Expert consensus and clinical guidelines on ME/CFS, which has been developed by 2021 in North American and European countries, reflect modern approaches to the diagnosis and therapy of this disease [128, 216, 224]. In 2020-2023 a number of publications appeared which indicate the clinical similarity of PCS (described at that time) with ME/CFS [158, 324, 329]. In 2023 Komaroff and Lipkin [180] conducted a comprehensive work on the systematization and comparison of the research into pathogenesis of ME/CFS and PCS, and compiled a roadmap summarising both positive and negative results regarding the role of each putative mechanism in the pathogenesis of ME/CFS and PCS (the total number of literature sources analysed by the authors is 559). In all of the review articles, an important place is occupied by findings indicating dysfunction of the body's regulatory systems – nervous, endocrine and immune, as well as the disturbances in microcirculation and energy metabolism. The analysis of review publications in Russian language devoted to ME/CFS has shown that the results described in English-language reviews, are mentioned (without a detailed consideration) only in few Russian-language articles on the issue of chronic fatigue associated with PCS published in 2021 [28, 35]. Original research works addressing the pathogenesis of ME/CFS and PCS are also only sporadic in the Russian-language literature. However, it is noteworthy that in most of them the subject of study was exactly neuro-immune-endocrine interactions [9, 15, 24, 27, 40, 42, 43]. From the clinical point of view, the prevalence of ME/CFS (according to its diagnostic criteria)

among patients with PCS has not been previously estimated in the Russian adult population.

The purpose of the study

was to clarify whether patients with chronic fatigue associated with PCS meet the diagnostic criteria of ME/CFS and to obtain new knowledge about the degree of similarity and possible differences in the pathogenesis of ME/CFS and PCS.

Research objectives

1. To study the prevalence of ME/CFS according to its diagnostic criteria among patients with chronic fatigue associated with PCS.
2. To determine the signs of dysautonomia based on the analysis of heart rate variability (HRV), blood pressure variability (BPV), and baroreflex function during spontaneous breathing and paced breathing in patients with chronic fatigue associated with PCS and in patients with ME/CFS not related to COVID-19.
3. To evaluate hypothalamic-pituitary-adrenal (HPA) axis activity with the measurement of the cortisol awakening response (CAR) in patients with chronic fatigue associated with PCS and in patients with ME/CFS not related to COVID-19.
4. To investigate microvascular endothelial function based on the analysis of post-occlusive reactive hyperemia (PORH) measured by Laser-Doppler flowmetry (LDF) in patients with chronic fatigue associated with PCS and in patients with ME/CFS not related to COVID-19.
5. To characterize the state of regulatory function of the immune system according to the data on the spectrum and intensity of autoreactive processes reflecting its interaction with cells of different organs and tissues in patients with chronic fatigue associated with PCS and in patients with ME/CFS not related to COVID-19.

6. To assess microbiome composition according to the data obtained by gas chromatography-mass spectrometry of microbial markers in venous blood of patients with chronic fatigue associated with PCS and in patients with ME/CFS not related to COVID-19.
7. On the basis of the study results, to assess the degree of similarity and the presence of differences in the pathogenesis of ME/CFS and chronic fatigue associated with PCS.

Scientific novelty of the study

For the first time in Russian population prevalence of ME/CFS among patients with chronic fatigue associated with PCS was determined according to the internationally accepted diagnostic criteria of ME/CFS.

For the first time in a single study a comprehensive assessment of several suggested pathomechanisms of ME/CFS and PCS (including dysfunction of three regulatory systems – nervous, endocrine and immune ones, and microcirculatory disorders) was carried out. The characteristics of these pathomechanisms and their interrelationships allowed to expand our understanding of the pathogenesis both of ME/CFS and PCS.

For the first time in a single study a simultaneous comparison of clinical picture and pathomechanisms of ME/CFS not related to COVID-19 and chronic fatigue associated with PCS was performed which should contribute to the formation of a more holistic understanding of PCS and its relationship with ME/CFS.

Theoretical and practical significance of the study

The high prevalence of ME/CFS, according to its diagnostic criteria, which was identified for the first time in the Russian population of patients with PCS, may facilitate the application of algorithms of medical care previously developed for patients with ME/CFS to those with PCS who meet the ME/CFS diagnostic criteria. This will

contribute to the development of a personalized approach to the medical management of PCS, particularly its fatigue dominant forms.

A comprehensive assessment of various potential pathomechanisms of ME/CFS and PCS in a single study made it possible to evaluate the contribution of each component and to identify potential links between different mechanisms, allowing them to be considered as links of a unified pathogenesis.

The clinical and pathophysiological focus of this study allowed us to formulate a number of practical recommendations regarding objective methods of examining patients with ME/CFS and PCS, as well as potential therapeutic approaches upon detecting specific abnormalities.

Some correlations identified between clinical features and pathophysiological findings in ME/CFS and PCS confirm the clinical significance of abnormalities detected through laboratory and instrumental methods of investigation.

Determination of similarities and differences in the pathogenesis of chronic fatigue associated with PCS and ME/CFS not related to COVID-19 is crucial for the understanding of the directions for further research in this field in order to develop methods of treatment based on the pathogenesis of these conditions.

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Research methodology

The research methodology included theoretical and empirical methods. The first, theoretical part of the dissertation consisted in the analysing literature on the issues of terminology and epidemiology of ME/CFS and PCS, their clinical relationship, modern views on their etiology and pathogenesis based on reproducible scientific research results.

In the second, empirical part of the work cross-sectional analysis methods were used to achieve the objectives of the study. At the first stage cohorts of patients were formed based on inclusion and exclusion criteria detailed in Chapter 2 from those who willed to participate in the study at the Laboratory of the Mosaic of Autoimmunity in St. Petersburg State University and at the Department of Hospital Therapy with the Course of Allergology and Immunology named after Academician M.V. Chernorutsky with the clinic in Pavlov First Saint Petersburg State Medical University.

At the second stage, the clinical, laboratory, and instrumental methods of examination, discussed in detail below in Chapter 2, were conducted.

The final stage consisted of processing the obtained data using appropriate statistical analysis methods, after which conclusions were drawn and practical recommendations were formulated.

Applicant's personal contribution

The applicant conducted an analysis of domestic and foreign literature, developed the research design and program. As a medical doctor, the applicant examined patients with ME/CFS and PCS, mastered and performed for each patient by herself all instrumental methods included in this work, and participated together with laboratory staff in the preparation of biomaterial and laboratory testing. The applicant performed by herself statistical analysis of the obtained data, formulated conclusions, and the main provisions presented for the dissertation defence.

The volume and structure of the dissertation

The thesis is set out on 195 pages of typewritten text and consists of an introduction, literature review, description of materials and methods of research, presentation of the results of own research, discussion of the results obtained, conclusions and practical

recommendations, includes 42 tables, 2 figures and 1 annex. The list of the references contains 331 bibliographic sources, including 48 in Russian and 283 in English.

The degree of reliability and approbation of the work

The reliability of the obtained results was ensured by the study of a sufficient number of patients, taking into account the homogeneity of the groups and their comparability in terms of sex and age characteristics, as well as the use of appropriate methods of statistical analysis. 16 journal articles were published on the topic of the study, 13 of which were published in journals indexed in the international reference databases Web of Science and Scopus, including:

- 8 articles describing the scientific results obtained during the work, some of which were included in the thesis:

1. Ryabkova VA, Gavrilova NY, Fedotkina TV, Churilov LP, Shoenfeld Y. Myalgic Encephalomyelitis/Chronic Fatigue Syndrome and Post-COVID Syndrome: A Common Neuroimmune Ground? // Diagnostics (Basel). 2022;13(1):66. doi: 10.3390/diagnostics13010066.

2. Ryabkova VA, Gavrilova NY, Poletaeva AA, Pukhalenko AI, Koshkina IA, Churilov LP, Shoenfeld Y. Autoantibody Correlation Signatures in Fibromyalgia and Myalgic Encephalomyelitis/Chronic Fatigue Syndrome: Association with Symptom Severity // Biomedicines. 2023;11(2):257. doi: 10.3390/biomedicines11020257.

3. Ryabkova VA, Rubinskiy AV, Marchenko VN, Trofimov VI, Churilov LP. Similar Patterns of Dysautonomia in Myalgic Encephalomyelitis/Chronic Fatigue and Post-COVID-19 Syndromes // Pathophysiology. 2024;31(1):1-17. doi: 10.3390/pathophysiology31010001.

4. Ryabkova VA, Gavrilova NY, Kanduc D, Churilov LP, Shoenfeld Y. Post-COVID syndrome and its immunopathological mechanisms. The role of autoimmunity // Russian Biomedical Research. 2021; 6(3):7-11.

5. Churilov LP, Kanduc D, Ryabkova VA. COVID-19: adrenal response and molecular mimicry // Isr Med Assoc J. 2021;23(10):618-619.

6. Gavrilova NY, Soprun LA, Lukashenko MV, Ryabkova VA, Fedotkina TV, Churilov LP, Shoenfeld Y. New Clinical Phenotype of the Post-Covid Syndrome: Fibromyalgia and Joint Hypermobility Condition. // Pathophysiology. 2022;29(1):24-29. https://doi.org/10.3390/pathophysiology29010003

7. Normatov M.G., Karev V.E., Kolobov A.V., Maevskaya V.A., Ryabkova V.A., Utekhin V.I., Churilov L.P. Post-COVID Endocrine Disorders: Putative Role Of Molecular Mimicry And Some Pathomorphological Correlates // Diagnostics. 2023;13(3):522.DOI: 10.3390/diagnostics13030522

8. Yevsyutina Y.V., Danilov A.B., Simonova A.V., Ryabkova V.A. Psychoneuroimmunological Markers of Post-COVID Syndrome // Clinical Pathophysiology. 2023; 29(3):43-50.

- 8 review articles:

1. Ryabkova VA, Churilov LP, Shoenfeld Y. Influenza infection, SARS, MERS and COVID-19: Cytokine storm – The common denominator and the lessons to be learned.// Clin Immunol. 2021;223:108652. doi: 10.1016/j.clim.2020.108652.

2. Ryabkova VA, Churilov LP, Shoenfeld Y. Neuroimmunology: What Role for Autoimmunity, Neuroinflammation, and Small Fiber Neuropathy in Fibromyalgia, Chronic Fatigue Syndrome, and Adverse Events after Human Papillomavirus Vaccination? //Int J Mol Sci. 2019;20(20):5164. doi: 10.3390/ijms20205164.

3. Shoenfeld Y, Ryabkova VA, Scheibenbogen C, Brinth L, Martinez-Lavin M, Ikeda S, Heidecke H, Watad A, Bragazzi NL, Chapman J, Churilov LP, Amital H. Complex syndromes of chronic pain, fatigue and cognitive impairment linked to autoimmune dysautonomia and small fiber neuropathy.// Clin Immunol. 2020;214:108384. doi: 10.1016/j.clim.2020.108384.

4. Makarova YA, Ryabkova VA, Salukhov VV, Sagun BV, Korovin AE, Churilov LP. Atherosclerosis, Cardiovascular Disorders and COVID-19: Comorbid Pathogenesis. // Diagnostics (Basel). 2023;13(3):478. doi: 10.3390/diagnostics13030478.

5. Shcherbak S. G., Anisenkova A. Yu., Mosenko S. V., Puzankova E. V., Mamaeva O. P., Vologzhanin D. A., Gavrilova N. Yu., Ryabkova V. A., Churilov L. P., Golota A. S., Kamilova T. A. “Long COVID”. Current state of the problem and prospects for study and treatment. Part 1. Clinical Pathophysiology, 2022; 28(3):3-21

6. Ryabkova VA, Churilov LP, Shoenfeld Y. COVID-19 and ABO blood groups. // Isr Med Assoc J. 2021;23(3):140-142.

7. Ryabkova VA, Churilov LP, Shoenfeld Y. Hyperstimulation of the immune system as a cause of autoimmune diseases. Annals of the Russian Academy of Medical Sciences. 2020;75(3):204-213.

8. Ryabkova VA, Bregovskaya AA, Soprun LA, Gavrilova NY, Churilov LP. Autoimmune manifestations of the post-COVID-19 condition. // Immunopathol Persa. 2022:e31339. doi:10.34172/ ipp.2022.31339.

- 4 chapters in book:

1. Ryabkova V.A., Churilov L.P. Disease course and pathogenesis of post-COVID-19 condition / in book: Autoimmunity, COVID-19, Post-COVID-19 Syndrome and COVID-19 Vaccination (Eds: Y. Shoenfeld & A. Dotan). – Amsterdam: Academic Press, 2023. – P. 759-771. – doi 10.1016/B978-0-443-18566-3.00006-2.

2. Soprun L., Gavrilova N., Ryabkova V.A., Lukashenko M., Kamaeva E. The post-COVID syndrome / in book: Autoimmunity, COVID-19, Post-COVID-19 Syndrome and COVID-19 Vaccination (Eds: Y. Shoenfeld & A. Dotan). – Amsterdam: Academic Press, 2023. – P. 747-758

3. Gavrilova N., Malkova A., Soprun L., Ryabkova V.A., Kamaeva E. Long-term assessment of autoantibodies in post-COVID syndrome. / in book: Autoimmunity, COVID-19, Post-COVID-19 Syndrome and COVID-19 Vaccination (Eds: Y. Shoenfeld & A. Dotan). – Amsterdam: Academic Press, 2023. – P. 772-779

4. Gavrilova N., Soprun L., Ryabkova V.A., Lukashenko M., Kamaeva E. The post-COVID syndrome / in book: Autoimmunity, COVID-19, Post-COVID-19 Syndrome and COVID-19 Vaccination (Eds: Y. Shoenfeld & A. Dotan). – Amsterdam: Academic Press, 2023. – P. 784-787

The main results of the study were reported and discussed at international and national scientific conferences with international participation. In particular:

- 11 papers were published in the proceedings of international and national scientific conferences:

1. Churilov, L. P., Ryabkova, V. A., Gavrilova, N. Yu., Poletaeva, A. A., The spectrum and intensity of natural autoimmunity in fibromyalgia and chronic fatigue syndrome – a potential key to therapy? // XVII International Scientific Congress "Rational Pharmacotherapy – Golden Autumn": Proceedings of the Congress / Edited by: Professor Hadjidis, A. K. St. Petersburg: Publishing House of St. Petersburg State Economic University, 2022. – 195-197 p.

2. Ryabkova, V. A., Gavrilova, N. Y., Poletaeva, A. A., Churilov, L. P. (2022). Abnormalities of the spectrum and intensity of autoimmunity in post-COVID-19 syndrome. // Proceedings of the Russian Scientific and Practical Conference "COVID-19: results and prospects" (Sestroretsk, 14 October 2022)/ University Therapeutic Bulletin, 2022, 4:32-33.

3. Ryabkova V.A., Poletaeva A.A., Koshkina I.A., Yevsyutina Y.V., Marchenko V.N., Trofimov V.I., Churilov L.P. Abnormalities in the levels of natural autoantibodies in post-COVID-19 syndrome and ongoing symptomatic COVID-19// All-Russian therapeutic congress with international participation Botkin readings Collection of abstracts. / Edited by: Mazurov V.I., Trofimov E.A. St. Petersburg: 2023. – c. 233-234.

4. Ryabkova VA, Churilov LP "Similar changes of microcirculation in post-COVID19 syndrome and myalgic encephalomyelitis/chronic fatigue syndrome". // 9th International Congress of Pathophysiology and 5th Congress of Physiological Sciences of Serbia with International Participation: final Program and Abstract Book, 04–06 July 2023, Belgrade,

Serbia / eds I. Srejović, I. Milosavljević; International Society of Pathophysiology. – Kragujevac: Fakultet medicinskih nauka Univerziteta u Kraguievcu, 2023. – P. 41

5. Ryabkova VA, Churilov LP, Gavrilova NY, Fedotkina TV, Poletaeva AA, Rodionova SV, Shoenfeld Y. Study of Human Microecology by Mass Spectrometry of Microbial Markers in the Blood of Patients with Myalgic Encephalomyelitis/ Chronic Fatigue Syndrome and Post-COVID-19-Condition // Eur J Rheumatol 2024;11(suppl 2):S133

6. Ryabkova V.A., Rubinsky A.V., Marchenko V.N., Trofimov V.I., Churilov L.P. Dysautonomia as a mechanism of pathogenesis of post-COVID-19 syndrome and chronic fatigue syndrome: the possibility of objectification of asthenia and a potential target of therapy// All-Russian therapeutic congress with international participation: Botkin readings. Collection of abstracts. Edited by: Mazurov V.I., Trofimov E.A. – St. Petersburg: 2024 – P. 194-195.

7. Ryabkova VA, Rubinsky AV, Marchenko VN, Trofimov VI, Churilov LP Features of autonomous dysfunction in patients with post-COVID-19 syndrome // New concepts of mechanisms of inflammation, autoimmunity and infections: Proceedings of IX International Scientific and Practical Conference (Kazan, 26-27 October 2023). Edited by R.Sh. Khasanov – Kazan: RIO KGMA, 2023. – c. 52-57.

8. Gavrilova N.Yu., Ryabkova V.A., Fedotkina T.V., Korovin A.E., Churilov L.P. Pathogenesis of disorders of autonomic regulation of systemic circulation in patients with chronic fatigue syndrome, postural tachycardia and fibromyalgia // State and prospects of development of modern science in the direction of "Biotechnical systems and technologies". Collection of articles of the III All-Russian scientific and technical conference. Anapa: Military-Innovation Technopolis "Era", 2021. – P. 192-195.

9. Ryabkova VA, Gavrilova NY, Korovin AE, Churilov LP, Schoenfeld I. Clinical and pathophysiological characteristics of chronic fatigue syndrome as a neuroimmune disease// State and prospects of development of modern science in the direction of "Biotechnical systems and technologies". Collection of articles of the III All-Russian scientific and technical conference. Anapa: VIT "Era", 2021. – P. 72-78.

10. Churilov L.P., Fedotkina T.V., Nikolaev A.V., Normatov M.G., Novitskaya T.A., Ryabkova V.A., Starshinova A.A. Infection and Autoimmunity: Triggering of Somatic Diseases // Immunology-2024. Abstract book of Conference (Harbin, 27 July 2024). Harbin, HMU Publisher, 2024. – P. 14

11. Ryabkova VA, Churilov LP. Chronic fatigue syndrome and post-covid syndrome: stressor adaptation disorders and approaches to their correction. In Proceedings of the XIX International Scientific Congress “Rational Pharmacotherapy - ‘Golden Autumn’. Edited by Professor Hadjidis, A. K. Spb: SpbGEU, 2024. – P. 183-186.

- the obtained results were also presented at the All-Russian conference in the form of an oral report (without publishing abstracts):

1. Ryabkova V.A., Poletaeva A.A., Sobolevskaya P.A., Korovin A.E., Churilov L.P. Abnormalities of natural autoimmunity in post-COVID-19 syndrome. Report at the XIII All-Russian School on Clinical Immunology "Immunology for Doctors", 29 January – 4 February 2023, Pushkin Mountains, Pskov region

The obtained new knowledge about the pathogenesis of ME/CFS and PCS, as well as the results of the analysis of modern international consensus recommendations on the diagnosis and therapy of these conditions (published in 2021) were introduced into the educational process and used in teaching at the Medical Institute of St. Petersburg State University in the elective course "Autoimmunology" (speciality "General medicine", 5th year). In this course for the first time modern ideas about ME/CFS and PCS were included in the training programme for medical doctors.

The main scientific results

Publications:

1. “Myalgic Encephalomyelitis/Chronic Fatigue Syndrome and Post-COVID Syndrome: A Common Neuroimmune Ground?” [212], pages 1-17 – *Some characteristics of ME/CFS and PCS as neuroimmune diseases are described and compared; high prevalence of dysautonomia and microcirculatory dysfunction in these*

conditions is shown; lack of correlation of fatigue severity with depression/anxiety and the presence of correlation between fatigue severity and neuroimmune disturbances in ME/CFS and PCS are noted. Author's contribution is 100%.

2. "Autoantibody Correlation Signatures in Fibromyalgia and Myalgic Encephalomyelitis/Chronic Fatigue Syndrome: Association with Symptom Severity" [73], pages 1-15 - *We described the methodology for the assessment of the regulatory function of the immune system, according to the data on the spectrum of natural AAb (as it reflects the interaction of the immune system with cells of different organs and tissues); we showed the presence of some shifts of the natural AAb serum levels in patients with ME/CFS, in particular, shifts in the level of AAb to gamma-aminobutyric acid (GABA) receptors were the most pronounced in this group of patients.* Author's contribution is 100%.

3. "Similar Patterns of Dysautonomia in Myalgic Encephalomyelitis/Chronic Fatigue and Post-COVID-19 Syndromes" [276], pages 1-17 – *The use of paced breathing at 12 breaths per minute was justified for the more accurate characterization of the separate contribution of disorders of the sympathetic and parasympathetic parts of the autonomic nervous system in the studies of HRV; the presence of a similar pattern of autonomic nervous system dysfunction in ME/CFS and PCS, its relationship to the clinical manifestations of these conditions and the potential therapeutic role of paced breathing were proved.* Author's contribution 100%.

4. "Post-COVID syndrome and its immunopathological mechanisms. The role of autoimmunity" [249], pages 7-11 – *Based on the analysis of the registry of patients with PCS, which we have established, we reported that the most common phenotype of PCS is chronic-fatigue-like one; high prevalence of ME/CFS among PCS patients, according to the diagnostic criteria of ME/CFS, was shown; the potential role of autoimmune mechanisms of the PCS pathogenesis and the need for a specialized center for dynamic observation and rehabilitation of such patients were substantiated.* Author's contribution is 100%.

5. "COVID-19: adrenal response and molecular mimicry" [96], pages 618-619 – *The*

hypothesis of autoimmune nature of the HPA axis dysfunction in post-infectious fatigue syndromes was substantiated. Author's contribution 30%.

The main provisions for defence

1. The prevalence of ME/CFS, according to its diagnostic criteria, among patients with chronic fatigue associated with PCS is 45.7%, necessitating increased awareness among physicians about ME/CFS, modern approaches to its diagnosis and therapy.
2. Patients with ME/CFS not related to COVID-19 and with the chronic fatigue associated with PCS exhibit a similar pattern of dysautonomia, characterized by reduced HRV, increased BPV, and decreased baroreflex sensitivity, and the severity of these signs correlates with the fatigue level.
3. Microcirculation disorders are characteristic of both groups of patients, but in ME/CFS not related to COVID-19 there is impairment in endothelium-dependent vasodilation, while patients with chronic fatigue associated with PCS shows a decrease in the minimum perfusion value during occlusion, which may indicate microcirculatory stasis.
4. Decreased reactivity of the HPA axis is typical for the group of patients with ME/CFS not related to COVID-19, in which it is closely linked to sleep disturbances. At the same time HPA reactivity does not appear to be a significant factor in the pathogenesis of chronic fatigue associated with PCS.
5. Both groups of patients exhibit signs of polyclonal activation of the adaptive immune system, and the analysis of natural autoantibody (AAb) serum profiles, according to the concept of the regulatory function of the immune system, suggests new pathomechanisms of ME/CFS and PCS.
6. The analysis of the microbiome composition in patients with chronic fatigue associated with PCS and in patients with ME/CFS not related to COVID-19 suggests alterations in the small intestinal microbiota composition, increased mucosal

permeability, and immune system dysfunction at the mucosal level, more pronounced in ME/CFS.

7. Thus, the comparison of findings characterizing the regulatory functions of the nervous, endocrine, and immune systems as well as microcirculation disorders in chronic fatigue associated with PCS, in patients with ME/CFS not related to COVID-19, and in healthy individuals revealed significant similarities in the pathomechanisms of PCS and ME/CFS, as well as some features more typical for each of these syndromes.

CHAPTER 1. CURRENT UNDERSTANDING OF MYALGIC ENCEPHALOMYELITIS/CHRONIC FATIGUE SYNDROME AND POST-COVID SYNDROME (LITERATURE REVIEW)

1.1 Issues of terminology and epidemiology of myalgic encephalomyelitis/chronic fatigue syndrome and post-COVID-19 syndrome

Fatigue is one of the most common symptom in general medicine [2, 294]. In Russian-language literature the term "asthenia" is used to describe pathological fatigue following normal activity or a sharp decline in working capacity accompanied by a decrease in the energy reserve necessary for normal functioning and attention [45]. Comparing this terminology with English-language literature, it can be noted that the term "asthenia" is hardly used in English-language publications. In the relevant context, English-speaking authors usually use the word "fatigue", sometimes with the specification "pathological fatigue" to distinguish between pathological and physiological conditions [323].

Thus, foreign authors divide fatigue into two distinct groups. Fatigue is considered pathological if it persists for more than one month, has no obvious causes (such as excessive physical activity or a recent acute respiratory viral infection etc.), and does not resolve spontaneously after the cessation of the causal factor [323]. In domestic literature, there is some uncertainty in terminology. In particular, classification of fatigue includes "physiological fatigue" which is distinguished from asthenic disorders and characterized by the following features: 1) short duration (no more than a few days); 2) association with increased loads and/or lack of sleep; 3) mild severity; 4) disappearance after rest [5]. However, at the same time domestic classification of fatigue includes a category of "reactive asthenia" which, on the one hand, by definition, cannot be considered a physiological reaction (since the definition of asthenia, as noted above, emphasizes that this term refers to pathological fatigue), but on the other hand, it is transient and characterized by an association with a certain trigger factor [47], which brings it closer to physiological fatigue. The cause of reactive asthenia, according to the authors who proposed this term, could be any activity associated with constant tension of adaptive

mechanisms, e.g. occupational factors (disruption of sleep and wakefulness etc), psychological stressful situations, recovery period after surgeries/injuries/acute infectious diseases, etc. [47]. Other authors add to this list some physiological states (pregnancy, lactation), toxic exposures, and deficit metabolic disorders (such as seasonal hypovitaminosis) [39]. The consequence of such terminological uncertainty is the appearance in scientific publications some statements contradicting the provided definitions, such as "a characteristic feature of the asthenic syndrome is that it passes after rest" [2].

Vasenina et al. [2] the role of physiological weakness/exhaustion as a protective mechanism, which indicates the depletion of energy resources and the need for their replenishment. Taking into account these authors' assertion that such protective weakness/exhaustion is a normal element of the recovery process after stress or illness, it can be assumed that reactive asthenia is regarded by them as a physiological phenomenon. To distinguish pathological fatigue (asthenia), they provide a time criterion of 1 month (which is consistent with the view of English-language specialists, see above) and justify it by stating that such a duration of weakness/fatigue either reflects the chronic progressive course of the pathological process underlying fatigue in this situation or indicates the inadequacy of compensatory mechanisms.

To avoid inaccuracies associated with different interpretations of the term “asthenia”, in this work we use the word “fatigue” in the sense corresponding to the majority of English-language publications (i.e. “pathological fatigue”).

According to the duration, fatigue is classified as acute (less than 1 month), subacute (1-6 months), and chronic (more than 6 months) [278]. And while acute fatigue in most cases is a physiological reaction, the likelihood of a benign nature and spontaneous resolution for chronic fatigue is significantly reduced. As a result, most studies focus on the chronic fatigue and hence it will the subject of the following review.

The prevalence of chronic fatigue in general population, according to a meta-analysis conducted in 2023, is 10.1% (95% CI, 8.2–12.5) in adults and 1,5% (95% CI, 0.5–4.7) in children and adolescence [294].

Given the polyetiological nature of chronic fatigue and its occurrence at various stages of almost any disease, it is necessary to distinguish chronic fatigue as a symptom and ME/CFS as an independent disease, a specific nosological form, as it is currently considered by the experts [2, 45, 222].

According to the aforementioned meta-analysis, the proportion of ME/CFS among all patients presenting with chronic fatigue is about 16% [294]. Another meta-analysis conducted by the same authors (Lim et al.) [288] estimated the prevalence of ME/CFS in the general population to be 0.89%. Notably, this prevalence was similar across different countries and parts of the world, indicating that lifestyle does not play a significant role in the development of this disease. Moreover, contrary to the previously popular idea of ME/CFS as a "yuppie flu," modern epidemiological studies indicate a higher prevalence of ME/CFS among individuals with lower family income as well as among rural dwellers compared to urban populations [217]. The meta-analysis also showed that women are 1.5 to 2 times more likely to suffer from ME/CFS than men [288]. In a Norwegian study involving more than 5000 patients with ME/CFS, two age peaks of incidence were identified: 10-19 and 30-39 years, and the second peak was predominantly observed in women. Other studies identified a single peak corresponding to ages 40-49 [312] or 25-44 years old [219].

It should be noted that the meta-analysis by Lim et al. [288] was conducted before the COVID-19 pandemic. After the first reports of long-lasting or manifesting following acute COVID-19 symptoms, several English-language publications which highlighted the similarity of these symptoms with the clinical picture of ME/CFS almost immediately appeared in the scientific literature [179, 196, 238, 325, 329]. The authors of these publications also pointed out that similar manifestations developed in a historical context among a large number of patients after other viral epidemics [282].

Fatigue is one of the most frequent symptoms reported by patients recovering from COVID-19. According to the recent meta-analyses, its prevalence is 32%, 36%, 47%, 41%, and 28% at 3-6, 6-9, 9-12, 12, and 24 months after the acute SARS-CoV-2 infection, respectively [242, 258]. It is easy to notice that even though the prevalence decreases after

the first year, in two years after the illness it remains almost three times higher among individuals who have had COVID-19 than in the general population [294].

To describe the wide range of manifestations following COVID-19, the WHO proposed the term "post-COVID-19 condition" on October 6th, 2021, and developed a clinical case definition by Delphi methodology, which implies the identification of consensus views based on a mixed iterative survey of experts, patients, and other stakeholders from various geographic regions, including Russia [50]. According to the clinical case definition, "post-COVID-19 condition occurs in individuals with a history of probable or confirmed SARS-CoV-2 infection, usually 3 months from the onset of COVID-19 with symptoms that last for at least 2 months and cannot be explained by an alternative diagnosis. Common symptoms include fatigue, shortness of breath, cognitive dysfunction but also others and generally have an impact on everyday functioning. Symptoms may be new onset following initial recovery from an acute COVID-19 episode or persist from the initial illness. Symptoms may also fluctuate or relapse over time" [50]. In ICD-10 this condition is coded as U09.9. Alongside the WHO definition, definitions proposed by other organizations are also widely used to describe post-COVID syndrome (see Chapter 1.2). In the English-language medical and healthcare literature, several synonymous terms are used in addition to the term used in the WHO document ("post-COVID-19 condition"): chronic COVID-19 syndrome, late sequelae of COVID-19, long COVID, long haul COVID, long-term COVID-19, post COVID syndrome, post-acute COVID-19, and post-acute sequelae of SARS-CoV-2 infection (PASC) [50]. In Russian literature, the most common term is PCS (post-COVID-19 syndrome). There is also inconsistency in understanding this term. Some researchers, in line with the WHO's 2021 definition of PCS, understand it as a combination of symptoms (the most common are fatigue, shortness of breath, and cognitive dysfunction) that cannot be explained by an alternative diagnosis [110]. Others include in PCS also organ-specific consequences, i.e. the development of certain pathological processes in individual organs accompanied by specific pathomorphological changes in these organs and related to the COVID-19 (e.g., development of pulmonary fibrosis or deep vein thrombosis and thromboembolic

complications, or myo/pericarditis) [319]. We believe that the term "post-acute sequelae of COVID-19 (PASC)" is more appropriate for this group.

It gradually became evident that PCS is a heterogeneous condition and includes different clinical scenarios, one of which is the development of ME/CFS [98]. Studies, which assess the prevalence of ME/CFS among patients with PCS, began to emerge. Various researchers obtained fairly similar results, according to which 43-58% of patients with PCS meet the diagnostic criteria for ME/CFS [54, 173, 193, 218, 220].

At the same time, the first studies on the prevalence of PCS after COVID-19 infection in the Russian population were conducted only in 2022 and remain limited in number [7, 11]. The prevalence of ME/CFS based on the diagnostic criteria for this condition among patients with PCS in the Russian population had not been previously evaluated.

1.2 Clinical presentation and diagnostic criteria for myalgic encephalomyelitis/chronic fatigue syndrome and post-COVID-19 syndrome

According to the definition, ME/CFS is characterized by the sudden or gradual onset of persistent, severe fatigue, which, along with other symptoms, worsens (often delayed) after previously well-tolerated activities (which is known as post-exertional malaise (PEM)), unrefreshing sleep, cognitive impairments, and autonomic dysfunction, which are not explained by any other disease, persist for at least 6 months, do not diminish with rest, and significantly affect the quality of life [181]. Consensus recommendations for the diagnosis and treatment of ME/CFS provide a typical natural history of the disease [128, 216]. Patients usually report an infectious episode prior to their initial symptoms after which they could not recover and continue to feel unwell for several months. Some patients may point to a non-infectious trigger (e.g., surgery, pregnancy, vaccination, etc.), or they may not associate the onset of the illness with any specific trigger. The natural course of ME/CFS can vary. In some patients, all symptoms appear within a few hours or days after the triggering event, while in others they develop gradually over weeks or months. The severity of the clinical manifestations often fluctuates throughout life, and

sometimes patients describe periods of remission; however, in most cases, symptom relapse follows the remission.

In addition to severe fatigue, patients may experience flu-like symptoms (fever, chills, arthralgia, myalgia, general malaise, sweating, headache), sleep disturbances, memory/attention problems (often described as "brain fog"), and worsening of symptoms in the upright position. The latter symptom may be a manifestation of the common comorbid condition of ME/CFS known as orthostatic intolerance, which is defined as a reduced tolerance to orthostatic stress (i.e. transitioning to maintain for a long time the upright posture). This often manifests not as syncope but as a general worsening of well-being and cognitive function, dizziness, palpitations, general weakness, and blurred vision [212]. Patients may also complain of increased sensitivity to sensory stimuli such as light, noise, touch, or changes in ambient temperature as well as pain in different parts of the body (muscle pain, joint pain, headaches). A pattern of widespread musculoskeletal pain meeting the diagnostic criteria for fibromyalgia is often observed. These symptoms typically have a significant impact on daily life, including education, work, household chores, and social activities; in severe cases even self-care is challenging [216]. Patients with ME/CFS may experience other symptoms but may not associate them with their illness or may have difficulty describing them. Notably, they often do not mention the key symptom of the disease – PEM [83]. PEM refers to the exacerbation of symptoms that can result from minimal or previously well-tolerated cognitive, physical, emotional, or social activities [101]. Symptom exacerbation typically occurs 12–48 hours after the activity and lasts for several days or even weeks. This symptom can be objectively measured using cardiopulmonary exercise testing with a two-day protocol: reduced workload at which the anaerobic threshold is reached on the second day of testing indicates PEM in ME/CFS patients [305]. Expert consensus documents published in 2021 list among other common symptoms infection-like immune manifestations (which include frequent and prolonged upper respiratory infections, chronic pharyngitis, and sinusitis, as well as tender cervical lymph nodes in addition to the aforementioned flu-like symptoms), new allergic reactions, food and alcohol intolerance, gastrointestinal dysfunction manifesting as

irritable bowel syndrome, weight loss or gain, dry eyes/mouth, or (less commonly) hypersalivation, emotional instability, and increased anxiety [128].

The diagnosis of ME/CFS, due to the lack of universally recognized biomarkers to date, is based on the assessment of clinical data by a competent clinician [128]. The standardization of the diagnostic approach is ensured by diagnostic criteria. Since 1988, when the term "chronic fatigue syndrome" was adopted for the disease, more than 20 sets of diagnostic criteria and clinical case definitions have been proposed. Some of them have shown low specificity (e.g., the Oxford criteria of 1991, the Australian criteria of 1990) [136, 288], and therefore are currently not recommended for clinical and research practice. On the contrary, the first historically proposed criteria (developed by the CDC and known as the Holmes criteria, 1988) may have too low sensitivity, identifying only a certain subgroup of patients suffer from severe ME/CFS with more pronounced infection-like symptoms [116]. In addition to a new onset of severe fatigue or easy fatigability in a person who has no previous history of similar symptoms that does not resolve with bedrest, and that is severe enough to reduce or impair average daily activity below 50% of the patient's premorbid activity level for a period of at least 6 months, these criteria require the exclusion of several other diseases as potential causes of this condition, and the presence of 8 out of 11 symptoms that appeared simultaneously with the onset of fatigue or thereafter; or 6 out of 11 symptoms + 2 out of 3 physical criteria documented by a physician [91]. The list of symptoms is the following:

1. Low-grade fever recorded by the patient (oral temperature 37.5-38.6°C) or chills;
2. Sore throat;
3. Painful lymph nodes (anterior/posterior cervical or axillary ones);
4. Unexplained generalized muscle weakness;
5. Muscle discomfort or myalgia;
6. Prolonged (24 hours or greater) generalized fatigue after levels of exercise that would have been easily tolerated in the patient's premorbid state;

7. Generalized headaches (of a type, severity, or pattern that is different from headaches the patient may have had in the premorbid state);
8. Migratory arthralgia without joint swelling or redness
9. Neuropsychologic complaints (one or more of the following: photophobia, transient visual scotomata, forgetfulness, excessive irritability, confusion, difficulty thinking, inability to concentrate, depression);
10. Sleep disturbance (hypersomnia or insomnia).
11. Description of the main symptom complex as initially developing over a few hours to a few days (this is not a true symptom, but may be considered as equivalent to the above symptoms in meeting the requirements of the case definition).

Three physical criteria include:

1. Low-grade fever (oral 37.6-38.6°C or rectal 37.8-38.8°C);
2. Non-exudative pharyngitis;
3. Palpable or tender lymph nodes (anterior/posterior cervical or axillary ones).

The low sensitivity of these criteria due to the necessity of confirming a large number of symptoms and their tendency to identify only a portion of ME/CFS cases (those with a clear connection to an infectious episode near the onset of symptoms) were noted by clinicians and researchers in the first years after the Holmes criteria were developed [93]. Concerns were also raised that the requirement of 8 or more symptoms might lead to the inclusion of individuals with psychiatric disorders, characterized by multiple unexplained somatic symptoms such as somatoform disorder [177].

Thus, out of more than 20 sets of diagnostic criteria proposed over thirty years, few have come into clinical and research practice, and only three sets of diagnostic criteria have been considered in expert consensus documents in 2020-2021 [128, 289] , which are:

- Fukuda Criteria (Fukuda or CDC 1994) [291],
- Canadian Consensus Criteria (CCC 2003) [215],
- Institute of Medicine/National Academy of Medicine (IOM/NAM 2015) [101].

The Fukuda criteria (CDC 1994) were initially developed as classification criteria for research purposes but firmly established in clinical practice as diagnostic criteria, and

represent a modification of the Holmes criteria. Like the Holmes criteria, they require the presence of chronic fatigue (that is of new onset, not alleviated by rest, and significantly reduces previous levels of occupational, educational, social, or personal activities), and ruling out its other potential causes.

However, physical examination findings were excluded from these criteria due to weak evidence that the three physical signs listed in the Holmes criteria are characteristic features of ME/CFS. Apart from that, the number of symptoms required to confirm ME/CFS was reduced to any 4 out of 8. These 8 symptoms include:

1. Significant impairment in short-term memory or concentration, substantial enough to markedly affect occupational, educational, social, or personal activities;
2. Sore throat;
3. Tender cervical or axillary lymph nodes;
4. Muscle pain;
5. Multijoint pain without joint swelling or redness;
6. Headaches of a new type, pattern, or severity;
7. Unrefreshing sleep;
8. Post-exertional malaise lasting more than 24 hours.

The 2021 expert consensus recommendations propose a modification to these criteria, namely the inclusion of PEM as a mandatory criterion rather than one of the optional additional symptoms [128]. This is because PEM is now considered a key characteristic of ME/CFS, distinguishing it from deconditioning and other diseases accompanied by severe fatigue [330].

The Canadian Consensus Criteria of 2003 (CCC 2003) are among the first diagnostic criteria for ME/CFS, rather than classification criteria, which means they were originally intended for diagnostic purposes in clinical practice. Similar to CDC 1994 they require the 6-month minimum duration of symptoms and the exclusion of a number of other diseases prior to a ME/CFS diagnosis. However, this set of criteria includes more specific mandatory symptoms for diagnosing ME/CFS, rather than the presence of a certain number of any symptoms from a suggested list (as the Fukuda or Holmes criteria). This is

likely due to accumulating evidence about different significance of symptoms for the establishing the diagnosis. According to the CCC, the mandatory symptoms, besides pathological fatigue, include PEM, sleep disturbances, pain (myalgia, arthralgia, or headaches of a new type/pattern/severity), and two or more neurological/cognitive symptoms. The diagnosis also requires the presence of at least one symptom in two of the following three domains: autonomic, neuroendocrine, and immune dysfunction.

The Institute of Medicine (IOM/NAM 2015) criteria were also developed as a diagnostic tool. The reduction in the number of mandatory symptoms facilitates their use in clinical practice. The required symptoms include pathological fatigue lasting more than six months, PEM, and unrefreshing sleep. An additional criterion requires the presence of at least one of the following manifestations: cognitive impairment or orthostatic intolerance. Unlike the CDC 1994 and CCC 2003 criteria, the IOM/NAM 2015 criteria do not provide a list of diseases that exclude the diagnosis of ME/CFS. However, it is noted that ME/CFS should not be diagnosed if treatment of a comorbid condition eliminates all symptoms. A detailed discussion and harmonization of views on diseases that exclude ME/CFS is provided in the article [127].

It should be noted that only the Fukuda/CDC 1994 criteria had been translated into Russian, and therefore they remained the only known option for most Russian-speaking doctors. At the same time, clinical guidelines and expert consensus documents on ME/CFS published over the past eight years have highlighted several shortcomings of this set of criteria and instead recommended CCC 2003 and IOM/NAM 2015 criteria for clinical practice [128, 216, 289]. The latter are considered the simplest and therefore the most convenient for use by primary care physicians [128].

Given these recommendations, we deemed it necessary to undertake the first translation of IOM/NAM 2015 and CCC 2003 criteria into Russian. The original versions of these criteria are presented in Tables 1 and 2 respectively. IOM/NAM 2015 criteria are supplemented with the symptom definitions, according to the NICE clinical guideline on ME/CFS (2021) [224]. The translated criteria are provided in the Russian version of the dissertation. The translation was undertaken in collaboration with the ME/CFS patient

community, ensuring that the final translation of each symptom was consistent with the patients' experiences.

Table 1 – Institute of Medicine/National Academy of Medicine 2015 diagnostic criteria for myalgic encephalomyelitis/chronic fatigue syndrome

Diagnosis requires that the patient have the following three symptoms:
1. A substantial reduction or impairment in the ability to engage in pre-illness levels of occupational, educational, social, or personal activities, that persists for more than 6 months and is accompanied by fatigue, which is often profound, is of new or definite onset (not lifelong), is not the result of ongoing excessive exertion, and is not substantially alleviated by rest.
2. Post-exertional malaise (the exacerbation of symptoms that can occur as a result of minimal or previously well-tolerated cognitive, physical, emotional, or social activity. The intensification of symptoms usually manifests 12-48 hours after the activity and persists for several days or even weeks)*.
3. Unrefreshing sleep (even after a full night's sleep, the patient does not feel rested; they report waking up with a feeling of fatigue, 'as if they hadn't slept at all,' regardless of the duration of sleep)*
At least one of the two following manifestations is also required:
1. **Cognitive impairment** (sometimes referred to as "brain fog"; may include word-finding problems, speech difficulties, slowed reaction times, short-term memory issues, as well as difficulties with concentration or multitasking)*
2. **Orthostatic intolerance** (a clinical condition in which symptoms such as dizziness, pre-syncope or syncope, impaired concentration, headache, blurry vision, palpitations, tremor, and chest pain occur or worsen upon standing and decrease (though not necessarily disappear) when sitting or lying down. Orthostatic intolerance can manifest as postural orthostatic tachycardia syndrome (POTS), characterized by an increase in heart rate of ≥30 beats per minute upon standing, or as orthostatic hypotension, characterized by a drop in blood pressure (systolic ≥20 mmHg or diastolic ≥10 mmHg) upon standing. Individuals with severe orthostatic intolerance may be unable to remain even when sitting upright)*.
* The frequency and severity of symptoms must be assessed. The diagnosis of ME/CFS should be questioned if the specified symptoms do not occur for the majority of the time with at least a moderate degree of severity.

Table 2 – Canadian Consensus Criteria 2003 for the diagnosis of myalgic encephalomyelitis/chronic fatigue syndrome (clinical working case definition)

A patient with ME/CFS will meet the criteria for fatigue, post-exertional malaise and/or fatigue, sleep dysfunction, and pain; have two or more neurological/cognitive manifestations and one or more symptoms from two of the categories of autonomic, neuroendocrine, and immune manifestations; and adhere to item 7.
1. Fatigue: The patient must have a significant degree of new onset, unexplained, persistent, or recurrent physical and mental fatigue that substantially reduces activity level.
2. Post-exertional Malaise and/or Fatigue: There is an inappropriate loss of physical and mental stamina, rapid muscular and cognitive fatigability, post-exertional malaise and/or fatigue and/or pain and a tendency for other associated symptoms within the patient's cluster of symptoms to worsen. There is a pathologically slow recovery period – usually 24 hours or longer.
3. Sleep Dysfunction[1]: There is unrefreshing sleep or sleep quantity or rhythm disturbances such as reversed or chaotic diurnal sleep rhythms.
4. Pain[1]: There is a significant degree of myalgia. Pain can be experienced in the muscles, and/or joints, and is often widespread and migratory in nature. Often there are significant headaches of new type, pattern or severity.
5. Neurological/Cognitive Manifestations: Two or more of the following difficulties should be present: confusion, impairment of concentration and short-term memory consolidation, disorientation, difficulty with information processing, categorizing and word retrieval, and perceptual and sensory disturbances – e.g. spatial instability and disorientation and inability to focus vision. Ataxia, muscle weakness and fasciculations are common. There may be overload[2] phenomena: cognitive, sensory – e.g. photophobia and hypersensitivity to noise – and/or emotional overload, which may lead to "crash"[3] periods and/or anxiety.
6. At Least One Symptom from Two of the Following Categories: **a. Autonomic Manifestations:** orthostatic intolerance – neurally mediated hypotension, postural orthostatic tachycardia syndrome (POTS), delayed postural hypotension; light-headedness; extreme pallor; nausea and irritable bowel syndrome; urinary frequency and bladder dysfunction; palpitations with or without cardiac arrhythmias; exertional dyspnea. **b. Neuroendocrine Manifestations:** loss of thermostatic stability – subnormal body temperature and marked diurnal fluctuation, sweating episodes, recurrent feelings of feverishness and cold extremities; intolerance of extremes of heat and cold; marked weight change – anorexia or abnormal appetite; loss of adaptability and worsening of symptoms with stress. **c. Immune Manifestations:** tender lymph nodes, recurrent sore throat, recurrent flu-like symptoms, general malaise, new sensitivities to food, medications and/or chemicals.
7. The illness persists for at least six months: It usually has a distinct onset[4], although it may be gradual. Preliminary diagnosis may be possible earlier. Three months is appropriate for children.
To be included, the symptoms must have begun or have been significantly altered after the onset of this illness. It is unlikely that a patient will suffer from all symptoms in criteria 5 & 6. The disturbances tend to form symptom clusters that may fluctuate and change over time. Children often have numerous prominent symptoms but their order of severity tends to vary from day to day.

Continuation of table 2

Exclusions: Exclude active disease processes that explain most of the major symptoms of fatigue, sleep disturbance, pain, and cognitive dysfunction. It is essential to exclude certain diseases, which would be tragic to miss: Addison's disease, Cushing's Syndrome, hypothyroidism, hyperthyroidism, iron deficiency, other treatable forms of anemia, iron overload syndrome, diabetes mellitus, and cancer. It is also essential to exclude treatable sleep disorders such as upper airway resistance syndrome and obstructive or central sleep apnea; rheumatological disorders such as rheumatoid arthritis, lupus, polymyositis and polymyalgia rheumatica; immune disorders such as AIDS; neurological disorders such as multiple sclerosis (MS), Parkinsonism, myasthenia gravis and B12 deficiency; infectious diseases such as tuberculosis, chronic hepatitis, Lyme disease, etc.; primary psychiatric disorders and substance abuse. Exclusion of other diagnoses, which cannot be reasonably excluded by the patient's history and physical examination, is achieved by laboratory testing and imaging. If a potentially confounding medical condition is under control, then the diagnosis of ME/CFS can be entertained if patients meet the criteria otherwise.
Co-morbid Entities: fibromyalgia syndrome, myofascial pain syndrome, temporomandibular joint syndrome, irritable bowel syndrome, interstitial cystitis, irritable bladder syndrome, Raynaud's phenomenon, prolapsed mitral valve, depression, migraine, allergies, multiple chemical sensitivities, Hashimoto's thyroiditis, Sicca Syndrome, etc. Such co-morbid entities may occur in the setting of ME/CFS. Others such as irritable bowel syndrome may precede the development of ME/CFS by many years, but then become associated with it. The same holds true for migraines and depression. Their association is thus looser than between the symptoms within the syndrome. ME/CFS and fibromyalgia syndrome often closely connect and should be considered to be "overlap syndromes".
Idiopathic Chronic Fatigue: If the patient has unexplained prolonged fatigue (6 months or more) but has insufficient symptoms to meet the criteria for ME/CFS, classify it as idiopathic chronic fatigue.
1) There is a small number of patients who have no pain or sleep dysfunction, but no other diagnosis fits except ME/CFS. A diagnosis of ME/CFS can be entertained when this group has an infectious illness type onset. 2) "Overload" refers to hypersensitivities to various types of stimuli that has changed from pre-illness status. 3) "Crash" refers to a temporary period of immobilizing physical and/or mental fatigue. 4) Some patients have been unhealthy for other reasons prior to the onset of ME/ CFS and lack detectable triggers at onset or have more gradual or insidious onset.

The controversy in contemporary understanding of the term "post-COVID-19 syndrome" has been previously noted. Describing the clinical picture of PCS, we do not include symptoms resulting from specific sequelae of COVID-19 (post-acute sequelae of COVID-19 (PASC)), which are often organ-specific, associated with the damaging effects of the virus during the acute phase of the infection and accompanied by pathomorphological changes in the corresponding organs, such as dyspnea related to pulmonary fibrosis or arrhythmias associated with myocarditis.

In addition to the clinical case definition of PCS proposed by the WHO, there are other definitions provided by health authorities in different countries. The main differences between these definitions concern the minimum length of time between symptom onset and persistence. Earlier definitions indicated symptom duration of 4 weeks following the initial signs of infection. However, a significant portion of these criteria has been revised, and as of 2024, national definitions of PCS in most countries specify a minimum term of 12 weeks for the symptom persistence [125].

Even before the WHO developed the clinical case definition for PCS, the UK National Institute for Health and Care Excellence (NICE) [223] proposed the following classification of COVID-19-related conditions in December 2020:

- **Acute COVID-19** (symptoms persisting for up to four weeks from the onset of the infection);
- **Ongoing symptomatic COVID-19** (symptoms continuing from 4 to 12 weeks);
- **Post-COVID-19 Syndrome** (symptoms developing during or after COVID-19 infection, persisting for more than 12 weeks, not explained by an alternative diagnosis, potentially fluctuating over time, and affecting multiple body systems).

Despite over 100 symptoms being described in the context of PCS [142], it seems reasonable to highlight the most significant ones based on the results of meta-analyses comparing the prevalence of each symptom at different time points after the illness.

A meta-analysis by Alkodaymi et al. [258] conducted in 2022 showed that at 3-6 months after a COVID-19 episode, the most common symptoms were fatigue, shortness of breath, sleep disturbances, depression, and reduced concentration. At 6-9 months, the prevalence of these symptoms either increased or remained unchanged, with the most significant increase in the prevalence of arthralgia, exercise intolerance and anxiety. According to another meta-analysis analysing studies conducted 12 months after COVID-19, the most common symptoms were shortness of breath with physical exertion, fatigue, impaired concentration, and arthromyalgia [247]. Finally, a meta-analysis summarizing results from twelve studies with assessments conducted 24 months after COVID-19

showed that the prevalence of key symptoms compared to earlier time points was lower, but fatigue, cognitive impairments, and sleep disturbances remained the most common symptoms, affecting more than 20% of individuals who had recovered from COVID-19 [242]. The results of these meta-analyses are summarized in Table 3.

Table 3 – Prevalence of major symptoms among COVID-19 patients at different time points after acute illness, according to meta-analyses [242, 247, 258]

Symptoms	3-6 months	6-9 months	12 months	24 months
Fatigue	32%	36%	31%	28%
Dyspnea	25%	25%	16%	9,4%
Shortness of breath with physical exertion/exercise intolerance	19%	45%	34%	–
Cognitive impairment	14%	15%	–	27,6%
- memory loss	–	–	18%	–
- concentration problems	22%	22%	32%	–
Headache	12%	14%	8%	8,9%
Arthralgia	14%	23%	9%	5,2%
Myalgia	12%	19%	8%	8,1%
Muscle weakness	–	–	25%	–
Chest pain	11%	12%	7%	4,25%
Palpitations	14%	14%	7%	4,15%
Anosmia (hyposmia)	9%	15%	6% (14%)	5,25%
Ageusia (dysgeusia)	8%	13%	9% (5%)	4,85%
Cough	15%	12%	7%	4%
Diarrhea	10%	5%	3%	2,65%
Nausea	8%	4%	3%	1,35%

Continuation of table 3

Symptoms	3-6 months	6-9 months	12 months	24 months
Depression	21%	23%	17%	18%
Anxiety	14%	23%	16%	13,4%
Sleep disturbances	24%	29%	18%	20,9%
Hair loss	9%	10%	12%	7,35%

Interestingly, that while the prevalence of all organ-specific symptoms (respiratory, cardiovascular, and gastrointestinal ones) decreases over time (generally in 12 months after an acute SARS-CoV-2 infection), a number of general and neurological symptoms (fatigue, exercise intolerance, cognitive and affective impairment, myalgia, sleep disturbances, anosmia, ageusia) become more frequent between 6 and 12 months, with subsequent reduction, though less pronounced than organ-specific symptoms. This observation may suggest different pathomechanisms underlying the emergence and persistence of different symptoms.

The WHO document “A clinical case definition of post COVID-19 condition by a Delphi consensus" dated October 6, 2021, includes a list of potential post-COVID-19 symptoms [50]. However, only three of them were included in the definition. This is because only for these three symptoms (fatigue, dyspnea, cognitive dysfunction) a consensus was achieved through the Delphi method – i. e., more than 70% of 460 researchers, patients, experts, and WHO staff from different countries including Russia rated 7 to 9 out of 9 points on a scale reflecting their confidence in the necessity to include each of these symptoms in the clinical case definition of PCS.

The Table 3 above also clearly demonstrates that fatigue/exercise intolerance is the leading symptom of PCS.

In this context, the results of the meta-analysis comparing the clinical picture of ME/CFS and PCS are of particular interest [329]. Out of 29 symptoms associated with ME/CFS and reviewed in this meta-analysis, all but five (which were lymph node tenderness; increased sensitivity to food, drugs, or chemicals; motor neurological

disturbances such as fasciculations and coordination issues; tinnitus; diplopia) were recorded in at least one study analysing the clinical manifestations of PCS. Key symptoms listed in almost all sets of diagnostic criteria for ME/CFS (such as fatigue with reduced daily activity, post-exertional malaise, chronic pain syndromes, cognitive disturbances, etc.) were documented in the majority of studies on PCS. The main difference between ME/CFS and PCS was that a follow-up period in almost all studies of PCS included in this meta-analysis (2021) was less than 6 months, whereas ME/CFS diagnostic criteria require the presence of chronic fatigue lasting at least 6 months. Additionally, several symptoms described in PCS are not characteristic of ME/CFS, namely: olfactory and taste disorders, hair loss, various skin rashes, dryness of the mouth and eyes, and eye redness [329].

1.3 Etiology of myalgic encephalomyelitis/chronic fatigue syndrome and post-COVID-19 syndrome: the role of trigger factors and body reactivity

The etiology of ME/CFS is unknown. Most researchers incline to the view of ME/CFS as a multifactorial disease. This point of view suggests that exposure to various stressors in predisposed individuals (including genetic predispositions) can trigger the development of symptoms [153, 235], which accounts for the relative polyetiology of ME/CFS. Stressors can include an episode of acute infection, psychological trauma, psycho-emotional overloads, significant life events, travel, pregnancy, surgical interventions, and vaccination [41, 196, 235]. Among these, acute infection is the most common trigger – prior to the COVID-19 pandemic, 63-74% of patients with ME/CFS indicated an acute infection as a factor that induced the onset of their symptoms [175, 235]. Patients most frequently reported a non-specific respiratory viral infection or a fever episode without respiratory or gastrointestinal symptoms; however, 35% of cases were associated with specific infectious agents (e.g., herpesviruses, parvovirus B19). Before COVID-19, infectious mononucleosis was the most common infectious disease associated with an increased risk of developing ME/CFS [168].

The association of the disease onset with the common symptoms of infection led to the hypothesis that ME/CFS is a manifestation of some chronic infectious disease, i.e. is associated with the persistence of pathogenic microorganisms in the human body. However, for example, it is known that viruses of the Herpesviridae family, and specifically human herpesviruses 4, 5, 6, and 7, which are most frequently discussed in the context of the etiology and pathogenesis of ME/CFS, are characterized by the ability to persist in the infected cells, which is widespread among healthy individuals and does not cause any symptoms. At the same time, these viruses are statistically more frequently detected in ME/CFS patients in the active phase rather than in the latent phase, as determined by the presence of viral DNA in the plasma of these patients [108, 301].

In the 1990s, enteroviruses were considered potential causative microorganisms for ME/CFS, as their RNA were detected in various samples (blood, stool, gastric mucosa biopsies, and muscle) from patients with ME/CFS significantly more often than in the control group. However, these results were not confirmed in subsequent studies [95].

Regarding PCS, it is noteworthy that SARS-CoV-2 RNA can be detected in the blood, stool, urine, and tissues of patients up to 7 months after acute COVID-19 [200]. Research is in progress to explore the association between the detection of viral RNA or other antigens after an acute infection with SARS-CoV-2 and the development of PCS, and preliminary results suggest this association [243, 300].

As with any multifactorial disease, an important role in the etiology of ME/CFS is attributed to the predisposition (possibly including genetic factors), which is related to the concept of reactivity of the human body – the background against which a stressor acts. Genealogical studies in ME/CFS remain controversial [222]. The data on the heritability of ME/CFS, the results from twin studies, and analyses of single nucleotide polymorphisms significantly vary depending on the cohort of patients. Although a considerable number of single nucleotide polymorphisms associated with ME/CFS have been identified by different researchers, reproducibility analysis did not confirm any associations [137]. The same applies to studies on the association of ME/CFS with HLA system genes. Lande et al. showed that HLA-C07:04 or HLA-DQB103:03 types are

present in 10% of patients with ME/CFS and are associated with the diagnosis [154]. Another study found an increased frequency of the HLA-DQA1*01 allele and a decreased frequency of the HLA-DRB1*11 allele in ME/CFS [69]. Carlo-Stella et al. found an increased frequency of the HLA-DRB1*1104 allele and a decreased frequency of the HLA-DRB1*1301 allele [210]. Underhill et al. did not find any association between ME/CFS and any HLA A, B, DRB, DQB, or DPB gene alleles [183].

There has been reported a link between ME/CFS (including ME/CFS associated with herpesvirus infection) and non-syndromic forms of connective tissue dysplasia, particularly the marfanoid and Ehlers-Danlos-like phenotypes, among both adults and adolescents [37, 78, 232, 280], although this has been disputed by some authors [172].

Several studies have evaluated non-genetic risk factors for the development of ME/CFS. According to the regression analysis performed by Lacedra et al., frequent colds in the medical history (OR = 8.26, $P \leq 0.001$) were the strongest factor associated with an increased risk of developing ME/CFS. Other significant risk factors in this study included loneliness (OR = 4.41, $P \leq 0.001$), lower income (OR = 3.71, $P \leq 0.001$), and family (but not personal) history of anxiety disorders (OR = 3.77, $P < 0.001$). According to Undehill, the presence of ME/CFS in a spouse is also a risk factor, and the author suggests that it may indicate the involvement of some infectious agent transmitted through intimate contact [316]. Finally, high level of psychological stress in daily life before the illness was also associated with the risk of developing ME/CFS according to Kato et al. [255].

Regarding the role of the human body reactivity in the development of ME/CFS, the most valuable results come from studies focusing on risk factors for developing ME/CFS after a specific infectious disease, because in this case individuals in the ME/CFS and control groups are exposed to the same etiological factor (the infectious agent). Hickie et al. [252] analysed a cohort of patients who developed ME/CFS following infectious mononucleosis, Q fever, and Ross River virus arthritis. The risk factor for developing ME/CFS was the severity of the infectious disease, but not gender, age, level of education, level of neuroticism, or the presence of pre-existing mental disorder (including anxiety and depressive ones) before or after the infection. In 2022 these findings were confirmed

on a larger group of patients [254]. Jason et al. [269] conducted a prospective cohort study which showed that adolescents who developed ME/CFS after infectious mononucleosis did not differ from their peers who fully recovered from the infection in terms of pre-morbid scores on depression, anxiety, autonomic dysfunction, stress levels, and coping skills. However, during the acute infection and at 6-month follow-up, ME/CFS patients significantly differed from the control group on these scales (except for coping skills).

Currently, risk factors for developing PCS are actively studied. A meta-analysis conducted in 2023, which included data from 41 research works and over 800,000 patients, revealed that the risk factors for developing PCS were female gender, age over 40 years, smoking, obesity, certain comorbid conditions (asthma, chronic obstructive pulmonary disease, ischemic heart disease, diabetes, anxiety and depressive disorders, immunosuppressive conditions), hospitalization during the acute phase of infection, and being in an intensive care unit (which reflects the severity of the illness). COVID-19 vaccination prior to the acute infection episode was associated with a lower risk of developing PCS [268].

In a single study conducted in the Russian population, female gender and arterial hypertension (but not the severity of COVID-19 or age) were identified as risk factors for developing PCS [257].

Interestingly, as it was previously noted for ME/CFS, PCS was also associated with undifferentiated connective tissue dysplasia: PCS was observed significantly more frequently in children and adolescents with these constitutional genetic features. Certain manifestations of PCS such as widespread myalgia and abdominal symptoms were more pronounced in cases of dysplasia [30, 31].

Thus, there are both similarities and differences between the risk factors associated with PCS and ME/CFS. As noted above, only half of the patients with PCS, according to the literature, meet the diagnostic criteria for ME/CFS. It should be noted that PCS is not a unique phenomenon – post-infectious syndrome with a similar clinical picture has been described following a number of other infectious diseases (both of viral and bacterial etiology) [317]. Remarkably, in these cases not all individuals with post-infectious

syndrome met the criteria for ME/CFS [133, 269]. Our comparison of the risk factors for developing PCS and ME/CFS confirms that, apparently, PCS and ME/CFS relate to each other as two partially overlapping conditions, and the symptoms of PCS, including chronic fatigue, do not necessarily represent manifestations of ME/CFS.

1.4 Key links of the pathogenesis of myalgic encephalomyelitis/chronic fatigue syndrome and post-COVID-19 syndrome

The pathogenesis of ME/CFS is not fully understood. However, significant data on the plausible pathomechanisms has accumulated over the past 20 years. To date the most significant of them are the following: dysautonomia; neuroinflammation/neuroglial dysfunction; reduced reactivity of the HPA axis to stressors; decreased efficiency of energy metabolism; disturbances in the micro- and macrostructure of sleep; immune dysfunction which is characterized by excessive activation of the immune system in the early stages of the disease (possibly compensatory to the primary immune response defect) followed by immune exhaustion; small fiber neuropathy; hypovolemia which results in the reduction of cardiac preload and cerebral hypoperfusion; endothelial dysfunction with hyperaggregation and hypercoagulation; oxidative stress; gut microbiome alterations [180, 222]. Nevertheless, it remains unclear how these pathological processes are interrelated, which of them are primary and/or key links of the pathogenesis, and how they lead to the clinical manifestations of ME/CFS. Depending on the answers to these questions and the identification of specific pathogenesis links as key ones, several theories of ME/CFS pathogenesis have been proposed.

There is view that the clinical picture observed in patients with ME/CFS reflects primary disturbances in energy metabolism. Since the 1990s, data on the reduced ability of cells to produce and use ATP molecules in ME/CFS have been accumulating. ATP production is impaired both at the stage of glycolysis and mitochondrial respiration [221, 318]. Reduced ATP production was associated with the severity of ME/CFS symptoms [295]. Considering the results of metabolomic research in ME/CFS which showed

decreased levels of 80% of the analysed metabolites in the blood of ME/CFS patients, it was hypothesized that chronic deficiency of energy resources (ATP molecules) leads to the development of ME/CFS. Thus, ME/CFS could represent an example of a genetically regulated and common in living nature hypometabolic state, characterized by a metabolic pattern similar to hibernation and anabiosis. This pattern ensures survival and persistence under stressor exposure but at the cost of significant inhibition of vital functions, leading to a decreased quality of life in patients with ME/CFS [202]. It should be noted that disturbances in energy metabolism (particularly mitochondrial dysfunction) may be secondary to the oxidative and nitrosative stress identified in ME/CFS (manifested by increased levels of free radical oxidation products and decreased levels of antioxidants) [222], as well as secondary to disturbances in the metabolism of simple and complex lipids, which include peroxisomal dysfunction in ME/CFS [203].

At the same time several researchers, recognizing the central role of energy deficiency in the pathogenesis of ME/CFS (which is characteristic of hypoxia), tend to shift the emphasis from the tissue hypoxia to the circulatory one. In recent years, evidence in favour of systemic circulatory hypoxia associated with generalized dysregulation of the vascular tone in macro- and microcirculatory vessels in ME/CFS has accumulated [326]. There is a predominance of vasoconstrictor influences at the level of skeletal muscles in ME/CFS resulting from sympathetic hyperactivity and reduced efficiency of functional sympatholysis due to dysfunction of M3-muscarinic and beta2-adrenergic receptors. This dysfunction may, in turn, be related to a genetic defect, desensitization or antagonistic activity of functional AAb against these receptors [212, 326]. It should be noted that sympathetic hyperactivity is not characteristic of all patients and, contrary to popular belief, is not always associated with psychological stress or affective spectrum disorders. Sympathetic hyperactivity may be secondary, for example, to the beta2-adrenergic receptor dysfunction or hypovolemia, which is characteristic of ME/CFS (see below). Chronic stimulation of alpha-adrenergic receptors leads to calcium sensitization and vascular remodelling characterized by hypertrophy of vascular smooth muscle cells which, in turn, enhances the vasoconstrictor effect of sympathetic hyperactivity. Beta2-

adrenergic receptors, which ensure functional sympatholysis in skeletal muscles, are more prone to desensitization under chronic activation [326].

There is evidence for both absolute and relative hypovolemia in ME/CFS. The primary mechanism of absolute hypovolemia is considered to be paradoxically low activity of the renin-angiotensin-aldosterone system in patients with ME/CFS [327]. Relative hypovolemia is associated with dysregulation of vascular tone in ME/CFS. Hypoxia at the skeletal muscle level (circulatory or tissue, as previously described) leads to the local production of molecules with vasodilatory activity (bradykinin, ATP, prostaglandins, hydrogen ions, adenosine etc.). It is hypothesized that due to systemic hypoxia in ME/CFS these autacoids, which normally act locally, may be produced in excessive quantities, enter the systemic circulation, and contribute to systemic vasodilation at the level of capacitance vessels. This disrupts the regulation of circulation, leading to a decrease in cardiac preload, cardiac output, and cerebral hypoperfusion observed in ME/CFS. These hemodynamic disturbances, in turn, not only cause ME/CFS symptoms related to the central nervous system (CNS) but also increase the activity of the sympathetic nervous system, thus forming a pathogenic vicious circle. This can be interpreted as a manifestation of the conflict between local and central regulatory patterns in ME/CFS, paralysing the effectiveness of both [8]. Another mechanism of inadequate vasodilation as a cause of relative hypovolemia could be small fiber neuropathy, which is quite common in ME/CFS [170]. Immunohistochemical studies show that these nerve fibres regulate microvascular tone primarily through sympathetic and parasympathetic cholinergic synapses on perivascular myocytes.

A widely accepted theory of ME/CFS pathogenesis focus on immunological dysfunction, which is reflected in one of the ME/CFS names – "chronic fatigue and immune dysfunction syndrome." According to this concept in its most general form, a trigger (most often an infectious agent) activates the innate immune response. Defects in the innate immune response contribute to the persistence of the infectious agent and chronic stimulation of the immune system, which is fraught with the development of autoimmune reactions. Alongside the pro-inflammatory response, compensatory anti-

inflammatory mechanisms are activated, and its persistent activation sooner or later leads to so-called functional exhaustion of immune cells. Consequences of immune dysregulation in ME/CFS include disruptions in the microbiome composition, chronic excessive systemic action of inflammatory mediators, and coagulopathy closely linked with chronic endotheliitis and peripheral vasoconstriction which contributes to the hypoxia [222].

Much research over the past thirty years has been dedicated to the state of the immune system in ME/CFS; however, data on the levels of various humoral factors of innate and adaptive immunity in the blood of patients and the deviations in the composition of immunocompetent cells subpopulations are often contradictory [38]. More consistent data pertain to the functional characteristics of T-lymphocytes and NK cells: a lot of studies have confirmed a decrease in the functional activity of these cells. Regarding T-lymphocytes, the metabolic profile of CD4+ and CD8+ T-cells in ME/CFS corresponds to the immune exhaustion. In these lymphocytes the activity of glycolysis and the mitochondrial membrane potential are reduced, while the activity of fatty acid oxidation is increased. These T-cells are also characterized by increased expression of inhibitory receptors, reduced proliferative capacity, and decreased cytokine secretion [198].

Some researchers assign a key role in the pathogenesis of ME/CFS to the disturbed cytokine regulation [38], pointing to the fact that cytokines affect the function of the endocrine and central nervous systems, representing a "sickness signal" for these systems. Therefore, cytokines could be considered a general regulatory link that connects immune homeostasis disorders and dysfunction of the nervous and endocrine systems in ME/CFS. Moreover, the excessive systemic action of cytokines, which are primarily short-range bioregulators, both in acute situations (shock and shock-like conditions) and in chronic ones (metabolic syndrome, connective tissue dysplasia, etc.), represents the basis of the conflict between local and systemic regulation resulting in insufficient oxygen delivery to tissues and cells (hypoxia) [12]. Specifically, injection of cytokines causes weakness and malaise in people without ME/CFS, and laboratory animals show significant behavioural changes that are alleviated by the administration of anti-cytokine drugs [38]. At the same

time, the accumulation of data on the levels of various cytokines in the blood of ME/CFS patients led to the conclusion of data inconsistency, as noted in a systematic review of cytokine studies in ME/CFS (2019) [55]. However, this inconsistency may be at least partially related to the different disease duration in different studies, as significant increase in both pro-inflammatory and anti-inflammatory cytokine levels has been observed in patients with early stage ME/CFS (first two years), but not in case of long-term condition [114]. The identified differences in the plasma cytokine profiles of patients at different stages of the disease suggest that in the early stages of infection-dependent ME/CFS the immune system actively tries to respond to the antigenic load associated with the infectious process and its consequences. However, as the disease progresses, immune exhaustion occurs [222]. Meanwhile, VanElzakker et al. [321] conclude in a critical review of methods to study neuroinflammation that the biological role of cytokines as short-range rather than systemic bioregulators, along with the numerous sources of fluctuations in their levels in biological fluids, makes it unlikely that a reproducible diagnostic cytokine profile for ME/CFS will ever be established.

Due to the detection of elevated serum concentrations of various AAb in patients with ME/CFS (including antinuclear antibodies, antibodies to double-stranded DNA, to neurons and endothelial cells, to adrenergic and muscarinic acetylcholine receptors) in several studies, a hypothesis of the autoimmune genesis of ME/CFS was proposed [213].

From a clinical perspective, immune dysfunction in ME/CFS, as it was noted back in the 1990s, manifests not only as a reduced resistance to infections (especially viral ones) but also as hypersensitivity reactions of various types, although the specific mechanisms underlying allergic reactions in ME/CFS have not yet been established. Together with shifts in many laboratory immunological parameters (though often in opposite directions according to different authors), these clinical signs of immune dysfunction have led to speculation that ME/CFS represents a manifestation of the immune system's reduced ability to adequately respond to the antigenic stress [38].

The combination of sleep disturbances, dysautonomia, cognitive symptoms, and nociplastic pain in ME/CFS has led researchers to assume that some pathological process

in CNS play a key role in the disease pathogenesis. Neuroimaging data (MRI spectroscopy and PET) in ME/CFS suggest that this process is neuroinflammation/dysfunction of neuroglia, which represents a universal response to injury and is now considered a common pathophysiological denominator uniting traumatic, neurodegenerative, and psychiatric disorders [82]. The significant role of astrocytes in controlling cerebral perfusion, including their influence on systemic hemodynamics, points to a possible connection between microglial dysfunction and dysautonomia in ME/CFS [87]. A ME/CFS animal model was developed by immunization of rats with viral double-stranded RNA analogue (polyinosinic-polycytidylic acid, polyI:C). In this model signs of increased blood-brain barrier permeability, astroglial activation, manifestations of neuroinflammation, hypercytokinemia, and serotonin metabolism disturbances were observed and led to ME/CFS-like manifestations in rats during behavioural tests [138]. Considering the above-mentioned concept that the impact of various stressors (which is by no means limited to psychological stressors) on the background of initially impaired body reactivity plays an important role in the pathogenesis of ME/CFS, it has been suggested that the epicenter of neuroinflammation (i.e. activation of microglia and astrocytes) in ME/CFS is the hypothalamus as a part of the autonomic and limbic systems whose dysfunction can explain the wide range of ME/CFS symptoms [186, 196]. It should be noted that as early as 1999, A.Sh. Zaichik and L.P. Churilov [10] first suggested that ME/CFS might represent a limited, slow-progressing autoimmune hypothalamitis affecting the body's stress resistance potential. In 2021, a high prevalence of AAb to the pituitary gland and hypothalamus in ME/CFS was shown, and the AAb titers correlated with the decreased adrenocorticotropic hormone/cortisol level reduction and disease severity [155]. In addition to this hypothesis, it can be noted that exposure to various stressors in animals or humans by itself causes activation of microglia and cytokine expression in the hypothalamus which expands the range of possible triggers of neuroinflammation in ME/CFS [228]. Activation of astro- and microglia in the CNS can be caused by direct brain injury, reactivation of infectious agents in CNS cells (which can

serve as microbial reservoirs), autoimmune reactivity to neural or glial antigens, cerebrovascular hypertension or hypoperfusion, recognition of damage-associated molecular patterns by immune cells in the CNS, excessive levels of norepinephrine or angiotensin II in systemic circulation, chronic psycho-emotional stress [299]. Moreover, neuroinflammation can be initiated by blood-brain barrier disruption, for example, due to the systemic action of inflammatory mediators causing endothelial dysfunction and coagulation disorders. Finally, information about local inflammatory processes in peripheral tissues can be transmitted to the CNS via the vagus nerve and activate glial cells (in case of organs innervated with vagus nerve) [299].

In this context, it is appropriate to mention the role of microbiome disturbances in ME/CFS. It is now well known that representatives of the gut microbiota can trigger or sustain disruptions in neuroglial functioning, and particularly neuroinflammation, both by transmitting signals through the vagus nerve and by affecting the CNS with various microbial metabolites and pathogen-associated microbial fragments. The latter is especially likely when gut permeability is increased, which is associated with microbiome disturbances [261]. Regarding microbiome composition in ME/CFS, a reduction in the proportion of short-chain fatty acid (particularly butyrate)-producing bacteria, such as Faecalibacterium and Bifidobacterium, has been identified [222]. Butyrate has important immunomodulatory effects, as it stimulates regulatory T-cells, inhibits the production of inflammatory cytokines, and induces the antimicrobial activity of macrophages [90]. Thus, a deficiency of this gut metabolite may contribute to the development of inflammation in the gut wall and increase its permeability. Elevated blood levels of one of the most well-known pathogen-associated microbial fragments – lipopolysaccharide (LPS) as well as anti-LPS IgM and IgA antibodies may reflect these processes in ME/CFS. LPS is an endotoxin, a structural component of the outer membrane of many gram-negative bacteria, and has pro-inflammatory, pro-coagulant, cytotoxic, and particularly neurotoxic effects [240]. It is considered a biomarker indicating the translocation of bacteria and toxins from the gastrointestinal tract into the systemic circulation [188]. A universal sign of dysbiosis is the reduction of gut microbiota diversity

and the increase in the proportion of bacteria from the Proteobacteria group (particularly Enterobacteriaceae), which are a major source of LPS [322]. This microbiome disturbance pattern is also characteristic of ME/CFS [222].

The concept of the key role of the hypothalamus in the pathogenesis of ME/CFS is well supported by data on the dysfunction of several hypothalamic-pituitary axes in this disease. Indicators of hypothalamic-pituitary axes dysfunction in ME/CFS include reduced basal levels of growth hormone, insulin-like growth factors, and growth hormone production in insulin-induced hypoglycemia test; euthyroid sick syndrome (low T3 syndrome); decreased blood cortisol levels and reduced responsiveness of the HPA axis to physical and psychological stressors, as well as to exogenous corticotropin-releasing hormone and adrenocorticotropic hormone in provocative tests, along with increased negative feedback (which corresponds to a higher density of glucocorticoid receptors at the pituitary and hypothalamic levels and their increased affinity for the ligands) [180, 211, 271, 279].

The aforementioned autonomic nervous system dysfunction, particularly in the context of vascular tone regulation disturbances, also aligns well with the concept of ME/CFS as a neuroinflammatory process and as a disease of impaired stress adaptation. There is substantial evidence supporting parasympathetic insufficiency and sympathetic predominance in ME/CFS [129]. This can be significant not only for the vascular tone regulation but also for the immune response regulation, considering the existence of the anti-inflammatory cholinergic pathway [81]. Thus, another vicious cycle is formed: neuroinflammatory process in the central autonomic nervous system causes its dysfunction, and the reduced parasympathetic activity contributes to the persistence of the neuroinflammatory process [209]. Overall, the most studied neuroimmune connections in ME/CFS are as follows: the CNS receives information from the immune system through the cytokine system and modulates immune system reactivity through the HPA axis, sympathetic and parasympathetic nerve fibres.

The pathophysiological mechanisms of PCS share much in common with ME/CFS [180]. Both syndromes are characterized with elevated levels of oxidative stress markers

(F2-isoprostanes, malondialdehyde), reduced levels of antioxidants (e.g., coenzyme Q-10), increased lactate levels, and reduced mitochondrial membrane potential in lymphocytes, indicating mitochondrial dysfunction [56, 200, 208, 302]. To date, however, cellular ATP production capacity has been directly assessed only in ME/CFS.

Regarding the state of micro- and macrocirculation: endothelial dysfunction, a tendency toward vasoconstrictive reactions due to hypersympathicotonia, hemostasis disorders (such as hyperaggregation and hypercoagulation), reduced cardiac preload, and signs of cerebral hypoperfusion are common for both ME/CFS and PCS [180, 287, 328]. Among the features of PCS, there is a more pronounced tendency towards hypercoagulation in PCS compared to ME/CFS, manifesting as microthrombs formation and elevated levels of D-dimer, von Willebrand factor, and factor VIII. Another feature of PCS is the activation of angiogenesis, evidenced by increased blood levels of angiopoietin-1, P-selectin, matrix metalloproteinase-1, ICAM-1, and vascular endothelial growth factors (VEGF) A and D [121, 200].

Cardiovascular complications during the acute phase of COVID-19 suggest that the consequences of myocarditis underlie some cases regarded as PCS. Despite the absence of cardiac MRI specifically in the PCS group, comprehensive cardiac MRI in individuals who had recovered from COVID-19 revealed signs of myocarditis (specifically, increased T2 signal corresponding to myocardial edema) in 60% of cases in 2-3 months after negative PCR test compared to 12% in the pre-pandemic control group [239].

Immune dysfunction plays a key role in the pathogenesis of PCS, according to most researchers [191, 200]. The general concept of immune dysfunction in ME/CFS (the gradual development of immune exhaustion due to chronic adjuvant-like activation of the immune response) is also relevant for PCS [271].

The phenomenon of immune exhaustion in severe COVID-19 can develop already during the acute infection and persist for several months thereafter [200]. Cytokines have gained significant attention in PCS. Compared to ME/CFS, there is greater consistency in research results regarding cytokine levels in the blood of patients with PCS, which may be due to a more homogeneous sample and testing at the early stages of the disease

(considering that in ME/CFS, as mentioned above, elevated cytokine levels are more common for the first two years of illness) [191]. Elevation of IL-1β, IL-6, TNF-α levels is typical for PCS, and it is more pronounced after infection with early SARS-CoV-2 strains (alpha, beta, gamma, and partially delta) compared to the omicron strain [260]. Cytokine regulation disorders in PCS are not limited to elevated levels of pro-inflammatory cytokines in the systemic circulation. It has been shown that the SARS-CoV-2 spike protein induces local production of pro-inflammatory cytokines by microglial cells [207]. At the same time, contradictory results have been obtained regarding the correlation between symptom severity and cytokine levels [180]. Another component of humoral immunity that has been extensively studied in PCS is AAb. Persistent increased levels of AAb – both classical pathogenic ones (antinuclear, antiphospholipid) and natural functional ones (to G-protein-coupled receptors) occur in PCS and have correlated with symptom severity in several studies. However, other studies did not confirm the association between antinuclear antibodies and antibodies to IFNγ with PCS, as prevalence of these AAb in patients with persistent PCS symptoms in a year after infection did not exceed that in the control group [200]. Regarding cellular immunity, it is noteworthy that elevated levels of activated CD8+ T lymphocytes and the development of T-cell exhaustion are common to both ME/CFS and PCS [180]. Reactivation of latent herpes infections as a sign of reduced immune system control is characteristic of ME/CFS, as mentioned above [108, 301]. For PCS developing after mild COVID-19 Epstein-Barr virus reactivation neither in the acute phase of COVID-19, nor after the initial infection is typical [126]. In studies of more heterogeneous groups, including patients who had moderate to severe COVID-19, Epstein-Barr virus reactivation during the acute infection correlated with the risk of developing PCS. However, by the time PCS developed, the reactivation had typically resolved, with the virus returning to its latent form [94]. Concluding the brief description of immune dysfunction in PCS, a tendency towards the type 1 hypersensitivities should be also mentioned [164].

Regarding the composition of the microbiota and intestinal wall permeability, the main features of ME/CFS have been described in PCS, including a decrease in butyrate-

producing flora, an increase in the number of LPS-producing bacteria, and elevated levels of LPS in the blood of patients as a marker of bacterial translocation [141, 187].

Evidence of the contribution of neuroinflammation to the pathogenesis of PCS has been obtained both from patient examinations (PET scans, assessing the level of glial fibrillary acidic protein (GFAP) in the blood) [80, 190, 227], and from studies on animal models of PCS. Specifically, in a mouse model of SARS-CoV-2 infection, activation of microglia and an increase in the cytokine CCL11 in the cerebrospinal fluid were demonstrated, even with a mild course of infection. Intraperitoneal administration of CCL11 to healthy mice was associated with cognitive dysfunction and impaired neurogenesis [205]. In hamsters infected with SARS-CoV-2, signs of neuroinflammation persisted after the viral clearance [204]. In non-human primates infected with SARS-CoV-2, signs of neuroinflammation (activation of microglia with characteristic morphological and immunohistochemical changes), neuronal apoptosis, microhemorrhages in the basal ganglia, and expression of the HIF-1a marker, indicating hypoxia, were observed even in animals with mild infection in brain regions where neuroinflammatory and degenerative phenomena were most pronounced [231].

In addition to the activation of neuroglia in patients with PCS and subjective cognitive impairments (brain fog), but not in patients with PCS without cognitive impairments, dynamic MRI with contrast enhancement revealed increased widespread blood-brain barrier permeability [80]. This group of patients, compared to the control group and the group of PCS patients without cognitive impairments, had higher blood levels of TGFβ, which was the only cytokine that correlated with the increased permeability of the blood-brain barrier. Interestingly, that among all cytokines the most data on the link between the elevation of cytokine level and clinical symptoms of ME/CFS refer to TGFβ. These data were obtained from studies both in patients and in the animal model of ME/CFS (which was developed through systemic administration of TGFβ to mice) [199]. TGFβ is elevated in the systemic circulation of individuals with Marfanoid phenotype connective tissue dysplasia [36], whose association with constitutional predisposition to both ME/CFS and PCS has been previously hypothesized (see above). A pattern of brain

hypometabolism (reduced 18F-fluorodeoxyglucose PET signal) is also common to both ME/CFS and PCS. In particular, brain hypometabolism was reported in the frontal cortex and brainstem – areas predominantly associated with the limbic system and reticular formation, which exert activating influences on higher brain regions [180, 229]. Although the inflammatory process is usually associated with hypermetabolism of fluorodeoxyglucose according to PET brain imaging, neuroinflammation/microglial activation, on the contrary, can be combined with a reduced cerebral glucose metabolism [99, 117], likely due to impaired energy metabolism in neurons and reduced glucose uptake by them in the context of the inflammatory environment created by glial cells.

Regarding hypothalamic-pituitary axis dysfunction in PCS, data presented in the literature remains contradictory [96]. Klein et al. [115] demonstrated that, similar to ME/CFS, patients with PCS showed decreased blood cortisol levels in a year after COVID-19. Additionally, they did not exhibit an expected increase in adrenocorticotropic hormone levels, which could be considered as a sign of secondary adrenal insufficiency due to reduced central reactivity of the HPA axis. The results of the study of Ach et al. [130] confirm lower blood cortisol levels and decreased HPA reactivity (in the insulin tolerance test) in patients with PCS examined 3-15 months after COVID-19. Similar results were obtained by them for the human growth hormone. Extensive information on insufficient HPA function as the basis of the pathogenesis of PCS and ME/CFS is provided in a recent review by Spanish authors [271]. However, in the study of Alijotas-Reig et al. [245] no changes in cortisol and adrenocorticotropic hormone levels were found in patients with PCS examined at the same times after acute infection as in the study by Ach et al. Euthyroid sick syndrome (also known as low T3 syndrome) is widely recognized in many critical conditions, particularly during the acute phase of severe COVID-19. It is interpreted as an adaptation that restrains basal metabolism and thus conserves substrate-energy resources during critical illness. However, it resolves successfully after the acute infection phase and seemingly does not significantly contribute to the development of PCS [251].

Regarding the autonomic nervous system, data from studies on HRV indicate that PCS is characterized by a dysautonomia pattern with reduced overall HRV and parasympathetic influences combined with increased sympathetic tone [266], which has been also described in ME/CFS [160]. However, in severe cases of ME/CFS, HRV analysis demonstrates a decrease in both parasympathetic and sympathetic components of autonomic regulation [67].

Analysis of the literature on modern concepts of ME/CFS and PCS therapy and new directions in this field is beyond the scope of our work.

Thus, the conducted literature review showed that ME/CFS and PCS are characterized by a relatively high prevalence among young and middle-aged individuals, which underlines the importance of research in this area. The clarification of terminology and key definitions, along with the presentation of modern internationally accepted diagnostic criteria, performed in this chapter of our work. should contribute to the unification of clinical understanding of these conditions.

Despite the apparent similarity of the main symptoms, only about 50% of PCS patients meet the diagnostic criteria for ME/CFS. We could not find an explanation for this fact in the available literature. However, based on the comparison of the clinical picture in PCS patients who do and do not meet the ME/CFS criteria, performed by Tokumasu et al. [97], and considering the current trend towards identifying clinical phenotypes of PCS, it can be assumed that patients with PCS who do not meet the ME/CFS criteria belong to the other phenotypes, namely: 1) mild PCS phenotype, with main manifestations of anosmia/dysosmia, ageusia/dysgeusia, and/or increased hair loss; 2) pulmonary phenotype, with the key symptom of persistent dyspnea, possibly due to the development of interstitial lung disease (fatigue is quite explicable in this case, but other typical symptoms of ME/CFS are absent). At the same time, the chronic fatigue-like phenotype of PCS remains undoubtedly the most common [244, 249]. Taking into account that when WHO developed the clinical case definition for PCS, consensus among experts was reached for three symptoms (fatigue, cognitive dysfunction, dyspnoea) – it can be

considered that the WHO definition of PCS implies primarily inclusion of patients with the chronic fatigue-like phenotype of PCS.

Regarding the mechanisms underlying the clinical picture of ME/CFS and PCS, there is evidence for the similarity of many pathogenetic pathways of these conditions. However, a direct comparison of ME/CFS not related to COVID-19 infection and PCS in order to identify the differences between them, has not yet been the subject of comprehensive research.

CHAPTER 2 MATERIALS AND RESEARCH METHODS

2.1 Clinical material

The study included 152 participants: 54 patients meeting the diagnostic criteria for ME/CFS with the onset of symptoms not related to COVID-19 (11 men and 43 women, mean age in the group was 37.91±9.51 years); 46 patients meeting the WHO clinical case definition for PCS (8 men and 38 women, mean age in the group was 37.41±9.33 years); and 52 apparently healthy individuals (13 men and 39 women, mean age in the group was 35.78±11.32 years).

Patients were examined at the Center for the Study of Autoimmune Diseases and Consequences of the Novel Coronavirus Infection named after Professor Y. Shoenfeld (at the Pirogov Clinic of High Medical Technologies at St Petersburg University), at the Clinic of the Research Institute of Rheumatology and Allergology of Pavlov First St. Petersburg State Medical University, and at the Medical Research Center "Immunculus."

Inclusion Criteria:

For all groups:

- age from 18 to 60 years;
- informed consent to participate in the study.

Additionally for the group of patients with ME/CFS:

- patient must meet three sets of ME/CFS diagnostic criteria simultaneously, as recommended by the European Expert Group on ME/CFS (EUROMENE) in the consensus document on the diagnosis and management of ME/CFS (2021). These criteria are modified Fukuda Criteria (Fukuda or CDC, 1994) [291], Canadian Consensus Criteria (CCC, 2003) [215] and criteria developed by Institute of Medicine/National Academy of Medicine, IOM/NAM, 2015) [101]

- absence at the time of the study any of the following diseases, if they can fully explain the clinical symptoms (according to the EUROMENE consensus recommendations on ME/CFS diagnosis, 2021 [128]):

- endocrine diseases/metabolic disorders: primary adrenal insufficiency, Cushing's disease and syndrome, hyper- and hypothyroidism, type 1 or 2 diabetes mellitus, hypercalcemia;
- rheumatic diseases: systemic lupus erythematosus, rheumatoid arthritis, polymyositis;
- hematologic diseases: iron deficiency anemia, hemochromatosis, idiopathic thrombocytopenic purpura;
- infectious diseases: HIV infection, hepatitis B and C, tuberculosis, Lyme disease (tick-borne borreliosis), giardiasis, helminthiasis, syphilis;
- neurological diseases: multiple sclerosis, narcolepsy, obstructive sleep apnea, restless legs syndrome, Parkinson's disease, myasthenia, vitamin B12 deficiency, cervical spine injuries, epilepsy;
- mental disorders: bipolar disorder, substance use disorder, schizophrenia, recurrent depressive disorder;
- gastrointestinal diseases: celiac disease, Crohn's disease, ulcerative colitis;
- cardiovascular diseases with chronic heart failure;
- respiratory diseases (chronic obstructive pulmonary disease, bronchial asthma) with respiratory failure;
- chronic heavy metal intoxication (lead, mercury);
- development of the symptoms as a manifestation of side effects of any medications;
- Overwork (working hours more than 50 per week);
- Overtraining syndrome in athletes;
- Body mass index (BMI) over 40 kg/m².

Additionally for the group of patients with PCS:

- compliance with the clinical case definition of PCS developed by WHO [50].

For the control group:

- no history of chronic diseases;
- absence of PCS and ME/CFS symptoms.

Exclusion criteria for all groups:

- presence of injuries or acute diseases (including acute respiratory infections) within the month preceding the study;
- pregnancy and lactation.

The study was approved by the local ethics committee of the I. P. Pavlov First St. Petersburg State Medical University of the Russian Ministry of Health and the ethics committee of St. Petersburg State University in the field of research involving humans. The study was conducted in accordance with the ethical standards of the Helsinki Declaration.

2.2 Research methods

2.2.1 General clinical examination

Based on the medical history, life history, and results of the Short Form-36 (SF-36) questionnaire for the measurement of the health-related quality of life and the DePaul Symptom Questionnaire Short-Form (DSQ-SF) for the assessment of ME/CFS symptoms [174] the following parameters have been analyzed for each study participant:

- duration of the primary illness;
- presence of co-morbidities;
- whether the individual meets the clinical case definition for PCS developed by WHO (October 6, 2021) [50];
- whether the individual meets three sets of diagnostic criteria for ME/CFS recommended by EUROMENE in the consensus document on the diagnosis and management of ME/CFS (2021) for use in research and clinical practice (2021) [128]: CDC 1994, CCC 2003 and IOM/NAM 2015.

The DSQ-SF is a short form of the DSQ, which was created and validated to assess patients' symptoms with three sets of diagnostic criteria for ME/CFS (CDC 1994, CCC

2003 and IOM/NAM 2015) [174]. It has been shown that the results of the DSQ can differentiate patients with ME/CFS from healthy individuals and from patients with other chronic illnesses [174]. Strand et al. [102] demonstrated 98% concordance between physicians' diagnosis of ME/CFS using the CCC, 2003 and the results of the DSQ. However, the original DSQ consists of 99 questions, so in 2019 a short form, the DSQ-SF, was developed. The DSQ-SF consists of 14 questions and also allows determination of compliance with the aforementioned three sets of diagnostic criteria and reliably differentiates ME/CFS patients from healthy individuals [286]. In the DSQ-SF each question addresses one symptom and consists of two parts: the assessment of frequency and severity of the symptom over the past 6 months (Annex A – DSQ-SF questionnaire; in the Russian text of the dissertation Annex A represents a translation of DSQ-SF into Russian made by the author of this dissertation together with her Russian-speaking scientific supervisor L.P. Churilov and community of patients suffering from ME/CFS).

2.2.2 Assessment of the severity of fatigue, anxiety and depression

Patients completed the Hospital Anxiety and Depression Scale (HADS) forms to evaluate the presence and severity of anxiety and depression. This scale has been repeatedly validated in various studies both in patients with somatic diseases and with mental disorders [308]. The questionnaire includes 7 questions for the assessment of anxiety and 7 question for the assessment of depression. Each question has 3 answer options, from which one must be chosen. The results are calculated by summing the points, with a maximum score of 21 for each subscale (anxiety and depression). The total score can correspond to the absence of anxiety/depression (0–7 points), subclinical anxiety/depression (8–10 points), or clinically significant anxiety/depression (≥11 points).

Patients also completed the Multidimensional Fatigue Inventory (MFI-20) to evaluate fatigue. This questionnaire consists of 20 statements reflecting different aspects of fatigue: General Fatigue, Physical Fatigue, Reduced Activity, Reduced Motivation, and

Mental Fatigue [298]. Each aspect corresponds to four statements. The patient rates their agreement with each statement on a scale from 1 to 5. Thus, the severity of fatigue in each aspect can range from 4 to 20 points. A score of 12 or more on any subscale corresponds to a clinically significant fatigue syndrome.

The assessment of physical activity (PA) levels was conducted with International Physical Activity Questionnaire Short Form (IPAQ) consisting of seven questions that evaluate the frequency and duration of walking, moderate and vigorous intensity PA, and periods of inactivity over the past 7 days [171]. If a time range was provided in response to the duration of PA, the mean value was used for calculation. MET (Metabolic Equivalent of Task) value was used for quantitative assessment of PA intensity levels. MET indirectly reflects the activity of metabolic processes in the body by calculating the oxygen consumption level for a given load. 1 MET corresponds to the resting metabolic rate: 1 MET = 3.5 ml O2/kg body weight/min. The following coefficients are used to calculate MET-min/week: low-intensity PA (walking) – 3.3; moderate-intensity PA – 4.0; vigorous-intensity PA – 8.0. The total energy expenditure represents the sum of MET-min/week from low-intensity PA, MET-min/week from moderate-intensity PA, and MET-min/week from vigorous-intensity PA.

Formulas for calculating MET-min/week from different types of PA [49]:

Low-intensity PA: 3.3 × walking duration (min) × number of days per week with walking

Moderate-intensity PA: 4.0 × moderate-intensity PA duration (min) × number of days per week with moderate-intensity PA

Vigorous-intensity PA: 8.0 × vigorous-intensity PA duration (min) × number of days per week with vigorous-intensity PA

2.2.3 Analysis of heart rate and blood pressure variability

The study of autonomic regulation of the cardiovascular system was conducted with the computerized complex "Spiroarteriocardiorythmograph-01" (manufactured by LLC

"INTOX" (Russia), registration license No. 29/03020703/5869-04 dated January 29, 2004) and included synchronous recording of electrocardiogram, arterial blood pressure, and spirometry during spontaneous breathing, as well as during paced breathing. Patients participating in the study did not take beta-blockers and calcium channel blockers to exclude the influence of medications on HRV and BPV parameters. On the day of the study the intake of coffee and alcohol were excluded; food intake and physical exertion were excluded within 2 hours before the study. The study was conducted in a seated position (the patient's arms were placed on special armrests at heart level). Three electrodes were fitted on the upper limbs of the patient for recording electrocardiogram in the first standard lead, a finger cuff with a photoplethysmographic sensor was fitted on the middle phalanx of the third finger of the left hand for non-invasive continuous measurement of arterial pressure using the Penaz unloading method, and a face mask with an ultrasonic spirometric sensor was used for recording the spirogram.

The study protocol consisted of recording electrocardiogram, blood pressure, and spirogram: 1) for 5 minutes during breathing with spontaneous breathing frequency; 2) for 2 minutes during paced breathing under the instructor's count at a rate of 12 breathing cycles per minute (12BR); 3) for 2 minutes during paced breathing under the instructor's count at a rate of 6 breathing cycles per minute (6BR). The spirometry data were used to confirm that patients adhered to the protocol for paced breathing.

The recorded data were processed using the spiroarteriocardiorythmograph software and included the assessment of HRV, systolic and diastolic BPV (SBPV and DBPV) in the frequency domain. Spectral analysis was performed using the discrete Fourier transform method. The following parameters were evaluated: total power in the frequency range ≤ 0.4 Hz (Total Power, TP), spectral power density in the high-frequency range (0.15–0.4 Hz) (High Frequency, HF), spectral power density in the low-frequency range (0.04–0.15 Hz) (Low Frequency, LF), and spectral power density in the very low-frequency range (≤ 0.04 Hz) (Very Low Frequency, VLF). These parameters were calculated separately for heart rate, SBP, and DBP. TP, VLF, LF, and HF for HRV were

calculated in ms² and had the subscript HR, while SBPV and DBPV were calculated in mm Hg² and had the subscripts SBP and DBP, respectively.

2.2.4 Analysis of baroreflex regulation

The assessment of baroreflex regulation was performed based on synchronous electrocardiogram and blood pressure recording for each heartbeat ("beat to beat") using two methods: the sequence method and the spectral analysis method [75].

Using the sequence method, segments with consistent changes of blood pressure and RR intervals over at least three consecutive cardiac cycles (the so-called "baroreflex activation episode") were identified, and regression coefficients were evaluated for these segments. Baroreflex sensitivity of the spontaneous arterial baroreflex (baroreflex sensitivity, BRS, ms/mmHg) is determined as the average of all obtained regression coefficients over a 5-minute recording of electrocardiogram and blood pressure at rest. This indicator was calculated for baroreflex activation episodes in response to both an increase and a decrease in systolic blood pressure (BRS_up and BRS_down).

The baroreflex effectiveness index (BEI, %) is determined as the ratio between the number of baroreflex activation episodes and the total number of unidirectional SBP changes over three cardiac cycles (increase or decrease) in a given time period. This indicator was also calculated for baroreflex activation episodes in response to both an increase and a decrease in SBP (BEI_up and BEI_down).

Using the spectral analysis method, BRS was determined separately in the HF and LF ranges both during the recordings with spontaneous breathing (5 minutes) and during paced breathing (12 and 6 breaths per minute) according to the formulas:

$$BRS\_LF = \sqrt{\frac{LF_{HR}}{LF_{SBP}}} \quad (1)$$

$$BRS\_HF = \sqrt{\frac{HF_{HF}}{HF_{SBP}}} \quad (2)$$

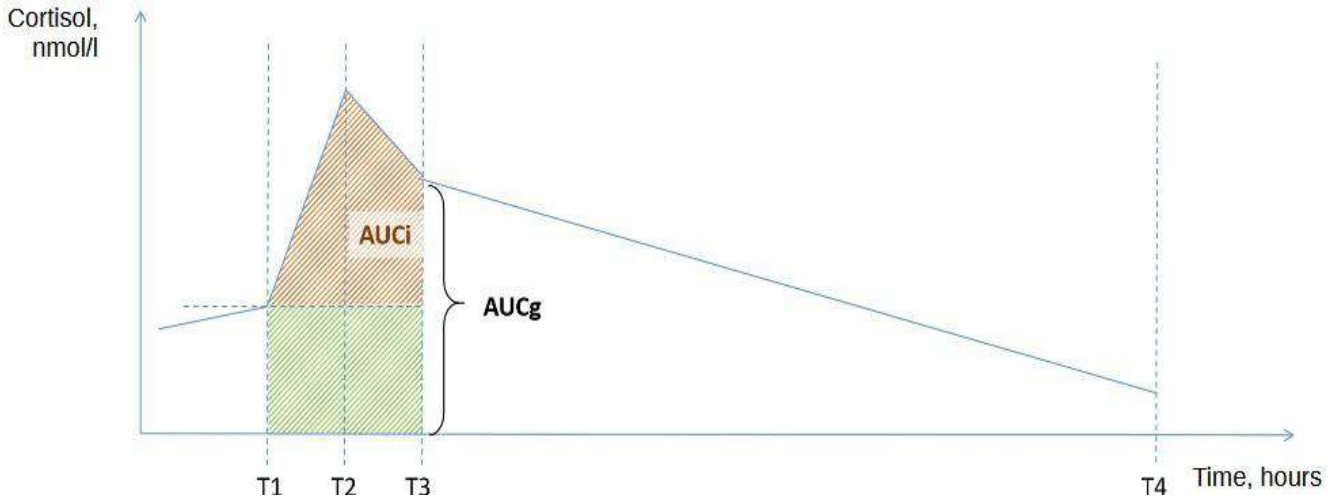
2.2.5 Analysis of the cortisol awakening response

The evaluation of the hypothalamic-pituitary-adrenal (HPA) axis function was based on the assessment of the cortisol awakening response (CAR) [68]. Patients participating in the study did not take glucocorticosteroid and mineralocorticoid drugs in any form, estrogen, progestogen, androgen, and their analogues in order to exclude the influence of these medications on cortisol level.

The determination of free cortisol in saliva was performed throughout the day: saliva samples were collected by the patients themselves at home on a weekend using special containers with a saliva collection swab (Salivette®) according to the following scheme, which is the most rational considering the study objectives and expert consensus guidelines for CAR assessment [68] (Figure 1):

- First sample (T1) – within the first 2 minutes after awakening;
- Second sample (T2) – 30 minutes after awakening;
- Third sample (T3) – 60 minutes after awakening;
- Fourth sample (T4) – at 11 pm (before going to bed; if the patient went to bed earlier, the sample could be obtained 1 hour earlier i. e. at 10 pm)

Figure 1 – Sampling protocol for the free cortisol measurement in saliva in order to assess the cortisol awakening response (CAR). AUCg – total area under the cortisol curve, AUCi – area under the cortisol curve above the awakening cortisol value



For two hours prior to sample collection, eating and tooth brushing were excluded. The containers with samples were stored at a temperature of +2…+8°C and transported to the laboratory the next morning after collection.

Cortisol levels were determined using the electrochemiluminescent immunoassay (ECLIA) method on Roche Hitachi Cobas 6000 and Roche Hitachi Cobas e411 equipment.

Patients were asked to indicate the time of sample collection on the containers and to record their bedtime on the night before the sample collection. After the analysis, patients were asked to answer the following questions:

1. On a scale from 0 to 100, how would you rate the quality of your sleep (depth of sleep + feeling of restfulness upon waking) on the night before the sample collection? 0 = superficial sleep, absolutely unrefreshing; 100 = restorative, deep sleep, with a feeling of vigour upon waking.
2. On a scale from 0 to 100, how fatigued did you feel on the day of the sample collection? 0 = no fatigue; 100 = maximum fatigue.

Based on the answers to these questions, the quality of sleep on the night before the analysis (visual analogue scale, VAS score) and the level of fatigue on the day of the analysis (VAS score) were assessed.

Following cortisol measurement, the following indices were calculated:

- total area under the cortisol curve [314]

$$AUCg = (T2 + T1)/2 + (T3 + T2)/2 \quad (3)$$

T_n – cortisol salivary level in the sample n

- area under the cortisol curve relative to baseline values [314]

$$AUCi = (T2 + T1)/2 + (T3 + T2)/2 - 2 \times T1 \quad (4)$$

- diurnal cortisol slope (nmol/l*hour)

$$DCS = (T2\text{-}T4)/(t4\text{-}t2) \quad (5)$$

t_n – time of the sample n collection

- dynamics of the cortisol level 30 minutes after awakening (%)

$$CAR_{0\text{-}30} = (T2\text{-}T1)/T1*100 \quad (6)$$

- dynamics of the cortisol level between 30 and 60 minutes after awakening (%)

$$CAR_{30\text{-}60} = (T3\text{-}T2)/T2*100 \quad (7)$$

2.2.6 Analysis of microvascular endothelial function

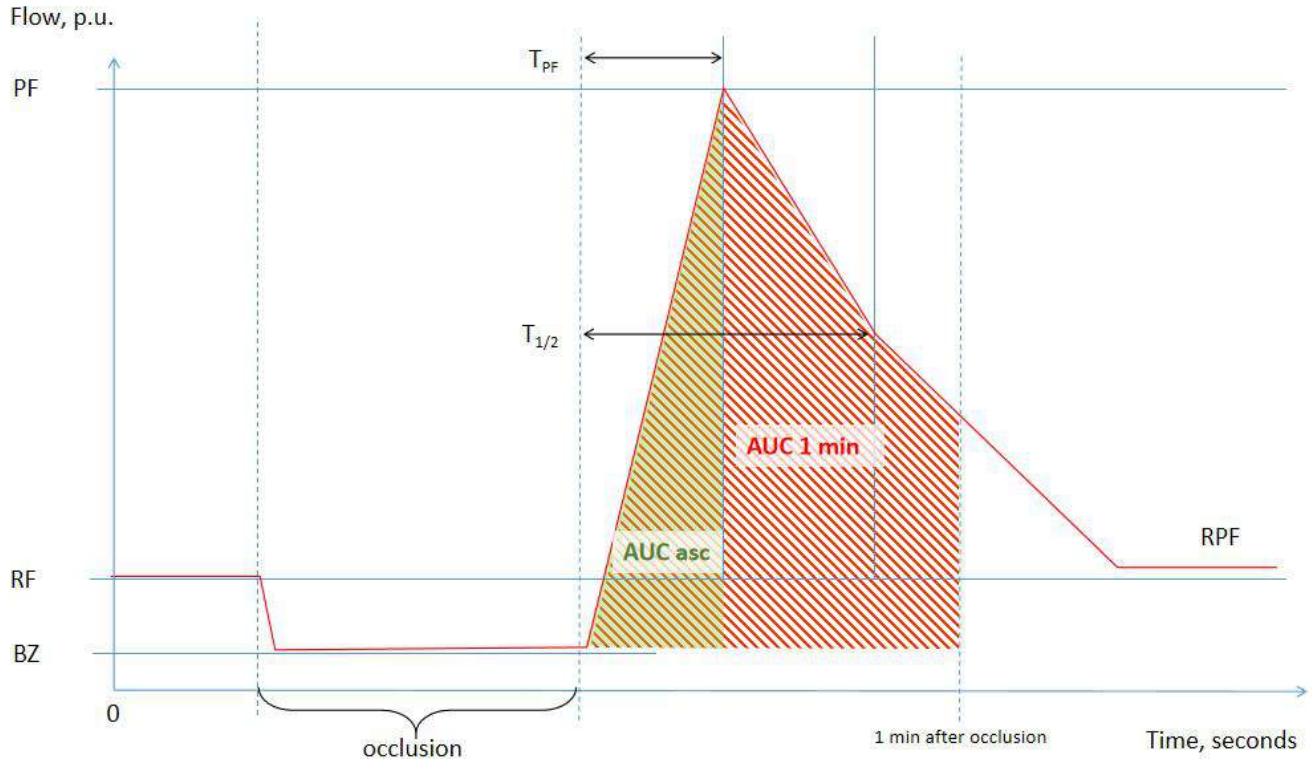
There are four typical forms of endothelial dysfunction: 1) vasomotor; 2) hemostatic; 3) adhesive; 4) angiogenic, which, however, are rarely isolated (predominantly in genetic disorders) [4]. The assessment of endothelial vasomotor function can be a method of choice, allowing for a comprehensive evaluation of endothelial functional state, as it is present in any acquired diseases accompanied by endothelial dysfunction and reflects not only the imbalance between vasoconstriction and vasodilation but also (indirectly) other forms of endothelial dysfunction [4]. To study the state of microvascular endothelial function, an arterial occlusion test was selected, which implies the assessment of endothelium-dependent vasodilation in response to short-term ischemia. Laser Doppler

flowmetry (LDF) was performed with the "LAZMA MC-1" peripheral blood and lymph flow analyser (NPP LAZMA, Russia). On the day of the study, coffee and alcohol consumption were excluded, and food and physical activity were avoided 2 hours before the test. During study patient lay on his/her back, arms placed on the couch alongside the body at heart level. A standard diameter cuff was placed on the patient's left arm, and the optical fiber probe of the device was fixed perpendicularly to the skin on the dorsal (outer) surface of the distal third of the forearm, 4–6 cm proximal to the wrist, using a stand and a special bandage. This area reflects better the overall state of microcirculation. corresponds to the Zakharyin-Ged zone for the heart, and does not contain arteriovenular anastomoses, allowing for a better assessment of nutritive flow [19]. Flow measurements were conducted in relative (perfusion) units (p.u.).

Before performing the test, blood pressure was measured on the brachial artery with the Korotkoff method to determine the pressure level in the brachial cuff to achieve arterial occlusion. The test consisted of recording: 1) baseline (basal) perfusion for 2 minutes; 2) a 3-minute period of arterial occlusion, achieved by rapidly inflating the cuff to 200 mmHg (or 60 mmHg above systolic blood pressure if initial systolic blood pressure >140 mmHg); 3) post-occlusion reactive hyperemia for 5 minutes after rapidly deflating the cuff.

The following parameters of the occlusion test were assessed: rest flow (RF, p.u.) – the flow determined in a rest state before arterial occlusion; biological zero (BZ, p.u.) – the flow determined during arterial occlusion; peak flow (PF, p.u.) – the flow determined after release of arterial occlusion; reperfusion flow (RPF, p.u.) – the flow after its stabilization during post-occlusion reaction; time to PF (TPF, s); rate of achieving PF (Vmax, p.u/s); half recovery time (T1/2, s); flow reserve (FR, %), and area under the reaction curve (AUC, p.u.*s), (Figure 2).

Figure 2: Main laser doppler flowmetry measurements of post-occlusive reactive hyperaemia



Rate of achieving PF (Vmax, p.u./s) was calculated as follows [267]:

$$Vmax = (PF\text{-}BZ) / T_{PF} \quad (8)$$

Flow reserve (FR, %), reflecting the range of possible changes of the microcirculatory blood flow, i. e. the number of functioning capillaries, was calculated as follows [19]:

$$FR = PF / RF * 100 \quad (9)$$

Area under the reaction curve relative to the biological zero (AUC, p.u.*s) was calculated by the trapezoidal rule separately for the ascending part of reaction curve (AUC asc) and for the first minute of reactive post-occlusive hyperaemia (AUC 1 min) (Figure 2):

$$AUC = \left(\sum_{i=1}^{n-1} \frac{(F_{i+1} + F_i - 2 * BZ)}{2} * t \right) \quad (10)$$

t – the signal sampling frequency, which is 0.05 seconds for the LAZMA MC-1 analyser;

F_i and F_{i+1} – flow values at two consecutive measurements.

To analyse the subjective assessment of discomfort in the arm using VAS during short-term ischemia, the subjects were asked to answer the following question after the test: «When you underwent the test to assess microcirculation, how would you rate the discomfort in your arm on a scale from 0 to 100 during the period when air was pumped into the cuff on your arm and blood flow in your arm was stopped? 0 – no discomfort, 100 – maximum pain».

2.2.7 Analysis of the natural autoantibodies serum profiles

To simultaneously assess the serum levels of several natural AAb, the enzyme-linked immunosorbent assay (ELISA) technology was used. ELI-Viscero-Test-24 and ELI-Neuro-Test-12 are based on ELISA technology and these test kits (manufactured by Immunculus, Moscow, Russia; registration number FSR 2009/04551 dated 23.03.2009 (ELI-Viscero-Test-24) and registration number FSR 2009/04554 dated 23.03.2009 (ELI-Neuro-Test-12)) were used in our study according to the manufacturer's instructions. The ELI-Viscero-Test-24 kit allows for the semi-quantitative determination of IgG AAb against 24 antigens (both organ-specific and non-organ-specific ones): ds-DNA, β2-GP I, Fc-Ig, Collagen, CoM-02, β1-AR, TrM-03, ANCA, KiM-S, LuM-S, GaM-02, ItM-07, ScM, HeS-08, HMMP, Insulin, Ins-R, TG, TSH-R, AdrM-D/C, Spr-06, S100, GFAP, MBP. The ELI-Neuro-Test-12 kit allows for the semi-quantitative determination of IgG AAb against 12 antigens of the nervous system: NF200, GFAP, S100, MBP, V-Ca-Chanel, N-Ach-R, Glu-R, GABA-R, DOPA-R, 5HT-R, μ-Opioid-R, β-Endorphin. The names of the antigens are provided in Table 4.

Table 4 – List of antigens in the ELI-Vicero-Test-24 and ELI-Neuro-Test-12 Kits

Antigen	Full name of the antigen
ds-DNA	Double stranded deoxyribonucleic acid
β2-GP I	β2-glycoprotein-I

Continuation of table 4

Antigen	Full name of the antigen
Fc-Ig	Fc-fragments of IgG
Collagen	Collagen
CoM-02	Membrane antigen of cardiomyocytes
β1-AR	β1-adrenergic receptors
TrM-03	Platelet membrane antigen
ANCA	Cytoplasmic antigen of neutrophils
KiM-S	Membrane and cytoplasmic antigens of renal glomerular cells
LuM-S	Membrane and cytoplasmic antigens of pulmonary alveolocytes
GaM-02	Membrane antigen of gastric epithelial cells
ItM-07	Membrane antigen of small intestine epithelial cells
ScM	Membrane antigen of large intestine epithelial cells
HeS-08	Cytoplasmic antigen of hepatocytes
HMMP	Membrane antigen of hepatocyte mitochondria
Insulin	Insulin
Ins-R	Insulin receptor
TG	Thyroglobulin
TSH-R	Thyrotropin receptor
AdrM-D/C	Membrane antigen of adrenal medulla cells
Spr-06	Membrane antigen of sperm and prostate cells
NF200	Neurofilament protein (200 kDa)
GFAP	Glial fibrillary acidic protein
S100	S100 protein
MBP	Myelin basic protein
V-Ca-Chanel	Voltage-dependent calcium channel
N-Ach-R	N-cholinergic receptors
Glu-R	Glutamate receptors
GABA-R	γ-aminobutyric acid receptors
DOPA-R	Dopamine receptors
5HT-R	5HT-receptors

Continuation of table 4

Antigen	Full name of the antigen
μ-Opioid-R	μ-opioid receptors
β-Endorphin	β-Endorphin

Serum samples of the study participants were analyzed. The reactions of each individual's serum sample with 24 antigens in the ELI-Vicero-Test-24 and ELI-Neuro-Test-12 were performed on two different 96-well plates. Additionally, each plate included reactions with a control pooled serum (with a known levels of the studied AAb, close to the average population values) with each of the antigens. All reactions for each serum sample were conducted twice. Standard ELISA procedures, as described in the manufacturer's instructions for the test systems, were performed. The evaluation of the obtained results was carried out according to the algorithm provided by the kit manufacturer.

First, the values of the relative immunoreactivity of the analysed serum sample with each of the studied antigens were calculated as a percentage of the immunoreactivity level of the control serum with the same antigen by the formula:

$$IR_n = \frac{OD_n * 100}{OD_{CSn}} - 100 \quad (11)$$

OD_n – average optical density for the duplicate reaction of the sample with the antigen n

OD_{CSn} – average optical density for the duplicate reaction of the control serum with the antigen n

Then, average individual immunoreactivity (AIR) of serum sample to 24 antigens in the ELI-Viscero-Test-24 and 12 antigens in the ELI-Neuro-Test-12 was calculated according to the formulas:

$$AIR_V = \frac{\sum_{1}^{24} IR_n}{24} \quad (12)$$

$$AIR_N = \frac{\sum_{1}^{12} IR_n}{12} \quad (13)$$

After this, the deviations in the relative immunoreactivity of the analysed serum sample with each of the studied antigens were calculated as a percentage of the AIR level, using the following formulas:

1) For the antigens of ELI-Viscero-Test 24

$$R_n = IR_n - AIR_V \quad (14)$$

2) For the antigens of ELI-Neuro-Test 12

$$R_n = IR_n - AIR_N \quad (15)$$

The obtained values allowed for the construction of the individual immunoreactivity profiles. Calculations were performed using specialized software provided with the test systems. According to the manufacturer, reference values of the AIR range from -25% to -5%. If the AIR of the serum sample was equal to or exceeded -5%, it was considered as polyclonal activation of the patient's immune system. If the AIR of the serum sample was below -25%, it was considered as polyclonal immunosuppression [33]. Shifts in relative immunoreactivity with any antigens above +10% or below -15% (for antigens in the ELI-Viscero-Test-24) and above +10% or below -20% (for antigens in the ELI-Neuro-Test-12) from the AIR were considered abnormal peaks, indicating existing or developing disturbances in the interaction of the immune system of the patient with the nervous system and different internal organs (Table 5 and Table 6).

Table 5 – Marker autoantibodies of the ELI-Viscero-Test-24 and the interpretation of abnormal peaks in relative immunoreactivity according to the methodological recommendations for the method's application [32]. AAb – autoantibodies

AAb	Interpretation of the abnormal levels of the AAb in the individual immunoreactivity profile
AAb to ds-DNA	1) Active infectious process (more often viral one) 2) Rarely – paraneoplastic reaction 3) Rarely – sign of systemic autoimmune process

Continuation of table 5

AAb	Interpretation of the abnormal levels of the AAb in the individual immunoreactivity profile
AAb to β2-GP I	1)Sign of antiphospholipid syndrome (more often associated with infectious processes) 2) Rarely – sign of systemic autoimmune process 3) Rarely – paraneoplastic reaction
AAb to Fc-Ig	1) Sign of chronic inflammatory process (any localization) 2) Rarely – sign of systemic autoimmune process 3) Rarely – paraneoplastic reaction
AAb to Collagen	Sign of scarring of any localization
AAb to CoM-02	Signs of pathological changes in the myocardium (of any nature)
AAb to β1-AR	1) Sign of changes in the heart's autonomic nervous system (often accompanied by arrhythmias) 2) Rarely – cardiomyopathy
AAb to TrM-03	Sign of thrombocytopathy (may be accompanied by increased or decreased blood coagulation)
AAb to ANCA	Sign of vascular inflammation (vasculitis, vasculopathies)
AAb to LuM-S	Sign of changes in the lungs (acute and chronic infectious/inflammatory diseases; tumor process)
AAb to KiM-S	Sign of changes in the kidneys (acute and chronic inflammatory kidney diseases; urolithiasis; tumor process)
AAb to HeS, HMMP	1) Sign of changes in the liver (acute and chronic infectious and inflammatory diseases; toxic changes; autoimmune or tumor process) 2) Inflammation of the gallbladder
AAb to GaM-02	Sign of changes in the stomach wall (gastritis, ulcer, tumor process)
AAb to ItM-07	Sign of changes in the small intestine wall (enteritis)
AAb to ScM	Sign of changes in the large intestine wall (colitis)
AAb to Insulin	Sign of changes in the pancreas (pancreatitis; tumor process) with involvement of the islet apparatus (risk group for type 1 diabetes development)
AAb to Ins-R	Sign of changes in the insulin receptor apparatus (in type 2 diabetes, metabolic syndrome; insulin resistance)
AAb to TG	Thyroiditis with anticipated thyroid hypofunction in the future
AAb to TSH-R	Thyroiditis with anticipated thyroid hyperfunction in the future (possible development of diffuse toxic goiter; Graves disease)
AAb to AdrM-D/C	Sign of changes in the adrenal glands; observed in prolonged stress, infectious, tumor, and autoimmune adrenal lesions

Continuation of table 5

AAb	Interpretation of the abnormal levels of the AAb in the individual immunoreactivity profile
AAb to Spr-06	1) In men: sign of changes in the prostate (prostatitis; tumor process) 2) In women: sign of endometritis or inflammatory process in other pelvic organs
AAb to S100	1) Affective changes (phobias, depression, aggressiveness) 2) Typically, antibodies to protein S100 are a consequence of papillomavirus infection (molecular mimicry) 3) Frequent cause of pregnancy complications and disturbances in the development of fetal central nervous system
AAb to GFAP	Signs of gliosis (e. g. after mechanical brain injury, cerebral ischemia). Gliosis leads to disturbances in brain electrical activity, up to seizure syndrome
AAb to MBP	1) Sign of neuritis, radiculitis, disc herniation 2) Less commonly – sign of the flare in demyelinating diseases

Table 6 – Marker autoantibodies of the ELI-Neuro-Test-12 and the interpretation of abnormal peaks in relative immunoreactivity according to the methodological recommendations for the method's application [32]. AAb – autoantibodies

AAb	Interpretation of the abnormal levels of the AAb in the individual immunoreactivity profile
AAb to NF200	Typical for degenerative changes in nerve fibers (axonopathies) of traumatic or other origins
AAb to GFAP	Typical for reactive astrogliosis; observed after traumatic brain injury, neuroinfection, general anesthesia, brain ischemia, and alcoholism
AAb to S100	1) Typical for changes in serotonergic structures (affective changes) 2) Marker of human papillomavirus past infection (molecular mimicry)
AAb to MBP	Typical for anti-myelin processes; however most often observed in traumatic or ischemic injuries of nerve bundles (e. g. radiculitis), less frequently in demyelinating diseases
AAb to V-Ca-Chanel	Typical sign of amyotrophic lateral sclerosis, cerebellar ataxia, Lambert-Eaton syndrome, and other neuromuscular junction disorders
AAb to N-Ach-R	Possible sign of myasthenic syndromes, cognitive impairments including Alzheimer’s disease
AAb to Glu-R	Sign of impaired regulation of the balance between excitation and inhibition processes in the central nervous system, often against the background of brain ischemia; can be accompanied by seizures and cognitive impairments
AAb to GABA-R	
AAb to DOPA-R	Sign of cognitive function disorders, volition and motivation; typical for schizophrenia, less often precedes the manifestation of Parkinson’s disease
AAb to 5HT-R	Sign of affective/motivation disorders, such as bipolar disorder

Continuation of table 6

AAb	Interpretation of the abnormal levels of the AAb in the individual immunoreactivity profile
AAb to μ-Opioid-R	Observed in bipolar disorder, anorexia, bulimia, and other eating disorders, substance use disorder
AAb to β-Endorphin.	

2.2.8 Gas chromatography-mass spectrometry of microbial markers in the blood

The characteristics of the microbiome composition were assessed using the gas chromatography-mass spectrometry of microbial markers in venous blood according to the original technique by Osipov G.A., certified by Roszdravnadzor (permission for the use of new medical technology FS No. 2010/038 dated 24/02/2010, issued by the Federal Service for Supervision of Healthcare and Social Development). This method is based on determining species-specific higher fatty acids, aldehydes, and alcohols from the cell walls of microorganisms in human blood, where they appear during the natural phagocytosis of the dead microorganisms. To determine the microbial markers, 6 ml of blood was obtained from the cubital vein into vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA). The time interval between blood collection and centrifugation did not exceed 30 minutes. Blood plasma was separated by centrifugation at 3,000 rpm for 10 minutes. The analysis implies direct extraction of higher fatty acids, aldehydes, and alcohols from the sample with a chemical procedure, the separation of these molecules on a high-resolution capillary chromatograph, and dynamic analysis of their composition using a mass spectrometer.

Blood analysis was performed on an "Agilent 7890" gas chromatograph with a "Agilent 5975C" mass-selective detector ("Agilent Technologies," USA). Chromatographic separation of the sample was performed on a capillary column with an HP-5ms methyl silicone grafted phase ("Agilent Technologies," USA) 25 meters long and with an internal diameter of 0.25 mm. The mass spectrometer was equipped with a computer and corresponding software for automatic analysis and data processing,

allowing for the reconstruction of microbial communities based on initial data (concentration of microbial markers in the blood). The results of such reconstruction are presented as the number of microorganism cells equivalent to the concentration of markers per ml of blood. According to G.A. Osipov, concentration of microbial markers in the blood represents the part of microorganism cells, information about which are present in the blood – this is 3.3 * 10^9 cells/ml (for the norm), which is lower than, for example, than the concentration of the microorganism cells in the mucosal layer of the small intestine (7.6 * 10^10 cells/g) [29]. Part of the information about the mucosa-associated microbiota of the intestine is considered to be lost in the analysis of microbial markers in the blood compared to measurements of microbiota directly in intestinal biopsy samples due to the removal of some dead microorganisms into faeces and the utilization of some microbial fatty acids for the renewal of host cells [29]. Additionally, the analysis of microbial markers in the blood allows for determining the total microbial load, endotoxin (LPS) and plasmalogen levels.

2.2.9 Methods of statistical analysis

Statistical processing of the obtained data was performed using the statistical analysis software packages Statistica (Software Inc., USA, version 10.0) and Jamovi (The jamovi project, Australia, version 2.3.28). The assessment of normality in the samples was conducted using the Shapiro-Wilk test. Qualitative data were expressed as frequencies (%). For the description of quantitative data the mean and standard deviation (M ±σ) were provided for normally distributed data; the median and interquartile range (Me [25;75]) were calculated for non-normally distributed data. Since the data within the study groups did not follow a normal distribution, the Kruskal-Wallis test with the Bonferroni-Dunn post hoc test was used to assess intergroup differences for continuous variables among three unrelated groups. For qualitative data, Pearson's $\chi 2$ (chi-square) test with Yates' correction and the z-test were used. The analysis of the relationship between variables was carried out by calculating Spearman's correlation coefficient. The critical significance

level of the null statistical hypothesis "p" in the study was set at 0.01 for Spearman's correlation coefficients and 0.05 for other statistical tests, as a result of the necessity to identify the most significant correlation coefficients.

CHAPTER 3. STUDY RESULTS

3.1 Some clinical characteristics of the examined individuals

A total of 152 individuals were examined, among whom were 32 (21.1%) men and 120 (78.9%) women. The average age of the examined individuals was 37.03±10.09 years old, with the youngest being 18 years old and the oldest – 60 years old.

The examined individuals belonged to three groups: patients with ME/CFS and symptom onset not related to COVID-19 – 54 people (35.6%), denoted as "ME/CFS" in tables; patients who met the WHO clinical case definition for PCS – 46 people (30.3%), denoted as "PCS" in the tables; and apparently healthy control individuals without symptoms of ME/CFS and PCS – 52 people (34.2%), denoted as "HC" in the tables. The groups were comparable in terms of age, sex, and BMI. The median duration of the illness in the ME/CFS not related to COVID-19 group and in the PCS group differed significantly ($p < 0.001$): in the ME/CFS group it was 7.00 [4.01; 13.00] years, while in the PCS group it was 1.46 [0.94; 1.76] years (Table 7).

Table 7 – Clinical and demographic characteristics of the examined individuals, Me [25;75]

	ME/CFS	PCS	HC	p*	p** (CFS/ HC)	p** (PCS/ HC)	p** (CFS/ PCS)
n	54	46	52	–	–	–	–
Gender, male/female (n/n) (%/%)	11/43 (20.4/79.6)	8/38 (17.4/82.6)	13/39 (25.0/75.0)	0.646 ***	–	–	–
Age, years	37.00 [30.75; 45.25]	35.50 [30.75; 42.50]	35.50 [27.00; 44.75]	0.647	–	–	–
BMI, kg/m2	22.48 [19.24; 27.56]	23.31 [20.80; 27.58]	21.60 [19.76; 25.29]	0.213	–	–	–

Continuation of table 7

	ME/CFS	PCS	HC	p*	p** (CFS/ HC)	p** (PCS/ HC)	p** (CFS/ PCS)
Disease duration, years	7.00 [4.01; 13.00]	1.46 [0.90; 1.77]	–	< .001	–	–	–
MFI-20 General Fatigue, score	19.00 [18.00; 20.00]	17.00 [14.75; 19.00]	7.00 [6.00; 8.00]	< .001	< .001	< .001	0.014
MFI-20 Physical Fatigue, score	17.00 [15.00; 19.25]	15.00 [13.00; 18.00]	6.00 [5.00; 8.00]	< .001	< .001	< .001	0.085
MFI-20 Reduced Activity, score	18.00 [16.00; 19.25]	15.50 [12.00; 18.25]	7.50 [5.00; 10.75]	< .001	< .001	< .001	0.009
MFI-20 Reduced Motivation, score	13.5 [11.00; 15.00]	12.00 [8.75; 14.25]	7.00 [5.25; 9.00]	< .001	< .001	< .001	0.046
MFI-20 Mental Fatigue, score	16.00 [13.00; 18.00]	13.50 [10.00; 16.00]	6.00 [5.00; 9.75]	< .001	< .001	< .001	0.012
HADS depression, score	10.00 [7.75; 13.00]	8.00 [5.00; 11.00]	3.00 [1.00; 4.00]	< .001	< .001	< .001	0.042
HADS anxiety, score	10.00 [6.00; 12.00]	10.00 [6.00; 13.00]	5.00 [2.00; 7.00]	< .001	0.001	0 .001	0.880
Physical activity level, MET-min/ week	1857.00 [590.25; 2841.00]	1506.00 [1039.50; 2814.00]	3027.00 [1606.50; 5399.00]	0.007	0.013	0.013	0.965

* – significance of differences in multiple comparisons (Kruskal-Wallis test)

** – significance of differences in pairwise comparisons (Dunn's test with Holm correction)

*** – $\chi 2$ test with Yates' correction

Note – BMI – body mass index; HADS – Hospital Anxiety and Depression Scale; HC – healthy controls; ME/CFS – myalgic encephalomyelitis/chronic fatigue syndrome; MET – Metabolic Equivalent of Task; MFI-20 – Multidimensional Fatigue Inventory; PCS – post-COVID-19 syndrome

Patients with ME/CFS and PCS had significantly higher scores across all domains of the MFI-20 scale compared to healthy individuals. All healthy controls scored <12 points in each domain of the MFI-20 scale that indicates the absence of fatigue. The groups of ME/CFS and PCS also significantly differed from each other: across all subscales of the MFI-20, except for the physical fatigue subscale, ME/CFS patients had higher scores.

In line with the comparable levels of physical fatigue in both patient groups, the level of physical activity, according to the IPAQ questionnaire, did not differ between the ME/CFS and PCS groups, but was significantly lower than in the control group.

Median levels of depression and anxiety in the ME/CFS and PCS groups were higher than in healthy controls. The severity of depression was somewhat higher in the ME/CFS group than in the PCS group. However, it should be noted that the values obtained on the anxiety and depression subscales of HADS in both patient groups corresponded to the subclinical levels of affective problems.

Correlation analysis of quantitative clinical and demographic features, which was conducted separately in the three study groups, showed that in the control group the severity of depression and anxiety did not correlate with each other. Positive correlations were found in this group between the level of depression and four domains of fatigue in the MFI-20 questionnaire (general fatigue, physical fatigue, reduced activity, mental fatigue) excluding the reduced motivation domain. Other positive correlations in this group were the following: between the anxiety and general fatigue; anxiety and physical fatigue; mental fatigue and the other fatigue domains (Table 8). Meanwhile, in the ME/CFS and PCS groups the severity of depression and anxiety correlated with each other, no statistically significant correlations were found between depression or anxiety and fatigue, and mental fatigue did not correlate with general and physical fatigue (Tables 9 and 10). It is also worth noting the absence of significant correlations between depression/anxiety/fatigue and age/BMI in both patient groups and in the control group.

Table 8 – Correlation coefficients between the studied clinical and demographic characteristics in healthy controls

	Age	BMI	HADS-D	HADS-A	GF	PhF	RA	RM	MF
Age		.252	.061	.042	.006	-.196	-.091	-.216	.030
BMI	.252		.021	.095	.023	.003	.102	.136	.036
HADS-D	.061	.021		.244	.496*	.373*	.561*	.314	.414*
HADS-A	.042	.095	.244		.612*	.374*	.235	.261	.334
GF	.006	.023	.496*	.612*		.595*	.612*	.568*	.522*
PF	-.196	.003	.373*	.374*	.595*		.504*	.563*	.416*
RA	-.091	.102	.561*	.235	.612*	.504*		.729*	.567*
RM	-.216	.136	.314	.261	.568*	.563*	.729*		.385*
MF	.030	.036	.414*	.334	.522*	.416*	.567*	.385*	

* – correlation coefficients with $p<0.01$;

Note – BMI – body mass index; GF – general fatigue; HADS-A – anxiety subscale of the Hospital Anxiety and Depression Scale; HADS-D – depression subscale of the Hospital Anxiety and Depression Scale; MF – mental fatigue; PhF – physical fatigue; RA – reduced activity; RM – reduced motivation

Table 9 – Correlation coefficients between the studied clinical and demographic characteristics in the group of patients with myalgic encephalomyelitis/chronic fatigue syndrome not related to COVID-19

	Age	BMI	HADS-D	HADS-A	GF	PhF	RA	RM	MF	DD
Age		.200	-.025	.020	-.015	-.053	.085	-.114	-.090	.261
BMI	.200		-.224	.103	.073	-.024	.036	-.037	-.145	.174
HADS-D	-.025	-.224		.379*	.270	.180	.285	.259	.084	.019
HADS-A	.020	.103	.379*		.047	-.132	.033	.098	.126	.087
GF	-.015	.073	.270	.047		.664*	.503*	.140	.054	.140
PF	-.053	-.024	.180	-.132	.664*		.398*	.159	.031	.244
RA	.085	.036	.285	.033	.503*	.398*		.411*	.212	.162
RM	-.114	-.037	.259	.098	.140	.159	.411*		.447*	.101
MF	-.090	-.145	.084	.126	.054	.031	.212	.447*		.019
DD	.261	.174	.019	.087	.140	.244	.162	.101	.019	

Continuation of table 9

*– correlation coefficients with p<0.01;

Note – BMI – body mass index; DD – disease duration; GF – general fatigue; HADS-A – anxiety subscale of the Hospital Anxiety and Depression Scale; HADS-D – depression subscale of the Hospital Anxiety and Depression Scale; MF – mental fatigue; PhF – physical fatigue; RA – reduced activity; RM – reduced motivation

Table 10 – Correlation coefficients between the studied clinical and demographic characteristics in the group of patients with post-COVID-19 syndrome

	Age	BMI	HADS-D	HADS-A	GF	PhF	RA	RM	MF	DD
Age		.163	-.052	-.145	-.182	-.157	-.203	-.131	-.165	-.085
BMI	.163		-.139	.045	-.322	-.235	-.229	-.169	.044	.021
HADS-D	-.052	-.139		.397*	.313	.267	.196	.373	.157	.134
HADS-A	-.145	.045	.397*		.128	.049	.107	.206	.221	.113
GF	-.182	-.322	.313	.128		.753*	.463*	.380	.189	.020
PF	-.157	-.235	.267	.049	.753*		.541*	.358	.316	.102
RA	-.203	-.229	.196	.107	.463*	.541*		.638*	.457*	.117
RM	-.131	-.169	.373	.206	.380	.358	.638*		.438*	.185
MF	-.165	.044	.157	.221	.189	.316	.457*	.438*		.125
DD	-.085	.021	.134	.113	.020	.102	.117	.185	.125	

*– correlation coefficients, p<0.01;

Note – BMI – body mass index; DD – disease duration; GF – general fatigue; HADS-A – anxiety subscale of the Hospital Anxiety and Depression Scale; HADS-D – depression subscale of the Hospital Anxiety and Depression Scale; MF – mental fatigue; PhF – physical fatigue; RA – reduced activity; RM – reduced motivation

When evaluating prevalence of ME/CFS, according to the clinical diagnostic criteria for this condition, it was found that 21 out of 46 (45.7%) patients with PCS met all three sets of ME/CFS diagnostic criteria. The results for each set of diagnostic criteria separately are presented in Table 11.

Table 11 – Prevalence of myalgic encephalomyelitis/chronic fatigue syndrome, according to three sets of clinical diagnostic criteria for this condition among patients with chronic fatigue associated with COVID-19

ME/CFS diagnostic criteria	CDC, 1994	CCC, 2003	IOM/NAM, 2015
Number of patients with PCS who met ME/CFS diagnostic criteria	30/46 (65,2%)	21/46 (45,7%)	25/46 (54,3%)

Note – CCC, 2003 – Canadian Consensus Criteria, 2003; CDC, 1994 – modified Fukuda/CDC criteria, 1994; IOM/NAM, 2015 – Institute of Medicine/National Academy of Medicine criteria, 2015; ME/CFS – myalgic encephalomyelitis/chronic fatigue syndrome; PCS – post-COVID-19 syndrome

Prevalence of ME/CFS among patients with chronic fatigue associated with PCS was the highest (65.2%) according to the CDC, 1994, while the CCC, 2003 appeared to be the most selective criteria as only 45.7% of PCS patients met these criteria.

3.2 Heart rhythm, systolic and diastolic blood pressure variability

A study of HRV, SBPV, DBPV involved 34 patients with ME/CFS, 29 patients with PCS, and 32 healthy individuals.

The results indicated that healthy individuals had similar HRV values across all three analysed frequency bands (HF, LF, and VLF), which is traditionally interpreted as a balanced autonomic regulation with equilibrium between parasympathetic (HF), sympathetic (LF), and subcortical (VLF) regulatory circuits (Table 12). The index reflecting the sympathovagal balance (LF/HF) corresponded to mild vagotonia [149].

In patients with ME/CFS compared to healthy individuals, as shown in Table 12, there was a significant reduction in VLF, LF, and HF of HRV, $p \leq 0.001$ which led to a significant decrease in the total power (TP, p=0.001). The most significant reduction (by 78.3%) was observed in the HF band, which is considered the vagal part of the spectrum, contributing to a shift in the sympathovagal balance towards sympathetic dominance, as indicated by the LF/HF index. However, sympathetic dominance was not pronounced, as

the LF/HF index in the ME/CFS group was not statistically different from that of the healthy controls.

In patients with PCS, a similar pattern of HRV changes was observed (a significant reduction in TP, p=0.006 due to a decrease in HRV power in the LF band, p=0.027, and in the HF band, p=0.012. At the same time power reduction was less pronounced in PCS than in the ME/CFS group (namely, in the vagal part of the spectrum it was 52.4%,) and in the VLF band it was not significant. Similar to the ME/CFS group, the sympathovagal balance index (LF/HF) was not significantly different between the PCS group and healthy controls.

There were no significant differences between the ME/CFS and PCS groups regarding any HRV indices (p>0.05).

Table 12 – Heart rhythm variability characteristics in patients and healthy controls, Me [25;75]

	ME/CFS	PCS	HC	p*	p** (CFS/ HC)	p** (PCS/ HC)	p** (CFS/ PCS)
n	34	29	32				
TP (ms^2)	852.45 [469.03; 1912.88]	1358.10 [834.15; 2687.15]	2709.05 [1483.38; 4454.36]	< .001	< .001	0.006	0.164
LF (ms^2)	367.10 [137.45; 635.13]	429.20 [279.65; 867.70]	759.30 [477.33; 2480.68]	< .001	< .001	0.027	0.191
HF (ms^2)	152.90 [91.93; 284.78]	335.00 [102.45; 717.10]	703.70 [311.00; 1394.80]	< .001	< .001	0.012	0.081
VLF (ms^2)	256.15 [192.03; 619.38]	469.20 [237.10; 814.60]	727.50 [431.85; 1014.80]	< .001	< .001	0.054	0.092
LF/HF	2.14 [1.20; 4.13]	1.46 [1.02; 4.01]	1.25 [0.71; 2.31]	0.08	–	–	–

* – significance of differences in multiple comparisons (Kruskal-Wallis test)

** – significance of differences in pairwise comparisons (Dunn's test with Holm correction)

Note – HC – healthy controls; HF – high frequency; LF – low frequency; ME/CFS – myalgic encephalomyelitis/chronic fatigue syndrome; PCS – post-COVID-19 syndrome; TP – total power; VLF – very low frequency

When analysing relationship between HRV indices and clinical features of the participants (presented in section 3.1), as well as with the level of their physical activity (IPAQ questionnaire score), no statistically significant correlations in the healthy group were found. A moderate negative correlation between age and LF HRV was observed in the PCS group, and a moderate negative correlation between BMI and LF HRV – in the ME/CFS group (Table 13).

Table 13 – Correlation coefficients between clinical characteristics and heart rate variability indices during breathing with spontaneous breathing rate in the study groups. Only correlation coefficients significant at $p<0.01$ are presented in the table

	Age	BMI	HADS-D	HADS-A	GF	PF	RA	RM	MF	IPAQ	DD
HC											
ME/CFS											
TP_{HR}											-.448
LF_{HR}		-.462									-.481
PCS											
LF_{HR}	-.477										

Note – BMI – body mass index; GF – general fatigue; HADS-A – anxiety subscale of The Hospital Anxiety and Depression Scale; HADS-D – depression subscale of The Hospital Anxiety and Depression Scale; HC – healthy controls; HR – heart rate; IPAQ – International Physical Activity Questionnaire; LF – low frequency; ME/CFS – myalgic encephalomyelitis/chronic fatigue syndrome; MF – mental fatigue; PCS – post-COVID-19 syndrome; RA – reduced activity; RM – reduced motivation; TP – total power

However, the most important findings were the moderate negative correlations in the ME/CFS group between disease duration and TP HRV, as well as between disease duration and LF HRV (Table 13). To assess the contribution of sympathetic and parasympathetic autonomic nervous system activity to these correlations, which may overlap within the LF HRV range during breathing with spontaneous breathing rate, a study of HRV during paced breathing was conducted (see section 3.3).

As shown in Table 14, no significant differences were found between SBPV and DBPV indices during spontaneous breathing in the study groups ($p>0.05$).

Table 14 – Characteristics of systolic blood pressure variability and diastolic blood pressure variability in the study groups, Me [25;75]

	ME/CFS	PCS	HC	p*
n	34	29	32	
SBPV				
TP ($mmHg^2$)	50.15 [23.38; 66.78]	42.20 [26.85; 65.80]	41.35 [22.15; 63.15]	0.763
LF ($mmHg^2$)	13.95 [6.45; 21.85]	13.50 [8.80; 23.30]	9.50 [6.30; 19.68]	0.550
HF ($mmHg^2$)	7.80 [4.38; 16.73]	8.40 [3.60; 11.45]	8.50 [4.48; 14.28]	0.987
VLF ($mmHg^2$)	20.30 [10.08; 31.83]	17.50 [7.80; 29.60]	12.90 [5.63; 33.60]	0.388
LF/HF	1.63 [0.77; 2.43]	1.79 [1.20; 2.92]	1.11 [0.65; 2.85]	0.314
DBPV				
TP ($mmHg^2$)	12.95 [7.38; 25.28]	12.80 [8.05; 25.35]	11.75 [7.60; 20.53]	0.738
LF ($mmHg^2$)	5.15 [2.93; 7.98]	5.30 [3.15; 8.70]	4.45 [2.25; 7.90]	0.882
HF ($mmHg^2$)	1.50 [0.90; 2.15]	1.10 [0.70; 2.50]	1.45 [0.70; 2.68]	0.945
VLF ($mmHg^2$)	5.75 [3.03; 12.15]	6.20 [2.80; 13.15]	4.45 [2.83; 13.48]	0.882
LF/HF	3.39 [1.84; 5.53]	3.91 [2.35; 6.83]	3.02 [2.17; 5.74]	0.885

* – significance of differences in multiple comparisons (Kruskal-Wallis test)
Note – DBPV – diastolic blood pressure variability; HC – healthy controls; HF – high frequency; LF – low frequency; ME/CFS – myalgic encephalomyelitis/chronic fatigue syndrome; SBPV – systolic blood pressure variability; PCS – post-COVID-19 syndrome; TP – total power; VLF – very low frequency

3.3 Effect of paced breathing (12 and 6 breaths/min) on heart rate and blood pressure variability

Paced breathing tests with breathing rate of 12 and 6 breathing cycles per minute (12BR and 6BR) were carried out on 34 patients with ME/CFS, 29 patients with PCS, and 32 healthy controls.

Significant changes of HRV and BPV indices were observed during paced breathing at 12 breaths/min (Tables 15, 16 and 17).

Table 15 – Dynamics of heart rate variability, systolic blood pressure variability, diastolic blood pressure variability, and respiratory rate variability in healthy controls during paced breathing at 12 breaths/min, Me [25;75]

	HC, spontaneous breathing rate	HC, 12 breaths/min	p
n	32	32	
HRV			
TP (ms^2)	2709.05 [1483.38; 4454.36]	1682.35 [1120.95; 3607.90]	0.012
LF (ms^2)	759.30 [477.33; 2480.68]	402.7 [237.35; 908.75]	< .001
HF (ms^2)	703.70 [311.00; 1394.80]	920.45 [439.75; 2023.70]	0.019
SBPV			
TP ($mmHg^2$)	41.35 [22.15; 63.15]	33.00 [18.85; 49.18]	0.531
LF ($mmHg^2$)	9.50 [6.30; 19.68]	5.75 [3.70; 10.85]	0.002
HF ($mmHg^2$)	8.50 [4.48; 14.28]	13.80 [8.38; 22.50]	< .001
DBPV			
TP ($mmHg^2$)	11.75 [7.60; 20.53]	9.35 [4.63; 14.55]	0.224
LF ($mmHg^2$)	4.45 [2.25; 7.90]	2.70 [1.73; 4.70]	0.005
HF ($mmHg^2$)	1.45 [0.70; 2.68]	1.45 [0.93; 3.15]	0.057
Respiratory rate variability			
TP (l/min^2)	576.10 [418.93; 809.75]	1303.65 [683.95; 2337.68]	< .001
LF (l/min^2)	39.45 [13.33; 105.73]	45.20 [22.50; 75.28]	0.708
HF (l/min^2)	449.85 [309.00; 569.55]	1189.25 [645.40; 2238,68]	< .001

Note – DBPV – diastolic blood pressure variability; HC – healthy controls; HF – high frequency; HRV – heart rate variability; LF – low frequency; SBPV – systolic blood pressure variability; TP – total power

In all study groups, a decrease of HRV power in the LF band and an increase of HRV power in the HF band were observed. It is important to note that during the 12BR test all study groups showed significant changes in respiratory rate variability characterized by a marked increase of HF power with the formation of a distinct peak corresponding to the respiratory rate. This confirms that the shift in HRV power to HF band is due to respiratory modulation of heart rate (mediated by the vagus nerve).

Table 16 – Dynamics of heart rate variability, systolic blood pressure variability, diastolic blood pressure variability, and respiratory rate variability in patients with myalgic encephalomyelitis/chronic fatigue syndrome during paced breathing at 12 breaths/min, Me [25;75]

	ME/CFS, spontaneous breathing rate	ME/CFS, 12BR	p
n	34	34	
HRV			
TP (ms^2)	852.45 [469.03; 1912.88]	998.90 [573.93; 1729.15]	0.288
LF (ms^2)	367.10 [137.45; 635.13]	226.55 [140.28; 399.68]	0.010
HF (ms^2)	152.90 [91.93; 284.78]	399.15 [162.38; 529.75]	< .001
SBPV			
TP ($mmHg^2$)	50.15 [23.38; 66.78]	49.30 [24.85; 65.45]	0.521
LF ($mmHg^2$)	13.95 [6.45; 21.85]	11.90 [5.93; 21.33]	0.156
HF ($mmHg^2$)	7.80 [4.38; 16.73]	14.74 [6.08; 32.05]	< .001
DBPV			
TP ($mmHg^2$)	12.95 [7.38; 25.28]	12.35 [7.48; 22.78]	0.388
LF ($mmHg^2$)	5.15 [2.93; 7.98]	4.10 [2.13; 6.90]	0.026
HF ($mmHg^2$)	1.50 [0.90; 2.15]	3.35 [1.45; 5.83]	< .001
Respiratory rate variability			
TP (l/min^2)	703.00 [523.63; 1122.15]	1763.45 [1247.75; 2613.40]	< .001
LF (l/min^2)	32.65 [11.20; 101.05]	51.10 [34.28; 81.90]	0.804
HF (l/min^2)	560.85 [421.90; 769.85]	1692.00 [1154.40; 2413.70]	< .001

Note – DBPV – diastolic blood pressure variability; HF – high frequency; HRV – heart rate variability; LF – low frequency; ME/CFS – myalgic encephalomyelitis/chronic fatigue syndrome; SBPV – systolic blood pressure variability; TP – total power

A decrease in HRV TP was found only in healthy controls, mainly due to the exclusion of VLF power, which cannot be assessed during the short signal recording during the test (2 minutes). SBPV TP and DBPV TP did not change in any group; however, dynamics similar to HRV (decrease in LF power, increase in HF power) was characteristic of SBPV and DBPV in healthy controls reflecting the normal involvement of the baroreflex in the formation of BPV. Meanwhile, there was no significant decrease in LF SBPV power in the ME/CFS and PCS groups, and no significant decrease in LF DBPV in the PCS group.

Table 17 – Dynamics of heart rate variability, systolic blood pressure variability, diastolic blood pressure variability, and respiratory rate variability in patients with post-COVID-19 syndrome during paced breathing at 12 breaths/min, Me [25;75]

	PCS, spontaneous breathing rate	PCS, 12 breaths/min	p
n	29	29	
HRV			
TP (ms^2)	1358.10 [834.15; 2687.15]	1506.80 [948.90; 2410.35]	0.370
LF (ms^2)	429.20 [279.65; 867.70]	272.60 [198.90; 329.50]	< .001
HF (ms^2)	335.00 [102.45; 717.10]	564.50 [253.85; 1095.35]	0.004
SBPV			
TP ($mmHg^2$)	42.20 [26.85; 65.80]	42.40 [26.00; 97.60]	0.673
LF ($mmHg^2$)	13.50 [8.80; 23.30]	9.60 [4.75; 23.10]	0.364
HF ($mmHg^2$)	8.40 [3.60; 11.45]	12.80 [6.40; 35.80]	< .001
DBPV			
TP ($mmHg^2$)	12.80 [8.05; 25.35]	13.90 [7.15; 20.60]	0.905
LF ($mmHg^2$)	5.30 [3.15; 8.70]	4.10 [1.85; 8.15]	0.218
HF ($mmHg^2$)	1.10 [0.70; 2.50]	2.60 [1.00; 6.40]	0.001
Respiratory rate variability			
TP (l/min^2)	722.90 [480.45; 993.45]	1647.10 [1020.70; 3323.25]	< .001
LF (l/min^2)	20.60 [7.20; 182.80]	57.50 [27.90; 112.10]	0.112
HF (l/min^2)	481.80 [350.90; 951.95]	1591.30 [988.10; 3197.25]	< .001
Note – DBPV – diastolic blood pressure variability; HF – high frequency; HRV – heart rate variability; LF – low frequency; SBPV – systolic blood pressure variability; PCS – post-COVID-19 syndrome; TP – total power			

During the 12BR test the previously identified differences in the main indices of the HRV between patients with ME/CFS and healthy controls persisted; however, in the PCS patient group the increase in HRV HF power led to the disappearance of differences in HRV HF and HRV TP values between groups of patients with PCS and healthy controls. At the same time, during the 12BR test differences in SBPV and DBPV emerged between the patient and healthy groups: namely, ME/CFS and PCS patients were characterized by higher SBPV power in the LF band (Table 18). In the ME/CFS group higher DBPV power in the HF band was also observed. There were no differences between the ME/CFS and PCS patient groups.

Table 18 – Comparison of heart rate variability, systolic blood pressure variability, and diastolic blood pressure variability indices between study groups during paced breathing at 12 breaths/min, Me [25;75]

	ME/CFS	PCS	HC	p*	p** (CFS/ HC)	p** (PCS/ HC)	p** (CFS/ PCS)
n	34	29	32				
HRV							
TP (ms^2)	998.90 [573.93; 1729.15]	1506.80 [948.90; 2410.35]	1682.35 [1120.95; 3607.90]	0.002	0.001	0.128	0.128
LF (ms^2)	226.55 [140.28; 399.68]	272.60 [198.90; 329.50]	402.7 [237.35; 908.75]	0.005	0.005	0.047	0.434
HF (ms^2)	399.15 [162.38; 529.75]	564.50 [253.85; 1095.35]	920.45 [439.75; 2023.70]	0.001	<0.001	0.100	0.100
SBPV							
TP ($mmHg^2$)	49.30 [24.85; 65.45]	42.40 [26.00; 97.60]	33.00 [18.85; 49.18]	0.223	–	–	–
LF ($mmHg^2$)	11.90 [5.93; 21.33]	9.60 [4.75; 23.10]	5.75 [3.70; 10.85]	0.018	0.027	0.046	0.817
HF ($mmHg^2$)	14.74 [6.08; 32.05]	12.80 [6.40; 35.80]	13.80 [8.38; 22.50]	0.972	–	–	–
DBPV							
TP ($mmHg^2$)	12.35 [7.48; 22.78]	13.90 [7.15; 20.60]	9.35 [4.63; 14.55]	0.071	–	–	–
LF ($mmHg^2$)	4.10 [2.13; 6.90]	4.10 [1.85; 8.15]	2.70 [1.73; 4.70]	0.158	–	–	–
HF ($mmHg^2$)	3.35 [1.45; 5.83]	2.60 [1.00; 6.40]	1.45 [0.93; 3.15]	0.041	0.039	0.190	0.470

* – significance of differences in multiple comparisons (Kruskal-Wallis test)
** – significance of differences in pairwise comparisons (Dunn's test with Holm correction)
Note – DBPV – diastolic blood pressure variability; ME/CFS – myalgic encephalomyelitis/chronic fatigue syndrome; HC – healthy controls; HF – high frequency; HRV – heart rate variability; LF – low frequency; SBPV – systolic blood pressure variability; PCS – post-COVID-19 syndrome; TP – total power

Correlation analysis revealed that there were correlations between certain indices of HRV, SBPV, and DBPV during the 12BR test and the clinical features of the study participants in the patient groups, but not in healthy controls (Table 19). When comparing

the correlations found during the 12BR test with the correlations identified previously during spontaneous breathing, it can be noted that the correlations of age (in the case of PCS) and BMI (in the case of ME/CFS) with variability indices were present in both breathing modes. However, shift of the parasympathetic component of variability to the HF band, which occurs during the 12BR test, results in the disappearance of negative associations between age/BMI and HRV LF power and emergence of the negative associations between age/BMI and BPV HF power.

Table 19 – Correlation coefficients between clinical characteristics and heart rate/blood pressure variability indices in the study groups during paced breathing at 12 breaths/min. Only correlation coefficients significant at $p<0.01$ are presented in the table

	Age	BMI	HADS-D	HADS-A	GF	PhF	RA	RM	MF	IPAQ
HC										
–										
ME/CFS										
HF_{SBP}		-.442								
TP_{HR}					-.444					
HF_{HR}					-.522					
PCS										
HF_{DBP}	-.497									
LF_{SBP}						.492				.520

Note – BMI – body mass index; DBP – diastolic blood pressure; GF – general fatigue; HADS-A – anxiety subscale of The Hospital Anxiety and Depression Scale; HADS-D – depression subscale of The Hospital Anxiety and Depression Scale; HC – healthy controls; HF – high frequency; HR – heart rate; IPAQ – International Physical Activity Questionnaire; LF – low frequency; ME/CFS – myalgic encephalomyelitis/chronic fatigue syndrome; MF – mental fatigue; PCS – post-COVID-19 syndrome; PhF – physical fatigue; RA – reduced activity; RM – reduced motivation; SBP – systolic blood pressure; TP – total power

In ME/CFS and PCS groups correlations were found between variability indices and fatigue severity (general fatigue and physical fatigue), but not with depression/anxiety. It is noteworthy that in the ME/CFS group such negative correlation was found for the HRV index that characterizes parasympathetic nervous system activity (HRV HF power), while in the PCS group a positive correlation was found for the index that characterizes

primarily sympathetic vasomotor activity (SBPV LF power). The latter index in the PCS group also correlated with the disease duration.

Significant changes were observed in HRV and BPV indices during paced breathing at 6 breaths/min. In all study groups (Tables 20, 21 and 22) an increase in HRV TP and HRV LF power were observed. In ME/CFS and PCS groups (but not in healthy controls) an increase in HRV HF power was also found. In all groups there was a significant increase in TP and LF power of SBPV and DBPV. In healthy controls a decrease in SBPV HF power was additionally noted. Changes in the respiratory rate variability in all groups were characterized by a marked increase in TP due to the LF power and a decrease in HF power confirming the shift of respiratory sinus arrhythmia at a breathing frequency of 0.1 Hz entirely into the LF band. This may explain the decrease in SBPV HF power in the healthy controls. At the same time, the increase in HRV HF power in ME/CFS and PCS groups may be associated with increased heart rate fragmentation [106].

Table 20 – Dynamics of heart rate variability, systolic blood pressure variability, diastolic blood pressure variability, and respiratory rate variability in healthy controls during paced breathing at 6 breaths/min, Me [25;75]

	HC, spontaneous breathing rate	HC, 6 breaths/min	p
n	32	32	
HRV			
TP (ms^2)	2709.05 [1483.38; 4454.36]	7007.30 [4443.65; 14608.75]	< .001
LF (ms^2)	759.30 [477.33; 2480.68]	6022.40 [3333.65; 11796.05]	< .001
HF (ms^2)	703.70 [311.00; 1394.80]	442.90 [183.50; 1643.08]	0.203
SBPV			
TP ($mmHg^2$)	41.35 [22.15; 63.15]	62.90 [39.38; 96.05]	0.004
LF ($mmHg^2$)	9.50 [6.30; 19.68]	39.25 [22.23; 69.70]	< .001
HF ($mmHg^2$)	8.50 [4.48; 14.28]	3.15 [1.93; 6.23]	0.001
DBPV			
TP ($mmHg^2$)	11.75 [7.60; 20.53]	15.30 [10.45; 27.23]	0.018
LF ($mmHg^2$)	4.45 [2.25; 7.90]	9.40 [5.05; 20.80]	< .001
HF ($mmHg^2$)	1.45 [0.70; 2.68]	1.10 [0.70; 3.38]	0.262

Continuation of table 20

	HC, spontaneous breathing rate	HC, 6 breaths/min	p
Respiratory rate variability			
TP (l/min^2)	576.10 [418.93; 809.75]	701.45 [492.33; 1332.78]	0.018
LF (l/min^2)	39.45 [13.33; 105.73]	588.45 [370.00; 937.15]	< .001
HF (l/min^2)	449.85 [309.00; 569.55]	116.55 [61.58; 183.60]	< .001
Note – DBPV – diastolic blood pressure variability; HC – healthy controls; HF – high frequency; HRV – heart rate variability; LF – low frequency; SBPV – systolic blood pressure variability; TP – total power			

Table 21 – Dynamics of heart rate variability, systolic blood pressure variability, diastolic blood pressure variability, and respiratory rate variability in myalgic encephalomyelitis/chronic fatigue syndrome during paced breathing at 6 breaths/min, Me [25;75]

	ME/CFS, spontaneous breathing rate	ME/CFS, 6 breaths/min	p
n	34	34	
HRV			
TP (ms^2)	852.45 [469.03; 1912.88]	5179.75 [1260.90; 8857.20]	< .001
LF (ms^2)	367.10 [137.45; 635.13]	4220.15 [921.70; 6590.25]	< .001
HF (ms^2)	152.90 [91.93; 284.78]	249.90 [98.28; 698.98]	0.006
SBPV			
TP (mmHg^2)	50.15 [23.38; 66.78]	77.20 [43.58; 136.63]	< .001
LF (mmHg^2)	13.95 [6.45; 21.85]	53.60 [22.05; 106.18]	< .001
HF (mmHg^2)	7.80 [4.38; 16.73]	5.35 [2.80; 10.85]	0.158
DBPV			
TP (mmHg^2)	12.95 [7.38; 25.28]	21.15 [11.45; 39.48]	0.021
LF (mmHg^2)	5.15 [2.93; 7.98]	14.25 [5.23; 26.93]	< .001
HF (mmHg^2)	1.50 [0.90; 2.15]	1.70 [1.10; 2.53]	0.227
Respiratory rate variability			
TP (l/min^2)	703.00 [523.63; 1122.15]	1133.80 [688.13; 1664.75]	0.001
LF (l/min^2)	32.65 [11.20; 101.05]	947.55 [591.03; 1304.13]	< .001
HF (l/min^2)	560.85 [421.90; 769.85]	157.40 [91.43; 249.58]	< .001
Note – DBPV – diastolic blood pressure variability; HF – high frequency; HRV – heart rate variability; LF – low frequency; ME/CFS – myalgic encephalomyelitis/chronic fatigue syndrome; SBPV – systolic blood pressure variability; TP – total power			

Table 22 – Dynamics of heart rate variability, systolic blood pressure variability, diastolic blood pressure variability, and respiratory rate variability in post-COVID-19 syndrome during paced breathing at 6 breaths/min, Me [25;75]

	PCS, spontaneous breathing rate	PCS, 6 breaths/min	p
n	29	29	
HRV			
TP (ms^2)	1358.10 [834.15; 2687.15]	5891.10 [2363.10; 11520.45]	< .001
LF (ms^2)	429.20 [279.65; 867.70]	4824.30 [1528.40; 9957.70]	< .001
HF (ms^2)	335.00 [102.45; 717.10]	536.10 [231.55; 1099.25]	0.009
SBPV			
TP ($mmHg^2$)	42.20 [26.85; 65.80]	84.40 [48.90; 157.35]	< .001
LF ($mmHg^2$)	13.50 [8.80; 23.30]	57.70 [27.30; 122.60]	< .001
HF ($mmHg^2$)	8.40 [3.60; 11.45]	5.20 [2.65; 8.95]	0.060
DBPV			
TP ($mmHg^2$)	12.80 [8.05; 25.35]	17.90 [12.70; 35.20]	0.010
LF ($mmHg^2$)	5.30 [3.15; 8.70]	12.60 [6.35; 27.00]	< .001
HF ($mmHg^2$)	1.10 [0.70; 2.50]	2.10 [1.40; 3.80]	0.183
Respiratory rate variability			
TP (l/min^2)	722.90 [480.45; 993.45]	1122.20 [724.45; 1865.40]	< .001
LF (l/min^2)	20.60 [7.20; 182.80]	933.80 [537.65; 1612.40]	< .001
HF (l/min^2)	481.80 [350.90; 951.95]	165.50 [97.20; 214.10]	< .001
Note – DBPV – diastolic blood pressure variability; HF – high frequency; HRV – heart rate variability; LF – low frequency; PCS – post-COVID-19 syndrome; SBPV – systolic blood pressure variability; TP – total power			

During the 6BR test the only difference (from ones identified during spontaneous breathing) that persisted between the groups of ME/CFS and healthy controls was in HRV LF power. At the same time HRV HF power (which in 6BR test was no longer influenced by respiratory sinus arrhythmia, i.e. parasympathetic activity) did not differ between ME/CFS patients and healthy individuals. When comparing patients with PCS and healthy controls, all significant differences in HRV indices identified during spontaneous breathing disappeared in 6BR test (Table 23). There were no differences between the ME/CFS and PCS groups.

Table 23 – Comparison of heart rate variability, systolic blood pressure variability, and diastolic blood pressure variability indices between study groups during paced breathing at 6 breaths/min, Me [25;75]

	ME/CFS	PCS	HC	p*	p** (CFS/ HC)	p** (PCS/ HC)	p** (CFS/ PCS)
n	34	29	32				
HRV							
TP (ms^2)	5179.75 [1260.90; 8857.20]	5891.10 [2363.10; 11520.45]	7007.30 [4443.65; 14608.75]	0.061	–	–	–
LF (ms^2)	4220.15 [921.70; 6590.25]	4824.30 [1528.40; 9957.70]	6022.40 [3333.65; 11796.05]	0.034	0.031	0.267	0.329
HF (ms^2)	249.90 [98.28; 698.98]	536.10 [231.55; 1099.25]	442.90 [183.50; 1643.08]	0.093	–	–	–
SBPV							
TP ($mmHg^2$)	77.20 [43.58; 136.63]	84.40 [48.90; 157.35]	62.90 [39.38; 96.05]	0.258	–	–	–
LF ($mmHg^2$)	53.60 [22.05; 106.18]	57.70 [27.30; 122.60]	39.25 [22.23; 69.70]	0.166	–	–	–
HF ($mmHg^2$)	5.35 [2.80; 10.85]	5.20 [2.65; 8.95]	3.15 [1.93; 6.23]	0.058	–	–	–
DBPV							
TP ($mmHg^2$)	21.15 [11.45; 39.48]	17.90 [12.70; 35.20]	15.30 [10.45; 27.23]	0.525	–	–	–
LF ($mmHg^2$)	14.25 [5.23; 26.93]	12.60 [6.35; 27.00]	9.40 [5.05; 20.80]	0.463	–	–	–
HF ($mmHg^2$)	1.70 [1.10; 2.53]	2.10 [1.40; 3.80]	1.10 [0.70; 3.38]	0.142	–	–	–

* – significance of differences in multiple comparisons (Kruskal-Wallis test)

** – significance of differences in pairwise comparisons (Dunn's test with Holm correction)

Note – DBPV – diastolic blood pressure variability; HC – healthy controls; HF – high frequency; HRV – heart rate variability; LF – low frequency; ME/CFS – myalgic encephalomyelitis/chronic fatigue syndrome; PCS – post-COVID-19 syndrome; SBPV – systolic blood pressure variability; TP – total power

In the correlation analysis of HRV, SBPV, and DBPV indices during the 6BR test with the clinical characteristics of the study participants, positive correlations were found between age and SBPV LF power in healthy controls and in patients with ME/CFS; BMI negatively correlated with HRV LF power in the ME/CFS and PCS groups and additionally with HRV HF power in the PCS group (Table 24).

Table 24 – Correlation coefficients between clinical features and heart rate/blood pressure variability indices in the study groups during paced breathing at 6 breaths/min. Only correlation coefficients significant at $p<0.01$ are presented in the table

	Age	BMI	HADS-D	HADS-A	GF	PhF	RA	RM	MF	IPAQ
HC										
LF_{SBP}	.465									
ME\CFS										
TP_{SBP}	.441									
LF_{SBP}	.452					.443				
TP_{HR}		-.452								
LF_{HR}		-.486								
TP_{DBP}						.513				
LF_{DBP}						.601				
PCS										
TP_{HR}		-.523								
LF_{HR}		-.547								
HF_{HR}		-.584								

Note – BMI – body mass index; DBP – diastolic blood pressure; GF – general fatigue; HADS-A – anxiety subscale of The Hospital Anxiety and Depression Scale; HADS-D – depression subscale of The Hospital Anxiety and Depression Scale; HC – healthy controls; HF – high frequency; HR – heart rate; LF – low frequency; ME/CFS – myalgic encephalomyelitis/chronic fatigue syndrome; MF – mental fatigue; PCS – post-COVID-19 syndrome; PhF – physical fatigue; RA – reduced activity; RM – reduced motivation; SBP – systolic blood pressure; TP – total power

However, the most interesting finding was the correlation between the severity of physical fatigue in the ME/CFS group and SBPV/DBPV indices in the LF band (in the PCS group similar correlations were identified but they did not reach the statistical significance at $p<0.01$ (p value ranged from 0.016 to 0.04)).

The opposite direction of changes in HRV and SBPV/DBPV in the 6BR test, as well as the observed pattern of correlations between variability indices and clinical characteristics, suggest a contribution of impaired baroreflex regulation to the pathogenesis of fatigue in ME/CFS. This contribution of this mechanism is likely to be especially significant in older age groups.

3.4 Baroreflex regulation

Assessment of baroreflex function was performed in 34 patients with ME/CFS, 29 patients with PCS, and 32 healthy controls.

Analysis of BRS with the sequence method showed that in patients with ME/CFS BRS was significantly lower compared to the healthy controls, both in cases of blood pressure increases and decreases ("up" and "down" sequences). In patients with PCS BRS was reduced only in "down" sequences (Table 25). At the same time, BEI did not differ significantly between the groups.

Table 25 – Comparison of baroreflex function among study groups assessed with the sequence method and spectral method during spontaneous breathing and paced breathing (at 12 and 6 breaths/min), Me [25;75]

	ME/CFS	PCS	HC	p*	p** (CFS/ HC)	p** (PCS/ HC)	p** (CFS/ PCS)
n	34	29	32				
BRSup (ms/mmHg)	4.42 [2.88; 6.28]	5.91 [3.54; 7.92]	7.40 [4.90; 14.03]	0.001	<0.001	0.131	0.131
BRSdown (ms/mmHg)	4.85 [2.93; 7.42]	5.24 [3.97; 8.48]	9.15 [6.42; 12.01]	<0.001	<0.001	0.006	0.317
BRSmean (ms/mmHg)	4.60 [3.12; 6.40]	5.99 [3.88; 8.48]	8.45 [5.25; 13.40]	<0.001	<0.001	0.048	0.153
BEIup (%)	0.57 [0.43; 0.80]	0.64 [0.44; 0.78]	0.70 [0.56; 0.88]	0.047	0.059	0.127	0.693

Continuation of table 25

	ME/CFS	PCS	HC	p*	p** (CFS/ HC)	p** (PCS/ HC)	p** (CFS/ PCS)
BEIdown (%)	0.45 [0.37; 0.62]	0.44 [0.29; 0.77]	0.49 [0.36; 0.82]	0.685	–	–	–
BRS_LF (ms/mmHg)	5.77 [4.01; 6.82]	6.19 [3.91; 7.67]	9.75 [7.24; 12.58]	<0.001	<0.001	0.001	0.359
BRS_HF (ms/mmHg)	4.58 [2.97; 6.47]	6.07 [3.28; 8.26]	8.48 [5.46; 14.94]	<0.001	<0.001	0.035	0.129
BRS_LF12 (ms/mmHg)	4.34 [2.99; 6.80]	4.92 [3.93; 6.29]	8.49 [5.75; 10.78]	<0.001	<0.001	0.001	0.672
BRS_HF12 (ms/mmHg)	8.04 [3.91; 11.86]	8.23 [5.70; 21.31]	12.71 [7.46; 21.01]	0.026	0.021	0.314	0.314
BRS_LF6 (ms/mmHg)	7.97 [3.85; 13.44]	8.11 [4.61; 11.54]	13.08 [7.79; 19.82]	0.009	0.018	0.020	0.953
BRS_HF6 (ms/mmHg)	8.04 [3.91; 11.86]	8.23 [5.70; 21.31]	12.71 [7.46; 21.01]	0.026	0.021	0.314	0.314

* – significance of differences in multiple comparisons (Kruskal-Wallis test)

** – significance of differences in pairwise comparisons (Dunn's test with Holm correction)

Note – BEI – baroreflex effectiveness index; BRS – baroreflex sensitivity (to the increases of systolic blood pressure – up, to the decreases of systolic blood pressure – down, and to all events – mean); HC – healthy controls; HF – high frequency; LF – low frequency; ME/CFS – myalgic encephalomyelitis/chronic fatigue syndrome; PCS – post-COVID-19 syndrome

When assessing BRS with the spectral method, which implies separate determination of BRS in HF and LF bands, it was found that patients with ME/CFS have significantly lower BRS values than healthy individuals in both frequency bands, both during spontaneous breathing and paced breathing at 6 and 12 breaths/min. Patients with PCS also had significantly lower BRS values than healthy individuals in LF and HF bands during spontaneous breathing. However, during paced breathing at 6 or 12 breaths/min lower values persisted only in LF band (Table 25).

Analysis of BRS dynamics in tests with paced breathing compared to spontaneous breathing revealed that during the 12BR test all groups showed a significant increase in BRS in the HF band, while in the LF band it decreased in healthy controls and PCS group.

During the 6BR test, BRS significantly increased in both HF and LF bands in all study groups (Tables 26, 27, and 28).

Table 26 – Dynamics of spontaneous baroreflex sensitivity during paced breathing at 12 and 6 breaths/min in healthy individuals, Me [25;75]

	HC, spontaneous breathing rate	HC, paced breathing	p
n	32	32	
12 breaths/min			
BRS_LF (ms/mmHg)	9.75 [7.24; 12.58]	8.49 [5.75; 10.78]	0.035
BRS_HF (ms/mmHg)	8.48 [5.46; 14.94]	12.71 [7.46; 21.01]	0.047
6 breaths/min			
BRS_LF (ms/mmHg)	9.75 [7.24; 12.58]	13.08 [7.79; 19.82]	0.002
BRS_HF (ms/mmHg)	8.48 [5.46; 14.94]	12.71 [7.46; 21.01]	0.047
Note – BRS – baroreflex sensitivity; HC – healthy controls; HF – high frequency; LF – low frequency			

Table 27 – Dynamics of spontaneous baroreflex sensitivity during paced breathing at 12 and 6 breaths/min in patients with myalgic encephalomyelitis/chronic fatigue syndrome, Me [25;75]

	ME/CFS, spontaneous breathing rate	ME/CFS, paced breathing	p
n	34	34	
12 breaths/min			
BRS_LF (ms/mmHg)	5.77 [4.01; 6.82]	4.34 [2.99; 6.80]	0.144
BRS_HF (ms/mmHg)	4.58 [2.97; 6.47]	8.04 [3.91; 11.86]	0.004
6 breaths/min			
BRS_LF (ms/mmHg)	5.77 [4.01; 6.82]	7.97 [3.85; 13.44]	< .001
BRS_HF (ms/mmHg)	4.58 [2.97; 6.47]	8.04 [3.91; 11.86]	0.004
Note – BRS – baroreflex sensitivity; HF – high frequency; LF – low frequency; ME/CFS – myalgic encephalomyelitis/chronic fatigue syndrome			

Table 28 – Dynamics of spontaneous baroreflex sensitivity during paced breathing at 12 and 6 breaths/min in patients with post-COVID-19 syndrome, Me [25;75]

	PCS, spontaneous breathing rate	PCS, paced breathing	p
n	29	29	

Continuation of table 28

	PCS, spontaneous breathing rate	PCS, paced breathing	p
12 breaths/min			
BRS_LF (ms/mmHg)	6.19 [3.91; 7.67]	4.92 [3.93; 6.29]	0.007
BRS_HF (ms/mmHg)	6.07 [3.28; 8.26]	8.23 [5.70; 21.31]	0.002
6 breaths/min			
BRS_LF (ms/mmHg)	6.19 [3.91; 7.67]	8.11 [4.61; 11.54]	0.001
BRS_HF (ms/mmHg)	6.07 [3.28; 8.26]	8.23 [5.70; 21.31]	0.002
Note – BRS – baroreflex sensitivity; HF – high frequency; LF – low frequency; PCS – post-COVID-19 syndrome			

The correlation analysis revealed several significant correlations between indices of baroreflex function and clinical characteristics in the study groups (Table 29).

Table 29 – Correlation coefficients between clinical characteristics and baroreflex function indices in the study groups. Only correlation coefficients significant at $p<0.01$ are presented in the table

	Age	BMI	HADS-D	HADS-A	GF	PhF	RA	RM	MF	DD
HC										
BRS_HF12	-.452									
BRS_LF6	-.633									
BRS_HF6	-.452									
BRS_ LF+HF6	-.520									
ME/CFS										
BRSup					-.559					
BRSmean					-.546					
BRS_HF					-.576					
BRS_LF+HF					-.531					
BRS_LF12					-.479					
BRS_HF12	-.444									
BRS_LF6	-.572									
BRS_HF6	-.444									
BRS_ LF+HF6	-.533									
PCS										
BRS_HF12		-.570								
BRS_ LF+HF12		-.565								

Continuation of table 29

	Age	BMI	HADS-D	HADS-A	GF	PhF	RA	RM	MF	DD
BRS_HF6		-.570								
BRS_ LF+HF6		-.557								

Note – BMI – body mass index; BRS – baroreflex sensitivity; DBP – diastolic blood pressure; GF – general fatigue; HADS-A – anxiety subscale of The Hospital Anxiety and Depression Scale; HADS-D – depression subscale of The Hospital Anxiety and Depression Scale; HC – healthy controls; HF – high frequency; LF – low frequency; ME/CFS – myalgic encephalomyelitis/chronic fatigue syndrome; MF – mental fatigue; PCS – post-COVID-19 syndrome; PhF – physical fatigue; RA – reduced activity; RM – reduced motivation; SBP – systolic blood pressure; TP – total power

The group of healthy controls in the 6BR test was characterized by negative correlations between BRS (both in HF and LF bands) and age of individuals. The same correlation was noted between BRS in LF band and age in the 12BR test.

In the group of patients with ME/CFS a negative correlation was found between BRS values during spontaneous breathing and severity of general fatigue, primarily due to the BRS in “up” sequences which reflected the degree of heart rate reduction in response to blood pressure increase. Spectral analysis of BRS confirmed this correlation and revealed that BRS in the HF band played a leading role in its formation. In the 12BR test the negative correlation of BRS with general fatigue shifted to the LF band, and BRS in the HF band showed a negative correlation with age. Negative correlations of BRS with age in the 6BR test in the ME/CFS group were similar to ones identified in the healthy controls.

In the PCS group there was no correlation between BRS and clinical characteristics of patients during spontaneous breathing rate. In 12BR and 6BR tests negative correlations were found between BRS and BMI, primarily due to BRS in the HF band.

3.5 Cortisol awakening response

HPA axis function based on the assessment of CAR was studied in 30 patients with ME/CFS, 25 patients with PCS, and 28 healthy controls. The analysis of standardized residuals and the use of the z-test with Bonferroni correction in the contingency table

showed that ME/CFS patients were significantly more likely to exhibit an absence of the physiological increase in salivary cortisol level 30 minutes after awakening (which is ≥50% from the baseline) compared to healthy individuals (Table 30).

Table 30 – Contingency table for identifying the association between the absence of a physiological cortisol awakening response and the presence of myalgic encephalomyelitis/chronic fatigue syndrome or post-COVID syndrome

		Number of individuals with CAR <50%	Number of individuals with CAR ≥50%	Total
HC*	Count	8	20	28
	Expected count	13.8	14.2	
	Adjusted standardized residual	-2.7	2.7	
ME/ CFS**	Count	21	9	30
	Expected count	14.8	15.2	
	Adjusted standardized residual	2.8	-2.8	
PCS***	Count	12	13	25
	Expected count	12.3	12.7	
	Adjusted standardized residual	-0.2	0.2	
Total		41	42	83

*p value in z-test with Bonferroni correction = 0.041

**p value in z-test with Bonferroni correction = 0.028

***p value in z-test with Bonferroni correction = 1.000

Note – HC – healthy controls; ME/CFS – myalgic encephalomyelitis/chronic fatigue syndrome; PCS – post-COVID-19 syndrome

The assessment of CAR revealed that ME/CFS group was characterized by lower salivary cortisol levels upon awakening, a smaller increase in salivary cortisol levels 30 minutes after awakening, and a lower area under the curve of cortisol levels relative to the baseline value (Table 31). At the same time, PCS group did not have statistically

significant differences in all characteristics of CAR and diurnal salivary cortisol dynamics compared to either healthy controls or patients with ME/CFS.

Table 31 – Comparison of cortisol awakening response characteristics and diurnal salivary cortisol dynamics between patient groups and healthy individuals, Me [25;75].

	ME/CFS	PCS	HC	p*	p** (CFS/ HC)	p** (PCS/ HC)	p** (CFS/ PCS)
n	30	25	28				
Salivary cortisol level upon awakening (nmol/l)	10.81 [7.33; 13.56]	7.28 [5.59; 10.67]	6.60 [4.54; 11.76]	0.026	0.025	0.434	0.154
Salivary cortisol level in 30 minutes after awakening (nmol/l)	11.55 [7.30; 16.08]	13.00 [10.50; 19.35]	14.15 [9.53; 18.05]	0.254	–	–	–
Salivary cortisol level in 60 minutes after awakening (nmol/l)	8.10 [6.25; 11.20]	9.70 [7.85; 13.55]	9.30 [5.32; 15.55]	0.409	–	–	–
Salivary cortisol level at 10-11 p.m. (nmol/l)	1.00 [1.00; 2.51]	1.00 [1.00; 1.82]	1.00 [1.00; 1.00]	0.147	–	–	–
Diurnal cortisol slope, (nmol/l*hour)	0.64 [0.37; 0.82]	0.39 [0.25; 0.68]	0.37 [0.23; 0.70]	0.135	–	–	–
AUCg, area under the curve of cortisol levels	624.38 [463.65; 869.89]	654.45 [556.28; 929.10]	685.50 [424.69; 903.68]	0.747	–	–	–
AUCi, area under the curve of cortisol levels relative to the baseline value	27.53 [-175.95; 170.36]	156.45 [-0.08; 435.60]	179.18 [91.61; 346.28]	0.013	0.017	0.623	0.059
$CAR_{0-30,}$ dynamics of the cortisol level in 30 minutes after awakening (%)	20.50 [-30.00; 75.50]	54.00 [10.00; 167.00]	73.00 [32.75; 163.25]	0.010	0.010	0.465	0.072

Continuation of table 31

	ME/CFS	PCS	HC	p*	p** (CFS/ HC)	p** (PCS/ HC)	p** (CFS/ PCS)
$CAR_{30-60,}$ dynamics of the cortisol level between 30 and 60 minutes after awakening (%)	-26.47 [-9.57; -42.21]	-28.17 [-18.88; -38.28]	-28.65 [-11.54; -45.89]	0.899	–	–	–
Night sleep quality in the night preceding the study day (VAS score)	49.50 [30.00; 70.00]	50.00 [40.00; 70.00]	80.00 [73.00; 94.00]	<0.001	<0.001	<0.001	0.410
Night sleep duration in the night preceding the study day (minutes)	510 [436; 566]	450 [380; 506]	483 [435; 560]	0.053	–	–	–
The severity of fatigue in the study day (VAS score)	67.50 [48.75; 71.25]	60.00 [50.00; 80.00]	30.00 [20.00; 52.50]	<0.001	0.001	0.001	0.812

* – significance of differences in multiple comparisons (Kruskal-Wallis test)

** – significance of differences in pairwise comparisons (Dunn's test with Holm correction)

Note – HC – healthy controls; ME/CFS – myalgic encephalomyelitis/chronic fatigue syndrome; PCS – post-COVID-19 syndrome; VAS – visual analogue scale

The assessment of sleep quality on the night preceding the study day as well as the severity of fatigue on the study day were, as expected, lower in patients with ME/CFS and PCS compared to the healthy controls. To evaluate the association of age, BMI, severity of fatigue, anxiety, depression, duration of night sleep and subjective assessment of its quality with the HPA axis function a correlation analysis was conducted in each study group (Table 32).

Table 32 – Correlation coefficients between clinical characteristics and hypothalamic-pituitary-adrenal axis function indices in the study groups. Only correlation coefficients significant at $p<0.01$ are presented in the table

	BMI	Night sleep quality	Night sleep duration
HC			
–			
ME/CFS			
Salivary cortisol level 30 minutes after awakening		.491	
Salivary cortisol level 60 minutes after awakening		.485	
AUCg		.466	
AUCi			-.490
CAR_{0-30}			-.515
PCS			
AUCi	-.634		
CAR_{0-30}	-.519		

* – significance of differences in multiple comparisons (Kruskal-Wallis test)

** – significance of differences in pairwise comparisons (Dunn's test with Holm correction)

Note – BMI – body mass index; HC – healthy controls; ME/CFS – myalgic encephalomyelitis/chronic fatigue syndrome; PCS – post-COVID-19 syndrome; VAS – visual analogue scale

In all study groups there were no associations between HPA axis function characteristics and age, severity of anxiety/depression/fatigue (across all domains of the MFI-20), and fatigue severity according to VAS on the study day. However, in ME/CFS group a positive correlation was found between cortisol levels (in 30 minutes and 60 minutes after awakening) and the quality of night sleep preceding the day of saliva sample collection. It is important to note that the positive effect of restorative night sleep on HPA axis function in ME/CFS patients cannot be attributed to the longer sleep duration – on the contrary, the duration of night sleep preceding the day of saliva collection negatively correlated with the increase in cortisol levels in 30 minutes after awakening (and accordingly – with the area under the cortisol curve relative to the baseline value).

Although the PCS group did not exhibit a significant decrease in HPA axis function, correlation analysis revealed a negative correlation between CAR and BMI in this group.

3.6 Microvascular endothelial function

Evaluation of microvascular endothelial function based on the results of LDF-based arterial occlusion test was performed in 35 patients with ME/CFS, 30 patients with PCS, and 30 healthy controls.

When analysing endothelium-dependent vasodilation, both patient groups showed lower values of PF during post-occlusive hyperemia compared to the healthy controls, however, this difference reached statistical significance only in the ME/CFS group. In PCS group, there was a decrease in BZ compared to the healthy controls. At the same time there were no significant differences between both patient groups and the group of healthy individuals regarding temporary characteristics of PORH (T_{PF}, $T_{1/2}$) and severity of the discomfort in the arm during the occlusion test (Table 33).

Table 33 – Comparison of microcirculation parameters between healthy controls, patients with myalgic encephalomyelitis/chronic fatigue syndrome and patients with post-COVID-19 syndrome, Me [25;75]

	ME/CFS	PCS	HC	p*	p** (CFS/ HC)	p** (PCS/ HC)	p** (CFS/ PCS)
n	35	30	30				
RF, p.u.	5.20 [4.20; 6.50]	5.15 [4.15; 5.90]	5.6 [4.88; 6.48]	0.178	–	–	–
BZ, p.u.	2.80 [1.50; 3.30]	2.55 [1.80; 3.10]	3.05 [2.50; 3.9]	0.014	0.103	0.013	0.307
PF, p.u.	12.28 [11.7; 14.00]	13.40 [11.1; 14.60]	14.45 [12.83; 15.68]	0.028	0.034	0.079	0.698
RPF, p.u	5.60 [4.10; 6.60]	5.00 [4.00; 6.13]	5.80 [4.45; 6.83]	0.171	–	–	–

Continuation of table 33

		ME/CFS	PCS	HC	p*	p** (CFS/ HC)	p** (PCS/ HC)	p** (CFS/ PCS)
$T_{PF, s}$		14.85 [12.05; 17.60]	13.48 [10.81; 16.68]	15.20 [11.51; 22.64]	0.350	–	–	–
Vmax, p.u./s		0.69 [0.54; 0.95]	0.77 [0.57; 0.96]	0.69 [0.51; 1.03]	0.611	–	–	–
$T_{1/2}$, s		13.70 [9.00; 21.20]	12.30 [7.18; 20.70]	14.75 [8.5; 24.00]	0.735	–	–	–
FR, %		239.57 [204.64; 267.44]	233.56 [213.59; 276.05]	245.28 [210.99; 274.91]	0.732	–	–	–
AUC, p.u.*s	asc	100.29 [63.70; 143.78]	94.92 [61.10; 122.13]	100.38 [72.67; 153.58]	0.354	–	–	–
	1 min	378.58 [318.28; 515.54]	405.99 [300.00; 487.37]	410.43 [325.07; 506.79]	0.850	–	–	–
Discomfort, VAS score		60.00 [45.00; 75.00]	50.00 [23.75; 70.00]	40.00 [14.50; 70.00]	0.112	–	–	–

* – significance of differences in multiple comparisons (Kruskal-Wallis test)

** – significance of differences in pairwise comparisons (Dunn's test with Holm correction)

Note – AUC asc – area under the curve for the ascending part of reactive curve; AUC 1 min – area under the curve for the first minute of reactive curve; BZ – biological zero; FR – flow reserve; HC – healthy controls; ME/CFS – myalgic encephalomyelitis/chronic fatigue syndrome; PCS – post-COVID-19 syndrome; PF – peak flow; RF – rest flow; RPF – reperfusion flow; T ½ – half recovery time; T_{PF} – time to peak flow; VAS – visual analogue scale; Vmax – rate of achieving peak flow

Analysis of the relationships between microcirculation parameters and the HRV/BPV indices revealed the following correlations in the patient groups and the control group (Table 34).

In the group of healthy controls significant correlations were found between the severity of discomfort in the arm during the occlusion test and BPV indices during spontaneous breathing and paced breathing at 12 breaths/min.

Table 34 – Correlations between microcirculation parameters and heart rate variability/blood pressure variability indices in the study groups. Only correlation coefficients significant at $p<0.01$ are presented in the table

	FR	BZ	$T_{1/2}$	RF	RPF	PF	T_{PF}	V_{max}	DComf	AUC asc	AUC 1 min
HC											
VLF_{HR}	.505			-.619							
TP_{SBP}									.486		
HF_{SBP}									.651		
TP_{SBP_12}									.495		
LF_{SBP_12}									.470		
HF_{SBP_12}									.532		
LF_{DBP_6}								.474			
HF_{DBP_6}							-.534	.495			
ME/CFS											
TP_{HR_12}											-.444
TP_{HR_6}						-.485					
LF_{HR_6}						-.443					
HF_{HR_6}				-.479	-.459	-.496					
PCS											
LF_{SBP}										-.529	
TP_{DBP}										-.504	-.501
TP_{HR_12}	.573										
LF_{HR_12}			-.535								-.535
HF_{HR_12}	.566										
TP_{SBP_12}										-.539	-.503
LF_{SBP_12}						-.530	-.532			-.629	-.645
LF_{DBP_12}											-.500
TP_{SBP_6}					-.479					-.597	-.608
LF_{SBP_6}										-.569	-.556
HF_{SBP_6}										-.599	-.563

Note – AUC asc – area under the curve for the ascending part of reactive curve; AUC 1 min – area under the curve for the first minute of reactive curve; BZ – biological zero; DBP – diastolic blood pressure; DComf – discomfort in the arm; FR – flow reserve; HC – healthy controls; HF – high frequency; HR – heart rate; LF – low frequency; ME/CFS – myalgic encephalomyelitis/chronic fatigue syndrome; PCS – post-COVID-19 syndrome; PF – peak flow; RF – rest flow; RPF – reperfusion flow; SBP – systolic blood pressure; T½ – half recovery time; TP – total power; TPF – time to peak flow; VAS – visual analogue scale; VLF – very low frequency; Vmax – rate of achieving peak flow

In the group of patients with ME/CFS significant negative correlations were observed between microcirculation parameters (both before and after arterial occlusion) and HRV indices in the 6BR test.

In the PCS group significant negative correlations were identified between the area under the post-occlusive hyperemia response curve, different BPV indices during spontaneous and paced breathing, and HRV in LF band during the 12BR test. As mentioned earlier, the latter reflects the intensity of sympathetic nervous system activity.

The results of the correlation analysis of baroreflex function and parameters of microcirculation (Table 35) in healthy controls and in patients with ME/CFS groups did not reveal significant correlations, except for the negative correlation in the healthy individuals between the intensity of the discomfort in the arm during arterial occlusion and BRS (which reflects blood pressure regulation). Meanwhile, in the PCS group, positive correlations were found between baroreflex regulation indices and microcirculation parameters such as FR and RF, which characterize the vasomotor function of the endothelium.

Table 35 – Correlations between microcirculation parameters and baroreflex function in the study groups. Only correlation coefficients significant at $p<0.01$ are presented in the table

	FR	BZ	$T_{1/2}$	RF	RPF	PF	T_{PF}	V max	DComf	AUC asc	AUC 1 min
HC											
BRS_LF									-.517		
BRS_HF									-.509		
BRS_LF+HF									-.518		
ME/CFS											
–											
PCS											
BRSup	.573										
BRSdown	.503										

Continuation of table 35

	FR	BZ	$T_{1/2}$	RF	RPF	PF	T_{PF}	V max	DComf	AUC asc	AUC 1 min
BRSmean	.585										
BEI_UP	.573	-.508									
BRS_LF+HF	.506										
BRS_LF12						.565					

Note – AUC asc – area under the curve for the ascending part of reactive curve; AUC 1 min – area under the curve for the first minute of reactive curve; BEI – baroreflex effectiveness index; BRS – baroreflex sensitivity; BZ – biological zero; Dcomf – discomfort in the arm; FR – flow reserve; HC – healthy controls; HF – high frequency; HR – heart rate; LF – low frequency; ME/CFS – myalgic encephalomyelitis/chronic fatigue syndrome; PCS – post-COVID-19 syndrome; PF – peak flow; RF – rest flow; RPF – reperfusion flow; T½ – half recovery time; T_{PF} – time to peak flow; VAS – visual analogue scale; Vmax – rate of achieving peak flow

At the next stage of the correlation analysis the relationships between microcirculation parameters and the clinical characteristics of the study participants were examined (Table 36)

Table 36 – Correlations between microcirculation parameters and clinical characteristics in the study groups. Only correlation coefficients significant at $p<0.01$ are presented in the table

	FR	BZ	$T_{1/2}$	RF	RPF	PF	T_{PF}	V max	DComf	AUC asc	AUC 1 min
HC											
BMI							-.472			-.501	
HADS-D									.536		
GF						-.475					-.463
RA					-.510						
MF											-.477
ME/CFS											
BMI							-.447	.447			
GF			.443								
DD							-.447	.442			

Continuation of table 36

	FR	BZ	$T_{1/2}$	RF	RPF	PF	T_{PF}	V max	DComf	AUC asc	AUC 1 min
PCS											
HADS-A	.495										

Note – AUC asc – area under the curve for the ascending part of reactive curve; AUC 1 min – area under the curve for the first minute of reactive curve; BMI – body mass index; BZ – biological zero; Dcomf – discomfort in the arm; DD – disease duration; FR – flow reserve; HADS-A – anxiety subscale of The Hospital Anxiety and Depression Scale; HADS-D – depression subscale of The Hospital Anxiety and Depression Scale; GF – general fatigue; HC – healthy controls; HF – high frequency; HR – heart rate; LF – low frequency; ME/CFS – myalgic encephalomyelitis/chronic fatigue syndrome; MF – mental fatigue; PCS – post-COVID-19 syndrome; PF – peak flow; RA – reduced activity; RF – rest flow; RPF – reperfusion flow; T½ – half recovery time; TPF – time to peak flow; VAS – visual analogue scale; Vmax – rate of achieving peak flow

In terms of BMI, negative correlations with T_{PF} were found both in healthy individuals and in patients with ME/CFS. Partial correlation analysis revealed that the correlations between BMI and Vmax and between duration of the disease and Vmax were false, as they did not remain significant after excluding the influence of a third variable (T_{PF}).

In the control group (but not in the patient groups) a positive correlation between the severity of the discomfort in the arm during arterial occlusion and the score on the depression subscale of the HADS was revealed. Negative correlations between the severity of fatigue and several parameters of microcirculation after arterial occlusion were also present in this group.

In the ME/CFS group the severity of fatigue correlated only with $T_{1/2}$ (which reflects the rate of the blood flow level return to baseline values after arterial occlusion).

In the PCS group the only significant correlation was between the score on the anxiety subscale of the HADS and FR (which reflects the baseline number of functioning capillaries and therefore increases when spastic phenomena in the microcirculatory bed are present before the arterial occlusion test).

3.7 Natural autoantibodies serum profiles

Analysis of the serum profiles of natural IgG AAb to the marker autoantigens which reflect the interaction of the immune system with cells of various organs and tissues, was performed in 27 patients with ME/CFS, 19 patients with PCS, and 20 healthy individuals. None of the participants had acute infectious diseases for three months preceding the study which is important to exclude the influence of acute infectious processes on the profiles of natural AAb.

According to the data obtained with ELI-Neuro-Test-12 and ELI-Viscero-Test-24, AIR level, which reflects the overall activity of humoral autoimmunity, was higher in ME/CFS and PCS patients compared to the healthy controls (Table 37). At the same time, according to the reference values provided by the manufacturer, the AIR values (as an indicator of the total content of natural AAb in the blood) in the vast majority of participants in all study groups corresponded to a reduction in the overall activity of the humoral immune system (i. e. polyclonal immunosuppression). Cases of polyclonal activation of the immune system were detected in both patient groups but absent in the control group.

Table 37 – Medians values of the average individual immunoreactivity in the study groups and the distribution of the participants based on the overall assessment of immune status according to these values

	ME/CFS	PCS	HC	p*	p** (CFS/ HC)	p** (PCS/ HC)	p** (CFS/ PCS)
n	27	19	20				
Normal AIR	9 (33.3%)	6 (31.6%)	4 (20%)				
Immuno-activation (AIR>-5%)	2 (7.4%)	2 (10.5%)	0				
Immuno-suppresion (AIR<-25%)	16 (59.3%)	11 (57.9%)	16 (80%)				

Continuation of table 37

	ME/CFS	PCS	HC	p*	p** (CFS/ HC)	p** (PCS/ HC)	p** (CFS/ PCS)
AIR, % Me [25; 75]	-27.00 [-36.00;-12.00]	-29.00 [-35.00;-15.00]	-38.00 [-45.75;-28.50]	0.013	0.017	0.038	0.831

* – significance of differences in multiple comparisons (Kruskal-Wallis test)

** – significance of differences in pairwise comparisons (Dunn's test with Holm correction)

Note – AIR – average individual immunoreactivity; HC – healthy controls; ME/CFS – myalgic encephalomyelitis/chronic fatigue syndrome; PCS – post-COVID-19 syndrome

When analysing the presence of correlations between AIR and clinical characteristics of the participants separately in each of the three study groups, only one significant correlation at the level of $p<0.01$ was identified: it was between AIR values and the severity of depression in the ME/CFS group ($r=0.508$; $p=0.007$).

Shifts in serum levels of at least one AAb outside the reference range, which reflects damage or changes in the functional state of cells in various organs and tissues and the immune system's response to these processes, were found in 17/20 (85%) healthy individuals, 17/19 (89.4%) PCS patients, and 23/27 (85.2%) ME/CFS patients. The median number of AAb with abnormal values in the ELI-Viscero-Test-24 was 3.00 [1.25; 5.00] in the healthy group, 4.0 [1.00; 6.00] in the PCS group, and 2.0 [1.00; 5.00] in the ME/CFS group. For neurotropic AAb (in the ELI-Neuro-Test-12), shifts in serum levels of AAb outside the reference range were found in 13/20 (65%) healthy individuals, 11/19 (57.9%) PCS patients, and 16/27 (59.3%) ME/CFS patients. The median number of AAb with abnormal values in the ELI-Neuro-Test-12 was 1.00 [0.00; 2.00] in the healthy and PCS groups, and 1.00 [0.00; 1.00] in the ME/CFS group, with no significant differences between the groups.

Comparison of the PCS and ME/CFS groups with the healthy controls regarding the number of individuals with abnormal shifts in the serum levels of AAb to each of the antigens in the ELI-Viscero-Test-24 and ELI-Neuro-Test-12 revealed that such shifts were significantly more prevalent among PCS patients than in healthy controls regarding AAb to adrenal medulla cells (AdrM-D/C), $p=0.031$, and tyroglobulin (TG), $p=0.047$. In

ME/CFS patients abnormal shifts of the AAb serum levels to GABA receptors (GABA-R), p=0.029, were more prevalent than in healthy controls. Conversely, abnormal shifts in the serum levels of AAb to the platelet membrane antigen (TrM) and neurofilament protein (NF-200) were significantly less prevalent (p=0.010 and p=0.027, respectively) in ME/CFS group compared to the healthy controls (Table 38). Compared to the PCS group, abnormal shifts of the AAb serum levels to GABA-R were also more common in patients with ME/CFS (p=0.006). At the same time abnormal shifts of the AAb serum levels to the glial fibrillary acidic protein (GFAP) were common in the PCS group compared to the ME/CFS group (p=0.008). When taking into account separately cases of increase (but not decrease) of AAb levels relative to the reference range, statistical significance was preserved only for the AAb to GABA-R in the ME/CFS group compared to the healthy co ntrols (p=0.029) and compared to the PCS patients (p=0.023); and for the AAb to GFAP between ME/CFS and PCS groups (p=0.008).

Table 38 – Number of patients with abnormal levels of AAb to the antigens of various organs and tissues in the study groups according to the ELI-Neuro-Test-12 and ELI-Viscero-Test-24

	ME/CFS	PCS	HC
n	27	19	20
dsDNA	6 (22.2%)	5 (26.3%)	5 (25.0%)
β2-GP	3 (11.1%)	3 (15.8%)	2 (10.0%)
Fc-IgG	2 (7.4%)	1 (5.3%)	3 (15.0%)
Collagen	5 (18.5%)	3 (15.8%)	5 (25.0%)
CoM-02	3 (11.1%)	4 (21.1%)	2 (10.0%)
β-AR	4 (14.8%)	5 (26.3%)	4 (20.0%)
TrM-03	0*	1 (5.3%)	5 (25.0%)
ANCA	2 (7.4%)	4 (21.1%)	3 (15.0%)
KiM-S	2 (7.4%)	2 (10.5%)	2 (10.0%)
LuM-S	10 (37.0%)	8 (42.1%)	10 (50.0%)
GaM-02	6 (22.2%)	0	3 (15.0%)
ItM-07	5 (18.5%)	6 (31.6%)	4 (20.0%)
ScM	7 (25.9%)	2 (10.5%)	7 (35.0%)

Continuation of table 38

	ME/CFS	PCS	HC
HeS-08	4 (14.8%)	2 (10.5%)	1 (5.0%)
HMMP	1 (3.7%)	4 (21.1%)	2 (10.0%)
Insulin	3 (11.1%)	3 (15.8%)	1 (5.0%)
Ins-R	1 (3.7%)	2 (10.5%)	1 (5.0%)
TG	4 (14.8%)	4 (21.1%)*	0
TSH-R	2 (7.4%)	5 (26.3%)	2 (10.0%)
AdrM-D/C	5 (18.5%)	8 (42.1%)*	2 (10.0%)
Spr-06	7 (25.9%)	2 (10.5%)	5 (25.0%)
NF200	0*	1 (5.3%)	4 (20.0%)
GFAP	0	5 (26.3%)	3 (15.0%)
S100	1 (3.7%)	2 (10.5%)	2 (10.0%)
MBP	0	3 (15.8%)	3 (15.5%)
V-Ca-Chanel	1 (3.7%)	0	0
N-Ach-R	0	0	0
Glu-R	1 (3.7%)	1 (5.3%)	3 (15.0%)
GABA-R	9 (33.0%)*	0	1 (5.0%)
Dopa-R	4 (14.8%)	2 (10.5%)	1 (5.0%)
5HT-R	2 (7.4%)	0	1 (3.6%)
μ-Opioid-R	2 (7.4%)	1 (5.3%)	1 (5.0%)
β-Endorphin	2 (7.4%)	2 (10.5%)	3 (15.0%)

* $p<0.05$ compared to the healthy controls

Note – HC – healthy controls; ME/CFS – myalgic encephalomyelitis/chronic fatigue syndrome; PCS – post-COVID-19 syndrome. Full names of the antigens in the ELI-Viscero-Test-24 and ELI-Neuro-Test-12 kits are presented in Table 4.

Based on the analysis of the shifts in the serum levels of AAb (in relation to the AIR), performed in each participant to each of the antigens in the ELI-Neuro-Test-12 and ELI-Viscero-Test-24 panels, it was found that the group of patients suffered from ME/CFS exhibit more pronounced shifts in the serum levels of AAb to the GABA-R compared to healthy controls (Table 39).

Table 39 – Median deviations (%) in the serum levels of natural autoantibodies of different antigenic specificities from the average individual immunoreactivity in the study groups, Me [25;75]

	ME/CFS	PCS	HC	p*	p** (CFS/ HC)	p** (PCS/ HC)	p** (CFS/ PCS)
n	27	19	20				
dsDNA	12.00 [7.00; 14.00]	10.00 [6.00; 14.00]	12.00 [5.25; 14.00]	0.610	–	–	–
β2-GP I	6.00 [3.00; 9.00]	7.00 [5.00; 11.00]	5.00 [3.25; 9.00]	0.345	–	–	–
Fc-IgG	4.00 [2.00; 6.00]	4.00 [2.00; 4.00]	5.50 [2.00; 12.75]	0.369	–	–	–
Collagen	6.00 [2.00; 10.00]	5.00 [2.00; 10.00]	7.00 [4.25; 12.00]	0.517	–	–	–
CoM-02	4.00 [1.00; 7.00]	3.00 [1.00; 10.00]	5.50 [2.25; 7.75]	0.597	–	–	–
β1-AR	6.00 [3.00; 9.00]	10.00 [4.00; 12.00]	8.50 [6.26; 10.5]	0.113	–	–	–
TrM-03	5.00 [2.00; 6.00]	4.00 [1.00; 8.00]	5.50 [2.00; 13.5]	0.691	–	–	–
ANCA	3.00 [2.00; 6.00]	4.00 [2.00; 8.00]	5.50 [2.25; 9.75]	0.356	–	–	–
KiM-S	2.00 [0.00; 5.00]	3.00 [1.00; 6.00]	4.50 [2.00; 6.75]	0.136	–	–	–
LuM-S	7.00 [3.00; 19.00]	11.00 [3.00; 30.00]	11.50 [3.50; 21.75]	0.649	–	–	–
GaM-02	6.00 [2.00; 13.00]	4.00 [1.00; 8.00]	7.00 [3.00; 11.75]	0.149	–	–	–
ItM-07	5.00 [3.00; 9.00]	5.00 [2.00; 13.00]	6.50 [1.25; 10.75]	0.908	–	–	–
ScM	8.00 [3.00; 13.00]	6.00 [3.00; 11.00]	9.00 [5.00; 16.00]	0.223	–	–	–
HeS-08	5.00 [2.00; 6.00]	7.00 [2.00; 13.00]	4.50 [2.00; 7.00]	0.550	–	–	–
HMMP	4.00 [1.00; 6.00]	5.00 [2.00; 11.00]	5.00 [3.00; 7.50]	0.346	–	–	–
Insulin	6.00 [2.00; 8.00]	5.00 [2.00; 10.00]	5.50 [3.00; 8.00]	0.989	–	–	–
Ins-R	4.00 [2.00; 8.00]	4.00 [2.00; 9.00]	5.50 [2.00; 9.75]	0.839	–	–	–

Continuation of table 39

	ME/CFS	PCS	HC	p*	p** (CFS/ HC)	p** (PCS/ HC)	p** (CFS/ PCS)
TG	3.00 [2.00; 8.00]	6.00 [3.00; 13.00]	6.00 [2.00; 7.75]	0.285	–	–	–
TSH-R	5.00 [3.00; 8.00]	7.00 [4.00; 14.00]	6.00 [2.50; 9.00]	0.325	–	–	–
AdrM-D/C	7.00 [2.00; 12.00]	8.00 [2.00; 21.00]	8.00 [4.50; 10.75]	0.676	–	–	–
Spr-06	5.00 [3.00; 19.00]	4.00 [2.00; 9.00]	6.00 [3.00; 13.50]	0.318	–	–	–
NF200	3.00 [2.00; 7.00]	6.00 [3.00; 8.00]	8.00 [2.25; 11.75]	0.188	–	–	–
GFAP	4.00 [2.00; 7.00]	6.00 [2.00; 13.00]	5.00 [1.25; 10.75]	0.170	–	–	–
S100	5.00 [1.00; 8.00]	2.00 [1.00; 7.00]	3.00 [2.00; 8.00]	0.580	–	–	–
MBP	2.00 [1.00; 3.00]	3.00 [1.00; 6.00]	2.00 [1.00; 7.50]	0.351	–	–	–
V-Ca-Chanel	6.00 [2.00; 8.00]	3.00 [2.00; 5.00]	4.00 [2.00; 6.75]	0.203	–	–	–
N-Ach-R	3.00 [1.00; 6.00]	2.00 [0.00; 5.00]	3.00 [1.25; 5.00]	0.345	–	–	–
Glu-R	4.00 [2.00; 7.00]	4.00 [2.00; 8.00]	6.00 [3.00; 12.50]	0.379	–	–	–
GABA-R	7.00 [3.00; 13.00]	5.00 [3.00; 7.00]	2.50 [1.00; 7.00]	0.017	0.021	0.499	0.104
Dopa-R	4.00 [3.00; 8.00]	4.00 [1.00; 7.00]	4.50 [2.00; 7.75]	0.635	–	–	–
5HT-R	4.00 [3.00; 7.00]	4.00 [1.00; 6.00]	4.00 [2.00; 7.00]	0.403	–	–	–
μ-Opioid-R	4.00 [1.00; 7.00]	2.00 [1.00; 6.00]	3.00 [1.25; 5.00]	0.748	–	–	–
β-Endorphin	5.00 [1.00; 10.00]	4.00 [1.00; 7.00]	3.50 [2.00; 8.75]	0.519	–	–	–

* $p<0.05$ compared to the healthy controls (HC)

Note – HC – healthy controls; ME/CFS – myalgic encephalomyelitis/chronic fatigue syndrome; PCS – post-COVID-19 syndrome. Full names of the antigens in the ELI-Viscero-Test-24 and ELI-Neuro-Test-12 kits are presented in Table 4.

Further analysis was conducted to investigate the association between clinical characteristics of the study participants (age, BMI, depression level, anxiety level, fatigue level across five domains of the MFI-20 questionnaire) and the presence of abnormal shifts of the serum levels of five AAb which exhibited significant differences between study groups (AAb to GABA-R, AdrM-D/C, TG, TrM и NF-200, see Table 38). Significant differences between subgroups with and without abnormal levels of these AAb were found only in ME/CFS patients: those with abnormal levels of AAb against GABA-R were characterized by more severe physical fatigue and depression (Table 40).

Table 40 – Comparison of clinical characteristics of patients suffered from myalgic encephalomyelitis/chronic fatigue syndrome (not related to COVID-19) with (+) or without (-) abnormal shifts in serum levels of autoantibodies to gamma-aminobutyric acid receptors

	AAb to GABA-R (+)	AAb to GABA-R (-)	p
Age, years	35,00 [28,50; 46,50]	36,00 [30,00; 47,00]	0,900
BMI, kg/m2	18.78 [17.88; 25.51]	22.48 [19.75; 27.31]	0.145
GF MFI-20, score	20.00 [19.00; 20.00]	19.00 [17.00; 20.00]	0.095
PhF MFI-20, score	20.00 [17.50; 20.00]	15.00 [14.50; 18.00]	0.011
RA MFI-20, score	20.00 [18.00; 20.00]	18.00 [16.00; 19.50]	0.075
RM MFI-20, score	14.00 [11.50; 14.50]	12.00 [9.50; 14.00]	0.458
MF MFI-20, score	14.00 [12.50; 17.00]	15.00 [12.00; 17.00]	0.874
HADS-D, score	13.00 [11.50; 16.50]	9.50 [7.00; 15.00]	0.027
HADS-A, score	10.00 [4.00; 10.50]	9.50 [6.00; 12.50]	0.403

Note – AAb – autoantibodies; BMI – body mass index; GABA-R – gamma-aminobutyric acid receptors; GF – general fatigue; HADS-A – anxiety subscale of The Hospital Anxiety and Depression Scale; HADS-D – depression subscale of The Hospital Anxiety and Depression Scale; MF – mental fatigue; MFI-20 – Multidimensional Fatigue Inventory; PhF – physical fatigue; RA – reduced activity

3.8 Gas chromatography-mass spectrometry of microbial markers in the blood

The assessment of microbial markers in venous blood with gas chromatography-mass spectrometry according to the method of Osipov G. A. was conducted in 27 patients with ME/CFS, 18 patients with PCS, and 20 healthy individuals. Concentrations of molecular markers from a wide range of microorganisms, total microbial load, endotoxin (LPS) and plasmalogen levels in the blood plasma were determined.

The data obtained on the molecular markers of various representatives of the human body's microbiome in the blood plasma of patients and healthy controls are presented in Table 41.

Table 41 – Median plasma levels of the molecular markers of various representatives of the human body's microbiome in the study groups, Me [25;75]

	ME/CFS	PCS	HC	p*	p** (CFS/ HC)	p** (PCS /HC)	p** (CFS/ PCS)
n	27	18	20				
Microorganisms, whose markers are present in the blood of >50% individuals in population							
Actinomyces spp	0.00 [0.00; 0.00]	0.00 [0.00; 0.00]	0.00 [0.00; 0.00]	0.798	–	–	–
Actinomyces viscosus	475.00 [347.00; 576.00]	460.50 [365.50; 549.50]	457.00 [358.25; 615.50]	0.912	–	–	–
Alcaligenes spp	28.00 [13.00; 43.00]	25.50 [8.75; 44.25]	0.00 [0.00; 24.75]	0.015	0.015	0.056	0.679
Bifidobacterium spp	3597.00 [2773.00; 4430.00]	3980.00 [2873.75; 4954.50]	3879.50 [2203.75; 4367.00]	0.420	–	–	–
Blautia coccoides	0.00 [0.00; 21.00]	0.00 [0.00; 9.00]	0.00 [0.00; 11.75]	0.443	–	–	–
Clostridium perfringens	9.00 [5.00; 16.00]	4.50 [3.00; 10.75]	8.00 [2.00; 12.75]	0.161	–	–	–
Clostridium propionicum	82.00 [66.00; 151.00]	84.50 [14.25; 113.25]	43.50 [0.00; 90.25]	0.079	–	–	–

Continuation of table 41

	ME/CFS	PCS	HC	p*	p** (CFS/ HC)	p** (PCS /HC)	p** (CFS/ PCS)
Clostridium ramosum	2127.00 [1717.00; 3787.00]	2555.00 [2144.00; 3068.25]	2715.50 [1643.25; 3599.00]	0.832	–	–	–
Clostridium tetani	293.00 [175.00; 511.00]	322.00 [169.25; 535.75]	301.50 [186.75; 512.75]	0.993	–	–	–
Corynebacterium spp	37.00 [2.00; 75.00]	92.50 [25.50; 116.50]	65.50 [43.50; 103.25]	0.042	0.100	0.800	0.140
Eggerthella lenta	268.00 [216.00; 312.00]	348.00 [245.00; 409.75]	311.50 [258.25; 417.50]	0.032	0.090	0.820	0.110
Eubacterium spp	4980.00 [3913.00; 5994.00]	5077.00 [4494.75; 7006.25]	4678.50 [3664.50; 6047.25]	0.544	–	–	–
Fusobacterium spp./Haemophilus spp.	0.00 [0.00; 0.00]	0.00 [0.00; 0.00]	0.00 [0.00; 0.00]	0.940	–	–	–
Lactobacillus spp	2427.00 [2093.00; 3147.00]	3047.50 [2122.50; 3706.00]	3383.00 [2561.25; 4001.75]	0.085	–	–	–
Nocardia spp.	407.00 [299.00; 602.00]	350.50 [247.25; 546.50]	406.00 [256.50; 724.75]	0.783	–	–	–
Nocardia asteroides	236.00 [184.00; 350.00]	275.00 [224.50; 452.75]	263.00 [196.00; 348.25]	0.481	–	–	–
Prevotella spp	25.00 [17.00; 32.00]	23.00 [18.25; 33.00]	21.50 [15.00; 26.75]	0.392	–	–	–
Propionibacterium acnes	5.00 [0.00; 37.00]	24.50 [6.00; 50.75]	17.00 [0.00; 44.25]	0.253	–	–	–
Propionibacterium freudenreichii	1728.00 [1087.00; 2069.00]	2323.00 [1452.25; 3057.50]	1914.00 [1427.75; 2323.25]	0.037	0.517	0.517	0.065
Propionibacterium jensenii	40.00 [6.00; 81.00]	66.50 [37.25; 115.75]	62.00 [0.00; 91.75]	0.406	–	–	–

Continuation of table 41

	ME/CFS	PCS	HC	p*	p** (CFS/ HC)	p** (PCS /HC)	p** (CFS/ PCS)
Pseudonocardia spp	0.00 [0.00;0.00]	0.00 [0.00;0.00]	0.00 [0.00;0.00]	0.652	–	–	–
Rhodococcus spp	33.00 [22.00; 48.00]	38.50 [24.00; 54.25]	44.50 [33.25; 61.25]	0.144	–	–	–
Ruminicoccus spp.	585.00 [410.00; 856.00]	434.00 [368.50; 703.50]	547.50 [337.25; 809.00]	0.472	–	–	–
Staphylococcus aureus	324.00 [254.00; 362.00]	257.50 [220.75; 313.00]	265.00 [170.50;381. 50]	0.169	–	–	–
Staphylococcus epidermidis	1.00 [0.00; 13.00]	6.50 [0.00; 16.75]	7.50 [0.00;23.50]	0.401	–	–	–
Streptococcus spp.	0.00 [0.00;0.00]	0.00 [0.00;0.00]	0.00 [0.00;0.00]	0.624	–	–	–
Streptococcus mutans	219.00 [153.00; 249.00]	180.50 [121.50; 228.75]	128.00 [100.50; 169.25]	0.002	0.001	0.047	0.271
Streptomyces spp.	168.00 [89.00; 216.00]	176.00 [125.00; 219.25]	153.00 [98.25; 220.25]	0.721	–	–	–
Veillonella spp.	0.00 [0.00; 0.00]	0.00 [0.00; 0.00]	0.00 [0.00; 0.00]	1.000	–	–	–
Microorganisms, whose markers are present in the blood of <50% individuals in population							
Bacillus cereus	0.00 [0.00;0.00]	0.00 [0.00;0.00]	0.00 [0.00;0.00]	0.239	–	–	–
Bacteroides fragilis	0.00 [0.00;0.00]	0.00 [0.00;0.00]	0.00 [0.00;0.00]	1.000	–	–	–
Campylobacter mucosalis	0.00 [0.00; 0.00]	0.00 [0.00;0.00]	0.00 [0.00;0.00]	1.000	–	–	–
Clostridium difficile	0.00 [0.00; 12.00]	0.00 [0.00; 8.50]	7.50 [0.00; 118.25]	0.161	–	–	–
Clostridium hystolyticum/Str. pneumonia	0.00 [0.00; 15.00]	0.00 [0.00; 11.75]	0.00 [0.00;0.00]	0.487	–	–	–

Continuation of table 41

	ME/CFS	PCS	HC	p*	p** (CFS/ HC)	p** (PCS /HC)	p** (CFS/ PCS)
Enterococcus spp	81.00 [41.00; 148.00]	72.00 [26.50; 115.50]	28.50 [9.50;84.25]	0.011	0.010	0.216	0.236
Flavobacterium spp	0.00 [0.00; 0.00]	0.00 [0.00;0.00]	0.00 [0.00;0.00]	1.000	–	–	–
Helicobacter pylori	0.00 [0.00; 0.00]	0.00 [0.00;0.00]	0.00 [0.00;0.00]	1.000	–	–	–
Kingella spp	19.00 [0.00; 39.00]	14.00 [0.00; 35.00]	6.00 [0.00; 20.75]	0.208	–	–	–
Acinetobacter spp	0.00 [0.00; 0.00]	0.00 [0.00;0.00]	0.00 [0.00;0.00]	0.301	–	–	–
Peptostreptococcus anaerobius 17642	0.00 [0.00; 0.00]	0.00 [0.00; 0.00]	0.00 [0.00; 0.00]	1.000	–	–	–
Peptostreptococcus anaerobius 18623	0.00 [0.00; 0.00]	0.00 [0.00; 0.00]	0.00 [0.00; 6.75]	0.165	–	–	–
Porphyromonas spp	0.00 [0.00; 0.00]	0.00 [0.00;0.00]	0.00 [0.00;0.00]	1.000	–	–	–
Pseudomonas aeruginosa	0.00 [0.00; 0.00]	0.00 [0.00; 0.00]	0.00 [0.00;0.00]	0.024	0.056	1.000	0.056
Enterobacteriaceae (E.coli and other)	0.00 [0.00; 0.00]	0.00 [0.00;0.00]	0.00 [0.00;0.00]	1.000	–	–	–
Microorganisms, whose markers are normally absent in the human blood							
Bacillus megaterium	0.00 [0.00;0.00]	0.00 [0.00;0.00]	0.00 [0.00;0.00]	1.000	–	–	–
Stenotrophomonas maltophilia	0.00 [0.00; 0.00]	0.00 [0.00;0.00]	0.00 [0.00;0.00]	1.000	–	–	–
Streptomyces farmamarensis	0.00 [0.00; 0.00]	0.00 [0.00;0.00]	0.00 [0.00;0.00]	1.000	–	–	–
Mycobacterium spp	0.00 [0.00; 0.00]	0.00 [0.00;0.00]	0.00 [0.00;0.00]	0.325	–	–	–
Chlamidia trachomatis	0.00 [0.00; 0.00]	0.00 [0.00;0.00]	0.00 [0.00;0.00]	1.000	–	–	–
Total microbial load	21282.00 [18532.00; 23760.00]	22348.00 [18813.25; 25956.50]	20734.50 [18230.50; 24177.75]	0.693	–	–	–
Plasmalogen, mcg/ml	36.73 [30.91; 42.01]	35.38 [31.44; 48.05]	34.24 [27.70; 39.09]	0.308	–	–	–

Continuation of table 41

	ME/CFS	PCS	HC	p*	p** (CFS/ HC)	p** (PCS /HC)	p** (CFS/ PCS)
Endotoxin, nmol/ml	0.30 [0.20; 0.40]	0.28 [0; 18; 0.37]	0.19 [0.17; 0.28]	0.048	0.050	0.310	0.400

* – significance of differences in multiple comparisons (Kruskal-Wallis test)

** – significance of differences in pairwise comparisons (Dunn's test with Holm correction)

Note – HC – healthy controls; ME/CFS – myalgic encephalomyelitis/chronic fatigue syndrome; PCS – post-COVID-19 syndrome

Patients with PCS and ME/CFS showed a significant increase in the levels of Streptococcus mutans molecular markers compared to the healthy controls. In the ME/CFS group there was also a significant increase in the levels of Alcaligenes spp. and Enterococcus spp. molecular markers, and a borderline significant (p=0.050) increase in the endotoxin (LPS) levels compared to the group of healthy individuals.

To determine the associations between the levels of microbial markers with significant differences between the study groups (Table 41) and clinical characteristics of the study participants, a correlation analysis was performed (Table 42).

Table 42 – Correlation coefficients between clinical characteristics and the levels of Streptococcus mutans, Alcaligenes spp, Enterococcus spp molecular markers in the study groups. Only correlation coefficients significant at p<0.01 are presented in the table

	Age	BMI	HADS-D	HADS-A	GF	PF	RA	RM	MF
HC									
–									
ME/CFS									
Str. mutans					,553				
PCS									
Alcaligenes spp.		,603				-,632	-,653		

Note – BMI – body mass index; GF – general fatigue; HADS-A – anxiety subscale of The Hospital Anxiety and Depression Scale; HADS-D – depression subscale of The Hospital Anxiety and Depression Scale; HC – healthy controls; ME/CFS – myalgic encephalomyelitis/chronic fatigue syndrome; MF – mental fatigue; PCS – post-COVID-19 syndrome; PhF – physical fatigue; RA – reduced activity; RM – reduced motivation

While no significant ($p<0.01$) correlations were found in the control group, there was a positive correlation between the severity of physical fatigue and levels of Streptococcus mutans molecular markers in the group of patients with ME/CFS. In the PCS group there was a positive correlation between BMI and levels of Alcaligenes spp. molecular markers, as well as negative correlations between fatigue (namely physical fatigue and reduced activity subscales of the MFI-20) and the levels of Alcaligenes spp. molecular markers. However, the correlation with BMI proved to be spurious, as it ceased to be significant after eliminating the influence of the correlation between the levels of Alcaligenes spp. molecular markers and physical fatigue in partial correlation analysis.

It is important to note a significant correlation between the endotoxin level and the levels of Alcaligenes spp. molecular markers in all study groups (although somehow weaker in the control group): $r=0.836$, $p<0.001$; $r=0.845$, $p<0.001$; $r=0.486$, $p=0.030$; for the groups of ME/CFS, PCS, and healthy controls, respectively.

SUMMARY

Our study focused on young and middle-aged patients who met the clinical case definition of PCS developed by WHO in 2021, and young and middle-aged patients who met the diagnostic criteria for ME/CFS with the onset of symptoms not related to COVID-19. The selection of these age groups was due to the fact that elderly and senior patients often suffer from chronic diseases that, even in the absence of any symptoms, affect the functioning of the body's regulatory systems (nervous, endocrine, and immune ones) as well as microvascular endothelial function, thereby limiting the interpretation of findings in the patient groups and casting doubt on their identification as being specifically associated with PCS and ME/CFS rather than with comorbid diseases.

To increase the homogeneity of the ME/CFS group, only patients who met all three sets of diagnostic criteria for ME/CFS recommended by the European ME/CFS Expert Group (EUROMENE) for research and clinical practice were included in these group. These are the modified Fukuda Criteria (CDC, 1994), the Canadian Consensus Criteria (CCC, 2003), and the Institute of Medicine/National Academy of Medicine Criteria (IOM/NAM, 2015). An important inclusion criterion for the ME/CFS group in our study, which was applied to ensure correct comparison between ME/CFS and chronic fatigue associated with PCS, was the absence of the link between the onset of symptoms in the ME/CFS group and acute COVID-19 infection. Thus, 87% of patients in the ME/CFS group in our study reported the onset of symptoms before the registration of the first COVID-19 cases in Russia (i.e. before March 2020); the remaining 13% of patients with ME/CFS, whose symptoms appeared during the pandemic, did not report any association between the symptom onset and COVID-19 infection.

The demographic characteristics of individuals included in the ME/CFS group (median age 37.00 [30.75; 45.25] years, female-to-male ratio 3.9:1) were in line with the data from international epidemiological studies, according to which the prevalence and incidence of ME/CFS peak in the age group of 30-50 years [313, 315] (a secondary, less significant peak was noted in the adolescence, ages 10-19 years) [313], and the female-to-

male ratio ranges from 2.5 to 5:1 [163, 313, 315]. The groups of healthy individuals, ME/CFS and PCS were age- and sex-matched which allow to compare results between the study groups. The median BMI in all groups corresponded to normal values, eliminating the influence of obesity as an important risk factor for endothelial dysfunction.

For further interpretation of the results, it is important to note that the median disease duration was significantly longer in the ME/CFS group compared to the PCS group (7.00 [4.01; 13.00] years and 1.46 [0.94; 1.76] years, respectively).

In accordance with the concept of pathological fatigue that worsens after minor physical/cognitive exertion as a key symptom of ME/CFS (so-called post-exertional malaise), ME/CFS patients in our study scored very high across all domains of the MFI-20 fatigue assessment scale. Notably, while the median scores for all domains of the MFI-20 scale in healthy individuals indicated the absence of fatigue syndrome (<12 points), the opposite situation was observed in the ME/CFS and PCS groups (≥12 points in all MFI-20 domains), suggesting the potential use of this scale for the ME/CFS and PCS screening.

The pattern of the fatigue syndrome, according to the MFI-20 scale, was similar in both patient groups and consistent with literature data for ME/CFS. In particular, fatigue syndrome in both patient groups was characterized by the highest score (16-17 points) in the general fatigue and physical fatigue subscales of MFI-20. Additionally, the median score in the physical fatigue domain was higher than that in the mental fatigue domain, and the lowest score was obtained in the reduced motivation domain [196]. These findings support the idea of predominantly physical rather than mental nature of fatigue in ME/CFS and PCS, and the absence of primary apathy and abulia typical of psychiatric disorders.

Furthermore, the discrepancy between the extremely severe fatigue (according to the MFI-20 scores) and only mild, subclinical anxiety/depression (according to the HADS scores) in both patient groups argues against the hypothesis of affective mental disorders as the cause of chronic fatigue in ME/CFS and PCS. Such subclinical anxiety and depression are typical as secondary phenomena for any chronic conditions significantly affecting quality of life [13, 16]. A patient with subclinical depression, by definition,

exhibits some symptoms of depression but does not meet diagnostic criteria for the major depressive disorder, and does not require pharmacological therapy [169]. Meta-analyses conducted in 2007 and 2021 demonstrated the effectiveness of psychotherapeutic approaches in subclinical depression in adults and adolescents compared to pharmacological treatment, that confirms the absence of significant endogenous mental pathology in patients with subclinical affective symptoms [52, 233].

Differences in the patterns of correlations between clinical and demographic characteristics when comparing patient groups and healthy individuals were identified, while these patterns were similar in the ME/CFS and PCS groups. This supports the clinical similarity between these conditions, which was observed during the COVID-19 pandemic by researchers and clinicians who had experienced with ME/CFS in the pre-pandemic period [107, 180, 329]. In our study the severity of depression/anxiety did not correlate with fatigue (in any domain of MFI-20) but correlated with each other in the ME/CFS and PCS groups; the opposite pattern was observed in healthy controls. These findings suggest the independence of psychopathological symptoms and fatigue syndrome in ME/CFS and PCS and distinguish these patients from those, for example, with depressive disorders [131, 212]. Interestingly, that age and BMI did not correlate with the severity of anxiety/depression/fatigue in any study group. This is likely due to the fact that the median BMI in all groups was normal, and one of the inclusion criteria for the study was age $\leq$ 60 years.

The first objective of our study was to determine the proportion of patients with PCS presenting with chronic fatigue who met the diagnostic criteria for ME/CFS which are used in global clinical and research practice for identifying and verifying the diagnosis of this condition. This issue is of significant practical importance, as ME/CFS (unlike PCS) has long been known to clinicians worldwide, listed in the ICD since 1969, and actively studied since the 1990s. The long history of study and clinical experience has led to the creation of international consensus recommendations on ME/CFS in 2021 which reflects current approaches to the diagnosis and therapy of this condition based on the clinical experience of experts [128, 216]. Therefore, the timely identification of ME/CFS cases

among PCS patients should facilitate the evidence-based prescription of pharmacological and non-pharmacological therapies for these patients.

As noted in the Chapter 1, more than 20 sets of diagnostic criteria and standard case definitions for ME/CFS have been developed worldwide for the last 40 years. For diagnosing ME/CFS (both in groups of PCS and ME/CFS not related to COVID-19) we used three sets of diagnostic criteria recommended by European experts in the international consensus guidelines of 2021 (modified CDC, 1994; CCC, 2003; IOM/NAM, 2015). In our experience, the Fukuda criteria (CDC, 1994) are the only ones familiar to domestic clinicians to date. At the same time the 2021 consensus guidelines for ME/CF highlighted several shortcomings of the Fukuda/CDC 1994 criteria, suggested their modification and recommended their use for clinical practice only for the screening purposes, with subsequent confirmation of the diagnosis using one of the other two sets of diagnostic criteria mentioned above [128, 216]. It was emphasized that the IOM/NAM 2015 criteria are the simplest ones, and therefore they were recommended for use by primary care physicians [128]. However, there is evidence that the specificity of the IOM/NAM 2015 criteria is lower than that of the CCC 2003 criteria, which may lead to overdiagnosis of ME/CFS [77]. Considering all the above facts, we classified cases as meeting the ME/CFS criteria only if they met all three sets of diagnostic criteria. With this approach, the prevalence of ME/CFS in the group of patients with the chronic fatigue associated with PCS was 45.7%. A comparison of the three sets of diagnostic criteria confirmed their specificity for the ME/CFS diagnosis determined in the pre-pandemic period [212]: prevalence of ME/CFS was the highest with the use of Fukuda/CDC 1994 criteria (65.2%), the lowest – with the use of CCC 2003 criteria (45.7%), and intermediate – with the use of IOM/NAM 2015 criteria (54.3%). Thus, due to its simplicity and ease of use, it seems optimal to use the IOM 2015 criteria at the primary care level for identifying suspected cases of ME/CFS, with confirmation of the diagnosis with the CCC 2003 criteria in complex cases.

As far as we know, the assessment of the prevalence of ME/CFS among PCS patients was conducted for the first time in the Russian population. However, our results were

comparable to the foreign data. In particular, Mancini et al. [320] using the Fukuda/CDC 1994 criteria found that 19/41 (46%) patients with unexplained dyspnea >3 months after COVID-19 met these criteria. In the study by Kedor et al.[54] 19/42 (45.2%) patients with the chronic fatigue in PCS met the CCC 2003 criteria, and Legler et al. [193] reported a value of 55/106 (51.9%) for these criteria. According to Bonilla et al [218] 45/105 (43%) of patients with PCS and symptoms lasting more than 6 months met the IOM/NAM 2015 criteria; Jason et al. [173] using these criteria obtained a value of 272/465 (58%). Only one study compared different sets of diagnostic criteria for ME/CFS [269]. In that study the authors separately analysed PCS groups with mild, moderate, and severe symptoms (a total of 299 patients). In the group of patients with mild symptoms, no one met the IOM/NAM, 2015 or CCC, 2003 criteria. In the group with moderate symptoms, the prevalence of meeting these criteria was 62.6% and 30.6%, respectively, and in the group with severe symptoms, it was 89% and 74.3%. Thus, the literature review showed that prevalence of ME/CFS among PCS patients assessed by other authors was similar to our results which indicates a good consistency of our understanding of PCS and ME/CFS with the understanding of physicians and researchers from other countries, despite the widespread opinion about the extreme non-specificity and heterogeneity of the clinical picture in these conditions.

The second objective of our study was to clarify the presence and characteristics of dysautonomia in patients suffered from chronic fatigue associated with PCS and from ME/CFS not related to COVID-19 [276].

Analysis of HRV indices revealed that both ME/CFS and PCS patients, compared to healthy individuals, showed a significant decrease in HRV TP due to a reduction of HRV power in all three frequency bands (VLF, LF, HF). The most significant decrease in the HF band which was observed in the ME/CFS and PCS groups indicates parasympathetic nervous system failure. It is important to emphasize that the simultaneous decrease in the absolute HRV power in LF, HF, and VLF bands cannot be explained by relative parasympathetic insufficiency against the background of absolute sympathetic overactivity. On the contrary, the identified pattern corresponds to a decrease in the

activity of all heart rate regulation circuits representing a pattern of generalized regulatory failure. This reflects the dysfunction of the autonomic nervous system as a component of the regulatory-integrating apparatus of the human body, which includes nervous, endocrine, and immune systems. This HRV pattern is also believed to reflect the depletion of the body's functional reserves [22]. According to these ideas and the concept of reduced functional reserves and adaptive capabilities of the body as the leading cause of the onset and development of diseases [14], there is evidence of the prognostic significance of reduced HRV regarding cardiovascular and overall mortality in general population, as well as clinical outcomes in stroke, oncological diseases, critical conditions, and after surgical interventions under general anesthesia [132, 146, 147, 148, 304].

Interpreting literature data regarding the identified HRV pattern in ME/CFS is somewhat challenging because a significant number of studies evaluating HRV in ME/CFS reported only relative (normalized) power values in the LF and HF ranges or, if absolute values were presented, they did not include data on TP and HRV power in the VLF band. In one of the four studies where absolute values of TP, LF, and HF were presented HRV pattern in patients with ME/CFS was identical to our results (an absolute decrease in TP, LF, and HF in the ME/CFS group compared to healthy individuals), although absolute values of HRV TP, LF and HF in patients with ME/CFS were 1.5-3 times higher than in our study [283]. In the second study TP, LF, and HF values in patients with ME/CFS were also higher than in our study, with significant differences from the healthy group found only for TP values [58]. In the third study, TP, LF, HF, and VLF values in ME/CFS patients were similar to ours, but the authors did not find significant differences between the patient and control groups (HRV indices in healthy controls were significantly lower than in our study) [57]. The fourth study differed in terms of experimental conditions: HRV measurements were taken not over 5 minutes during wakefulness, but during an 8-hour overnight sleep. The authors showed a decrease in LF, VLF, and TP in the ME/CFS group, but the HF value (reflecting parasympathetic activity) during nighttime sleep did not differ between ME/CFS patients and healthy individuals [151]. Another four studies only presented absolute power values in the LF and HF bands.

In two of them, both indices were significantly lower than in the control group and were similar to our results [262, 306]. In the third study, the HRV indices were significantly higher than ours and did not differ between ME/CFS and control groups [63]. In the fourth study, the HF value in the ME/CFS group were consistent with ours and was lower than in the healthy group, but the LF value was higher than ours and did not differ from the control group [197]. One reason for these differences could be the duration of the illness. A complete HRV pattern characteristic of ME/CFS (decrease in HRV power in all frequency ranges) may develop gradually, and in our study the degree of the TP and LF reduction correlated with the duration of the illness. It is also important to note that in all these studies, the ME/CFS was diagnosed with CDC, 1994 criteria which, according to EUROMENE recommendations, should only be used as a screening tool since they do not include PEM as a mandatory criterion, while PEM is one of the key symptoms, distinguishing ME/CFS from other conditions with similar clinical presentations [128]. In our study, to enhance the representativeness of the sample, we applied three sets of diagnostic criteria simultaneously as outlined in the EUROMENE recommendations. Future use of this approach in HRV research as well as the practice of mandatory inclusion of absolute values for TP and spectral power across all ranges (HF, LF, VLF), will help confirm the significance of the generalized regulatory insufficiency pattern as an indicator of autonomic nervous system regulatory dysfunction (dysautonomia) in ME/CFS.

Our findings regarding differences in HRV parameters between patients with PCS and healthy individuals generally align with those obtained by other researchers. According to Aranyo et al. [162] both PCS patients with sinus tachycardia (average heart rate >90 bpm over 24 hours) and asymptomatic individuals who survived COVID-19 exhibited reduced HRV power across all frequency bands (HF, VLF, LF) in 24-hour HRV assessments conducted three months after COVID19 infection compared to individuals never infected with SARS-CoV-2. Jiang et al. [64] compared 5-minute HRV in female patients four months after COVID-19 with a control group of asymptomatic individuals who survived COVID-19. They showed significant reduction in HRV spectral power across all

frequency bands (HF, VLF, LF). Mooren et al. [74] conducted 24-hour HRV monitoring in PCS patients with the average symptom duration of 8.4 months and found significant reductions of HRV in the LF and HF bands compared to a historical control group (2016). Day-night analysis performed by these authors indicated that PCS patients did not experience the physiological nocturnal increase in parasympathetic activity (HF power) observed in healthy individuals. Acanfora et al. [161] also reported reduced HRV total power and spectral power in the VLF and HF bands (but not in the LF band) in PCS patients compared to controls, though they did not specify the duration of PCS and whether individuals in the control group had been infected by SARS-CoV2. The reduction in HF power, indicative of parasympathetic activity during breathing at ≥ 10 breaths per minute, was the most reproducible result. For example, Junior et al. [86] found reduced HRV HF but not VLF or LF in PCS patients (with average symptom duration of 3.6 months) during 24-hour HRV analysis. Asarcikli et al. [145], reported similar results in PCS patients with symptoms lasting 3-4 months, compared to a historical control group noninfected by SARS-CoV2 without cardiovascular diseases but with complaints of palpitations. However, considering the presence of palpitations in the control group, some autonomic dysfunction in these individuals cannot be ruled out.

A distinctive feature of our work is the examination of PCS patients in the long-term period (median duration of symptoms in this group was 17.52 months). The persistence of the pattern of reduced HRV total spectrum power and its components (most pronounced in the HF band), which has been described in the above-mentioned studies of PCS patients in the early post-infectious period, indicates a persistent nature of dysautonomia (dysfunction of the autonomic nervous system) in PCS and raises questions about the benign and transient nature of health disturbances in these patients.

The classical works of R.M. Baevsky [1], the founder of the cardiorythmographic approach in the physiology and pathology of adaptation, attribute the dynamics of HRV in adaptive and pre-nosological processes as a universal indicator reflecting the neuroendocrine aspects of compensatory-adaptive reactions to any extreme or pathogenic impacts. Meanwhile, from modern perspectives, it should be recognized that the

categories of activation, tension, and overstrain also pertain to the third component of the regulatory-integrating apparatus of the body (see above) – the immune system. Infections with SARS-CoV-2 and other pathogens associated with chronic fatigue cause hyperstimulation and overstrain of the immune system, which imply the increase of cytokines in the blood and other pathogenetic links of the infectious process. The issue of immune system overstrain as the basis for pre-nosological states, analogous to R.M. Baevsky's concept based on studying the stages of cardiovascular system reactions in stress adaptation and maladaptation, has already been raised in the literature [34]. In particular, Autoimmune/Autoinflammatory Syndrome Induced by Adjuvants (ASIA) can be considered as a pre-nosological condition, i. e. overstrain of the regulatory-integrating apparatus of the body (see above) due to immune system hyperstimulation. In a recent comprehensive study by Ruiz-Pablos et al [271] both PCS and ME/CFS are described as the result of the transformation of premorbid ASIA to the manifestation of distress-associated pathology.

In our study correlation between HRV indices and age was identified only in the PCS group; no correlation between HRV indices and physical activity level (according to the IPAQ questionnaire) was found in any group. These findings do not align with the literature data (including studies of healthy individuals) which indicate significant negative correlations between HRV indices and both age and physical activity level [256]. This discrepancy may be due to the fact that the median age of the patients in all three groups of our study was in the range 30-40 years, whereas the most pronounced decrease in HRV parameters occurs in the first and second decades of life [62]. In addition, correlation between subjective physical activity level (questionnaire) and HRV indices was previously established by Rennie et al [120] in men but not in women who constituted 78.7% of our study population. Therefore, objective methods for the assessment of the physical activity levels (such as accelerometry) can be recommended for the future HRV studies, especially if female patients are involved.

A negative correlation between HRV indices and BMI was identified only in the ME/CFS group. According to the literature, there are currently conflicting data regarding

the relationship between HRV indices and BMI in healthy individuals [166]. More pronounced differences can be expected when comparing individuals with normal BMI and those with obesity [166], however, in our study, the median BMI in all groups corresponded to normal values.

Reduction of HRV TP and power in the LF band correlated in our study with the disease duration in the ME/CFS group, suggesting a progressive nature of dysautonomia in this disease. According to Mooren et al. [74], HRV power in the LF and HF bands negatively correlated with disease duration in PCS.

We did not find significant associations between HRV indices during spontaneous breathing and the severity of the key syndrome of ME/CFS and PCS – fatigue, as well as anxiety and depression severity. A literature review did not reveal any studies evaluating the presence of correlations between PCS symptoms and HRV values (LF, HF, VLF, TP). Escorihuela et al [263] showed correlations between HRV HF and LF reduction and the severity of fatigue (across all domains of the FIS-40 questionnaire), anxiety and depression (HADS questionnaire), autonomic dysfunction symptoms (COMPASS-31 questionnaire), and decreased sleep quality (Pittsburgh questionnaire) in ME/CFS patients, and the correlations were stronger for the HF band. In the group of male ME/CFS patients the same authors found no differences in HRV indices from healthy individuals, and there were no significant correlations within the patient group between HF, LF, and the severity of anxiety, depression, fatigue (except for the physical fatigue domain), autonomic symptoms (except for gastrointestinal ones), or sleep problems [63]. Boissoneault et al. [89] showed that the severity of fatigue in female ME/CFS patients correlated with the HRV power reduction in all frequency bands (VLF, LF, HF). We hypothesized that the lack of significant correlations in our study is due to the mixed-gender composition of the groups.

Short-term BPV during spontaneous breathing in our study did not differ between the patient groups and healthy individuals. Short-term BPV is influenced by Traube-Hering and Mayer waves and significantly less studied compared to HRV, partly due to the limited availability of equipment that can measure beat-to-beat blood pressure. Data on

BPV in ME/CFS are contradictory: Duprez et al. [189] found a reduction in SBPV and DBPV (TP, LF and HF power values) in patients with ME/CFS in the supine position, which disappeared in orthostasis. Frith et al. [160] showed an increase in DBPV (TP, LF and HF) in patients with ME/CFS in the supine position, as well as an insufficient increase in SPBV during the orthostatic challenge test in comparison with the control group. According to Wyller et al. [331], adolescent ME/CFS patients showed a reduction in SBPV in the HF band, and there were no differences between the ME/CFS group and healthy individuals during lower body negative pressure testing (analogous to the orthostatic test). Short-term BPV in PCS has not been previously studied.

Given the dependence of the power spectrum of HRV and BPV on the breathing rate, we hypothesized that a more accurate reflection of the autonomic nervous system activity in the form of HRV indices for the subsequent detection of correlations between dysautonomia and patient symptoms could be achieved in paced breathing at 12 breaths/min.

The rationale for this hypothesis is as follows. The concept of a direct association between the frequency measurements of HRV and BPV (in LF and HF bands) with the sympathetic and parasympathetic parts of the autonomic nervous system, respectively, has been recently reconsidered. An alternative approach is the so-called two-oscillator model of heart rate regulation by the autonomic nervous system. According to this model, HRV and BPV patterns are viewed in terms of the contributions of two primary mechanisms that generate HRV and BPV within the frequency range which can be assessed in short-duration electrocardiogram recordings [140]. These mechanisms are respiratory sinus arrhythmia (RSA) and sinus arrhythmia associated with the activity of a slow-wave (0.1 Hz) oscillator in the brainstem's vasomotor centre and the activity of baroreceptors in the aortic arch and carotid sinus (Mayer wave sinus arrhythmia, MWSA) [140]. Since RSA is primarily driven by fluctuations in vagal tone during the breathing cycle, the frequency of this oscillator during calm breathing is 0.2-0.3 Hz. When the breathing rate drops below 0.15 Hz, the reflection of RSA in the HRV power spectrum shifts into the LF band, and the association between HRV in the HF band and

parasympathetic nervous system activity is lost. Therefore, for an adequate assessment of the parasympathetic division of the autonomic nervous system, it is necessary for the subject to breathe at ≥ 0.15 Hz (optimally 0.2 Hz, i.e., 12 breaths/min) during the study [29 6]. In this case RSA (i. e., vagal tone as an indicator of parasympathetic nervous system activity) will be reflected in the HF band, while the LF power will represent the activity of the autonomic oscillator in the vasomotor centre and the baroreceptor mechanism as the primary sources of HRV and BPV in this frequency band (Mayer waves) [65].

The observed lack of a reduction in BPV LF, despite the reduction in HRV power in this range during the 12BR test in ME/CFS and PCS patients (unlike healthy individuals, who showed similar dynamics between BPV and HRV, i. e. a decrease in the LF power and an increase in the HF power) – indicates increased vasomotor sympathetic activity. This is likely due to the activity of the slow-wave oscillator in the brainstem neural network, which sends impulses to the sympathetic preganglionic neurons in the thoracolumbar region of the spinal cord, and suggests a potential impairment of baroreflex function in the ME/CFS and PCS groups. The increased vasomotor sympathetic activity in these patients is further supported by significant differences from the control group regarding SBPV – there was an increase in the LF spectral power in the patient groups.

The 12BR test helped clarify the mechanisms underlying the associations between variability indices and age (in PCS) or BMI (in ME/CFS) which were identified during spontaneous breathing. Specifically, the negative associations of DBPV and SBPV HF power with these characteristics suggest that the increase in age (in PCS) and body mass (in ME/CFS) is associated with reduced stroke volume fluctuations during the respiratory cycle, which may in turn be due to reduced depth of breathing movements and, consequently, reduced fluctuations in the venous return.

Finally, of particular interest is the correlation that emerged in the 12BR test between variability indices and chronic fatigue (general and physical fatigue domains of MFI-20) in the ME/CFS and PCS groups. In the ME/CFS group, this negative correlation was found with HRV HF power which reflects parasympathetic activity in the 12BR test. In the PCS group a positive correlation was found between the fatigue severity and SBPV LF

power which primarily reflects sympathetic vasomotor activity in the 12BR test. This index in the PCS group also correlated with disease duration, suggesting that increased sympathetic vasomotor activity might be a pathogenic factor that facilitates prolonged persistence of symptoms after COVID-19. It is also noteworthy that HRV indices correlated not with severity of depression/anxiety, but exclusively with the severity of fatigue (which also did not correlate with depression/anxiety) in PCS and ME/CFS patients. This suggests that it is the dysautonomia (with some differences in PCS and ME/CFS), rather than depression/anxiety, that significantly contributes to the primary symptom of these patients – chronic fatigue. The relationships between fatigue, anxiety/depression, and HRV/BPV in ME/CFS and PCS has not been thoroughly studied before. However, this issue may be of great importance, especially for differential diagnosis.

A HRV pattern observed in anxiety disorders, according to the literature, differs from what we found in PCS and ME/CFS, and is characterized by the increased power in the LF band [66].

At the same time, the HRV pattern characteristic of PCS and ME/CFS patients in our study during spontaneous breathing is similar to the HRV pattern of major depressive disorder, as noted in meta-analyses from 2019 and 2023 [51, 150]. However, recent studies points to an important difference that can be observed during stress testing: while patients with depressive disorder (similar to healthy individuals), typically show an increase in the initially low HF power after exertion [275], ME/CFS patients do not exhibit this increase; instead, a decrease in HRV power in this range (which reflects parasympathetic activity) may even be observed after exertion [264]. This difference may reflect PEM – a significant worsening of symptoms, particularly fatigue, following physical and/or mental exertion that is disproportionate to the level of exertion and persists for more than 24 hours. This phenomenon is a key characteristic of ME/CFS and is not typical of patients with major depressive disorder.

Given the identified signs of parasympathetic failure in PCS and ME/CFS patients, a simple test with paced breathing at 6 breaths/min was conducted to determine whether

these changes are reversible i.e. of functional nature. When breathing at 6 breaths/min (0.1 Hz), RSA shifts into the LF range, and the two main mechanisms underlying HRV/BPV (RSA and the activity of the slow-wave oscillator in the brainstem vasomotor centre) resonate. This imposes a sinusoidal rhythm of 0.1 Hz and maximum amplitude to HRV and BPV. Breathing at a 4.5-6.5 breaths/min, which produces the maximum amplitude of HRV and BPV, is known as resonance breathing or breathing at the resonant frequency. During resonance breathing, the parasympathetic nervous system activity is maximal, which causes a sharp increase in the HRV TP (due to an increase in the spectral power within the LF range, which includes the 0.1 Hz frequency) [6, 273].

In our study both healthy individuals and patients with ME/CFS and PCS showed a significant increase in TP and LF power of HRV, SBPV, and DBPV in the 6BR test. In the ME/CFS group dysautonomia appears to be more persistent, as the activation of the parasympathetic nervous system during resonance breathing in this group did not lead to the normalization of the HRV indices. In patients with PCS this increase was more pronounced, resulting in the disappearance of significant differences between the PCS and control groups in all HRV indices, which could be interpreted as a normalization of t he autonomic nervous system function in the PCS group during resonance breathing.

According to the literature, resonance breathing techniques have therapeutic effects, particularly in anxiety and depressive disorders, arterial hypertension, and fibromyalgia [53, 84].

At the same time it becomes apparent from 6BR test that the spectral power of HRV in the HF band is not wholly due to RSA, which reflects parasympathetic activity. When RSA shifts into the LF band (in the case of paced breathing at 6 breaths/min), HRV in the HF band does not drop to zero. The main contribution to this residual power is heart rhythm fragmentation [106], which is thought to be linked to disruptions in the electrophysiological properties of sinoatrial node cardiomyocytes [144].

Correlation analysis of HRV, BPV, and clinical characteristics of the study participants revealed in the ME/CFS group a positive association of the physical fatigue severity with SBPV and DBPV (but not HRV) indices in the LF band. To interpret this

relationship it is important to note a positive correlation between age and SBPV LF power which was found both in ME/CFS and healthy controls (although the age of patients was not associated with the severity of physical fatigue in the ME/CFS group). Since this correlation was not observed in the 12BR test, which somewhat separates the influence of the sympathetic and parasympathetic divisions of the autonomic nervous system, it is most likely that age-$SBPV_{LF}$ correlation reflects not the activation of the sympathetic nervous system but rather the second source of BPV in the LF band – namely baroreceptors in the major arteries, which acts as a damping factor for spontaneous blood pressure fluctuations [23]. It is known that surgical inactivation of baroreceptors in the carotid sinus and aortic arch leads to a significant increase in blood pressure fluctuations [23]. To test the hypothesis of the association between age, baroreflex failure (as one of the factors contributing to the increased spontaneous blood pressure fluctuations) and fatigue we conducted an in-depth examination of baroreflex function in all study groups.

Reduced baroreflex function was identified in both patient groups; however, the BRS patterns suggested some differences between PCS and ME/CFS patients. The pattern of baroreflex dysfunction exclusively in episodes of spontaneous blood pressure decrease ("down" sequences), but not during spontaneous blood pressure increase ("up" sequences) has been reported by other authors in PCS and observed also in our patients with PCS [76]. Other authors interpreted this pattern as a manifestation of increased parasympathetic nervous system activity compensatory to the elevated peripheral vascular resistance in PCS patients, as indicated by higher diastolic blood pressure and lower pulse pressure at rest in this group compared to the controls. However, in our study HRV analysis did not provide evidence for parasympathetic overactivity in patients with PCS. We believe it is more accurately to interpret the observed BRS pattern as relative preservation of parasympathetic activity in PCS (particularly compared to ME/CFS, which is characterized by a clear parasympathetic failure).

In ME/CFS statistically significantly lower BRS values were observed both in "up' and "down" sequences, indicating more severe baroreflex dysfunction in this group. BEI was not reduced in either patient group suggesting the integrity of the anatomical substrate

of baroreflex (unlike, for example, cases of surgical denervation of the carotid sinus) and pointing instead to the functional failure of baroreflex in ME/CFS and PCS.

The significant increase in BRS across all study groups and even the partial normalization of baroreflex function in PCS patients (but not in ME/CFS patients) during paced breathing support the hypothesis of less impaired mechanisms of hemodynamic regulation in the PCS group. It also suggests a potential therapeutic effect of resonance breathing regarding hemodynamic regulation. A meta-analysis on the effects of resonance breathing on cardiovascular health performed in 2023 confirmed the acute impact of this method, particularly the activation of the parasympathetic nervous system, which leads to the reduction in blood pressure and heart rate immediately following a 5-minute breathing session [274].

Correlation analysis confirmed a positive association between age and baroreflex failure during paced breathing in both healthy individuals and patients with ME/CFS. In the ME/CFS group a link between reduced baroreflex function and fatigue severity was also identified. Since age itself did not correlate with the severity of fatigue in any MFI-20 domains in this group, the results suggest a probable pathophysiological link between baroreflex failure and fatigue in ME/CFS. The results of the correlation analysis also support the hypothesis of reduced parasympathetic activity as a significant characteristic of dysautonomia in ME/CFS: fatigue severity correlated with the BRS reduction in “up” (but not in “down”) sequences – indicating an inability to reduce heart rate in short-term blood pressure regulation.

Thus, baroreflex dysfunction is a normal age-related process, and assessments of BRS in paced breathing tests could be more sensitive for its detection. However, the premature development of baroreflex failure in ME/CFS is apparently related to dysautonomia, primarily due to parasympathetic insufficiency. In such patients, baroreflex dysfunction (along with reduced HRV during the 12BR test and increased BPV during the 6BR test) can serve as an objective reflection of the patient complaints about constant fatigue and pathological tiredness correlating with their severity.

In the group of patients with PCS baroreflex dysfunction lost its association with age (i. e. was equally common across different age groups). This may suggest that COVID-19 had a negative impact on baroreflex function in younger and middle-aged subjects (who were the focus in our study). It is also worth noting negative correlations between BRS and BMI in the PCS group indicating that higher BMI may be a predictor of baroreflex failure in PCS. The mechanisms underlying the relationship between BRS and BMI are currently unclear; however, it is hypothesized that increased body weight is associated with the development of insulin resistance and sympathetic hyperactivity, which ultimately leads to a decrease in BRS [112]. In the PCS group baroreflex dysfunction did not correlate with the severity of fatigue in our study, which may be due to a greater contribution of other mechanisms to the development of chronic fatigue in PCS.

The next objective of the study was to clarify the presence of reduced HPA axis activity (as a manifestation of endocrine system dysfunction in ME/CFS and PCS) and its potential contribution to the development of chronic fatigue in these conditions.

To this end, an assessment of cortisol awakening response (CAR), widely used in the studies of neuroendocrine regulation in various diseases [68] was employed. This method allows to investigate the HPA axis integrity, similar to tests used for diagnostics of central adrenal insufficiency (e. g. insulin tolerance test, metyrapone stimulation test, and corticotropin-releasing hormone stimulation test).

According to our findings, patients with ME/CFS were significantly more likely to exhibit an absence of a physiological CAR (i. e. an increase in salivary cortisol of ≥50% from the baseline value in 30 minutes after awakening) compared to healthy individuals. In particular, CAR was absent in 70% of patients ME/CFS compared to 28.6% of healthy controls. Correspondingly, AUC for cortisol levels relative to baseline values over the first 60 minutes after awakening (AUCi) was also reduced in the ME/CFS group. However, the area under the curve for cortisol levels relative to zero over the first 60 minutes after awakening (AUCg) remained within normal limits, indicating that the overall amount of cortisol produced by the adrenal glands in the first hour after awakening

was intact, and it is presumably dynamics of cortisol secretion which was disrupted in ME/CFS.

This result aligns with findings of Nater et al. [60] who showed preserved AUCg and reduced AUCi in ME/CFS patients compared to healthy individuals. Two other studies reported a reduction in AUCg in ME/CFS patients compared to the control group [272, 307]. Another study found a reduction in AUCg only in the subgroup of ME/CFS patients who had experienced severe childhood psychological trauma, but not in those without negative childhood experiences [119]. However, in three other studies CAR in ME/CFS patients did not differ from healthy controls [104, 105, 156]. It has been suggested that this inconsistency may be due to variations in the diagnostic criteria for ME/CFS applied in different studies, prevalence of comorbid anxiety and depressive disorders among patients, severity of sleep disturbances, levels of physical activity, and medication use – as all of these factors could influence HPA axis activity. At the same time it is noteworthy that even if no reduction of CAR was found by some authors, other signs of reduced HPA axis activity were detected in their studies: Gaab et al. [156] identified increased cortisol suppression in the dexamethasone suppression test in ME/CFS patients supporting the hypothesis of possible increased sensitivity of glucocorticoid receptors (which mediate the negative feedback mechanism in the HPA axis) to endogenous corticosteroids as one of the mechanisms underlying hypocortisolism in ME/CFS. Rimes et al. [105] showed that AUC for the cortisol levels throughout the day (i. e. the total daily cortisol output), was lower in ME/CFS patients compared to the control group. A similar result was obtained by Herane et al. [104].

Dysfunction of HPA axis in ME/CFS characterized by hypocortisolism, as noted in Chapter 1, has been extensively reported over the past thirty years with various research methods examining different aspects of HPA axis dysfunction. These findings were summarized in a recent review by Ruiz-Pablos et al. [271] who interpreted ME/CFS as hypocortisolism resulting from the immunopathological damage to the HPA axis in ASIA syndrome.

Despite common view of hypocorticolism in ME/CFS, when comparing findings of different research groups that used the same method for the detection of HPA axis dysfunction, contradictory results frequently emerged. In particular, the assessment of CAR performed in our study may not be the most optimal method for detecting HPA axis dysfunction in ME/CFS, as although this method revealed statistically significant differences between the ME/CFS and control groups, the absence of physiological cortisol increase upon awakening in the healthy group was relatively frequent (around 30% according to our results and the literature [293]), and its clinical significance remains unclear. At the same time CAR was preserved in about 30% of patients with ME/CFS in our study. No correlation was found between fatigue severity and CAR measures in the study groups.

One of the unique aspects of our study was that participants were instructed to collect samples on their day off, when they could sleep as much as they wanted, whereas in other studies evaluating CAR in ME/CFS sample collection was conducted on a workday, or participants were required to wake up at a specific time by an alarm clock. The choice of a day off in our study was related to our clinical experience and literature data, which indicate that around 50% of ME/CFS patients do not work due to the disease symptoms as they are no longer able to meet their employers' demands [216]. At the same time it is known that CAR is typically more pronounced on a workday than on a day off [68]. This fact may lead to false differences between the ME/CFS and control groups when assessing cortisol levels on a workday, as it will not be in fact a workday for many ME/CFS patients. Carrying out the study on a day off so that all participants were in the same conditions might explain the higher cortisol levels upon awakening in the ME/CFS group compared to healthy individuals in our study, which has not been reported by other authors. In their studies healthy individuals may have lower cortisol levels than ME/CFS patients because the sample collection day was a workday for healthy individuals but a day off for ME/CFS patients.

Although sleep duration at the night preceding the sample collection did not differ between the groups in our study, a significant negative correlation between sleep duration

and CAR was found in the ME/CFS group. In this group, a correlation between cortisol levels and sleep quality (which was significantly lower in ME/CFS and PCS patients compared to the control group) was also observed: cortisol levels in 30 and 60 minutes after awakening correlated with the patients' subjective assessment of sleep quality. Our results align with the literature, indicating that the shorter the sleep duration on the night preceding the study day, the more pronounced CAR tends to be [182, 292]

Thus, in efforts to correct HPA axis dysfunction in ME/CFS particular attention should be given to sleep quality rather than a simple recommendation to increase its duration, which might otherwise lead to a further CAR reduction.

Unlike ME/CFS patients, PCS patients did not show any deviations in the CAR measures from those of the healthy controls. These findings suggest that the duration of the illness, which was significantly shorter in the PCS than in the ME/CFS group, plays an important role in the development of HPA axis dysfunction. Correlation analysis revealed a negative association between CAR and BMI in the PCS group. This correlation suggests that elevated BMI may be a risk factor not only for the development of PCS itself, as has been reported [297], but also for the HPA axis dysfunction in these patients. We performed bioinformatic analysis of antigenic mimicry between proteins of different coronaviruses (including SARS-CoV2) and human antigens relevant to the HPA axis function, and identified the presence of common pentapeptides between the receptor of adrenocorticotropic hormone and SARS-CoV2 proteins, suggesting the induction of pathological autoimmunity as one of the mechanisms of HPA axis dysfunction after viral infection [96].

Interestingly, that using a different method (cumulative cortisol levels in hair), Vroegindeweij et al [194] in a recent study, which involved adolescents and young adults with ME/CFS, PCS, post-viral fatigue triggered by Q fever and juvenile idiopathic arthritis, also found reduced cortisol levels compared to healthy subjects only in ME/CFS and Q-fever fatigue syndrome, but not in PCS. Hair cortisol levels negatively correlated with fatigue severity and sleep disturbances.

Assessment of the microcirculation allow us to reveal certain features of microcirculatory dysfunction both in PCS and ME/CFS not related to COVID-19. Endothelial dysfunction is characterized by reduced endothelium-dependent vasodilation, which can be estimated from the characteristics of PORH at the microcirculatory level in the forearm skin after a short arterial occlusion of the brachial artery. Thus, the reduced peak blood flow in the post-occlusion period in the ME/CFS and PCS groups compared to healthy individuals, as observed in our study, apparently reflects endothelial dysfunction in these conditions. A progressive nature of endothelial dysfunction can be suggested considering the unidirectional changes of the post-occlusion peak flow in both PCS and ME/CFS groups, though significant differences compared to healthy individuals were only achieved in the latter group, which was characterized by a significantly longer disease duration. The absence of differences in the AUC of the PORH, which also reflects endothelium-dependent vasodilation, was probably due to the bidirectional changes in the "temporal" indices of the PORH (T_{PF}, $T_{1/2}$) among ME/CFS patients compared to healthy individuals.

Our findings are consistent with those of other researchers. The first study assessing PORH in ME/CFS was published in 2012 and reported endothelial dysfunction at the microcirculatory level in patients with ME/CFS (in particular, a reduction in the AUC of the PORH in the patient group compared to the control group) [184].

In 2021, these results were reproduced in two other cohorts of ME/CFS patients, who also had a reduced PORH compared to healthy individuals, according to the LDF data [124, 262].

At the time of writing of this chapter, the assessment of PORH in PCS patients has been performed in two studies: in the study by Charfeddine et al. [285], 49.7% of PCS patients met the criteria for the presence of endothelial dysfunction based on a combined assessment of PORH with LDF and skin thermography. Unfortunately, this study lacked a healthy control group. In the study by Jamieson et al. [201], no significant difference was found in the PORH indices between patients with PCS and healthy subjects; however, the authors used a different method of PORH assessment – near-infrared spectroscopy.

At the same time, our data suggest that microcirculatory dysfunction in PCS may have some unique features. In the PCS group (but not in the ME/CFS group), a lower BZ value (which is based on the skin LDF signal recorded during arterial occlusion) was observed, indicating a possible tendency toward venular blood pooling in this group of patients. It is known that another significant component determining a non-zero signal during occlusion is the Brownian motion of macromolecules in the interstitial fluid, which is linearly dependent on the tissue temperature [178]. However, the role of this factor in our study is probably less significant, as, according to our data, perfusion levels remained stable during the 3-minute arterial occlusion while tissue temperature during occlusion, as demonstrated by Charfeddine et al. [285], linearly decreases from the onset of occlusion for at least 6 minutes.

The severity of endothelial dysfunction in PCS in our study was clearly associated with increased BPV and the HRV index that reflects the activity of the sympathetic nervous system (LF_{HRV_12}). Additionally, only in the PCS group correlations between some indices which characterize endothelial and baroreflex function have been found. These findings are consistent with the current understanding of BPV sources. The key role in raising BPV belongs to the baroreflex failure, which in turn is mediated by increased vascular wall stiffness, the effects of angiotensin II, sympathetic nervous system hyperactivity, endothelial dysfunction, and nitric oxide deficiency [23]. Considering current views on the increased BPV as a reflection of vascular ageing, it can be hypothesized that in some young and middle-aged patients who report persistent symptoms (>3 months) after acute COVID-19 this infection or its consequences may accelerated vascular ageing [23]. The relationship between anxiety levels and flow reserve index in the arterial occlusion test in the PCS group may represent the association between anxiety and the baseline sympathetic vasomotor hyperactivity, which leads to the reduction in the number of functioning capillaries (and consequently, higher flow reserve, which reflects the extent of possible increase in blood flow in post-occlusion period, and thus – the initial number of functioning capillaries).

In the ME/CFS group, correlation analysis showed a link between endothelial dysfunction and HRV in the 6BR test. Higher HRV HF power in this test was associated with lower blood flow indices (both RF and PF), which may suggest a connection between impaired microcirculation and heart rate fragmentation. Heart rate fragmentation, as revealed during the 6BR test, was characteristic of the ME/CFS group, manifesting as increased HRV in the HF band. Disturbed blood pressure regulation results in more pronounced blood pressure fluctuations, which in turn trigger pre-capillary vasoconstriction as an initially protective mechanism that, however, leads to reduced tissue perfusion [253].

However, correlation analysis in this group of patients indicated the independence of the baroreflex and endothelial dysfunction, unlike in the PCS group.

Regarding healthy individuals, a noteworthy finding was the relationship between the severity of discomfort in the arm during arterial occlusion and BPV metrics. This association suggests that the subjective estimation of the discomfort during the occlusion test may serve as a simple and accessible marker of endothelial dysfunction or its potential risk in apparently healthy individuals.

In contrast, chronological age, whether of patients or healthy individuals, in our study did not show significant correlations with any PORH parameters which characterize microcirculation.

Interestingly, the severity of discomfort in the arm during arterial occlusion also correlated in the healthy controls (but not in the patient groups) with the depression subscale score (HADS), despite the absence of depression in healthy subjects according to the obtained scores. It has been previously suggested that patients with major depressive disorder exhibit heightened sensitivity to pain stimuli during algometry testing; however, further analysis revealed that this was entirely due to associated somatic factors such as sleep disturbances and reduced physical activity in these patients [241]. In our study no increased sensitivity to pain stimuli was found in patients with PCS or ME/CFS, despite significantly higher depression subscale scores in patients compared to healthy controls, which argues against the hypothesis of a connection between the patients' symptoms and

depressive disorders. The absence of a relationship between the severity of discomfort during the test and depression severity in these groups indirectly supports the involvement of other factors in individual pain sensitivity and development of chronic pain syndromes, which are widely prevalent among ME/CFS and PCS patients.

In the group of healthy individuals several significant correlations were also found, indicating an association between endothelium-dependent vasodilation and the scores on the fatigue subscales. In contrast, in the ME/CFS and PCS groups, where pathological fatigue was present in all patients and endothelium-dependent vasodilation was also reduced compared to the healthy subjects, the relationship between these indexes was lost. This finding suggests that endothelial dysfunction may be one of the early link in the pathogenesis of ME/CFS, potentially related to complaints of episodic fatigue in clinically healthy individuals at the preclinical stage when symptoms do not reach the severity of ME/CFS. As other pathogenic mechanisms become involved, despite the persistence and increased severity of endothelial dysfunction in such patients, its relationship with the symptoms severity becomes less apparent. It is also possible that in ME/CFS patients with more pronounced symptoms the progression of endothelial dysfunction leads to reduced reactivity of microcirculatory vessels and prolonged post-occlusion hyperemia response, which could explain correlation between general fatigue severity and the $T_{1/2}$ index in this group.

Amid increased interest among researchers in the potential connection between microcirculation disorders in acute COVID-19/PCS and the immune system activation and autoimmune reactions, a similar connection was suggested for ME/CFS as well [135]. For instance, D. Berg et al. [92] as early as 1999, compared ME/CFS with antiphospholipid syndrome, pointing out similar patterns of impaired blood rheology and hemostasis in these conditions.

In our study the assessment of the immune system activation was based on the AIR, which reflects the average levels of natural AAb of various specificities in the blood. The decreased AIR across all three groups compared to reference values may be related to changes in the normal values of AIR in the population since the establishment of reference

values by the manufacturer in 2009. At the same time, when comparing AIR values between the study groups, increased (auto-)immune activity in ME/CFS and PCS patients compared to healthy individuals was identified. There were no significant differences between patients with ME/CFS and PCS.

When discussing the connection between dysfunction of nervous and immune systems in these conditions, it is important to note the significant correlation between AIR values and the severity of depression in the ME/CFS group. It is known that depressive syndrome is common in many autoimmune and chronic inflammatory diseases, which are characterized by increased levels of pro-inflammatory cytokines [185]. The identified correlation may indicate a contribution of functional AAb (possibly against CNS antigens) to the development of depressive syndrome in ME/CFS patients or suggest damage to the nervous system at the cellular/tissue level (as reflected by the increased production of natural AAb) as a mechanism for the development of depression in this condition.

We attempted to identify disturbances in the regulatory function of the immune system in PCS and ME/CFS, indicated by changes in natural AAb serum profiles (i. e. significant alterations in the blood levels of natural AAb with certain specificities). Such changes in natural AAb serum profiles were somewhat unexpectedly found also in the majority of healthy controls (in 85% of subjects according to the ELI-Vicero-Test-24 and in 65% of subjects according to the ELI-Neuro-Test-12), which was comparable to the PCS and ME/CFS groups. Thus, isolated deviations in these tests without considering the clinical picture should not be considered as definitely pathological.

At the same time several autoantigens were identified, to which abnormal levels of AAb were significantly more frequently observed in PCS (thyroglobulin and membrane antigen of the adrenal medullary cells) or in ME/CFS (GABA receptors) compared to the control group. Our data are partially supported by the literature: according to Rojas et al. [72], abnormally high levels of AAb against thyroglobulin were found in 14% of patients with PCS compared to the pre-pandemic control group. In another study, individuals who had recovered from COVID-19, regardless of whether symptoms persisted, showed

higher levels of AAb against thyroglobulin compared to the pre-pandemic healthy control group [165]. The thyroid gland is commonly affected during acute COVID-19, serving as a target for lymphocytic infiltration. Moreover, subacute lymphocytic thyroiditis, De Quervain's granulomatous giant cell thyroiditis, and chronic autoimmune Hashimoto's thyroiditis often flare up after COVID-19 or are triggered by this infection [48, 70]. According to available literature, autoimmune reactions against adrenal medulla have not been previously studied in PCS. Given our findings regarding HRV and microcirculation, which suggest a potential role of increased sympathetic vasomotor activity as a pathogenic factor in the prolonged persistence of symptoms after COVID-19 (see above), the enhancement of autoimmunity against this critical element of the sympathoadrenal system in PCS can be interpreted as a compensatory reaction of the immune system against the neuroendocrine component of the neuroendocrine-immune regulatory network, aimed at suppressing sympathetic influences. This interpretation is supported by numerous data collected in a recent article by Cadegiani [85], indicating that both during COVID-19 and after mRNA vaccinations against SARS-CoV-2, there is expression of mRNA and coronavirus spike protein in chromaffin cells of the adrenal medulla, and moreover, this expression enhances the activity of enzymes producing noradrenaline. Some PCS symptoms and post-vaccination complications overlap with the clinical picture of hypercatecholaminemia.

Regarding AAb to GABA receptors, it is worthy to note that high titers of these AAb are associated with a specific form of autoimmune encephalitis, as well as with neuropsychiatric systemic lupus erythematosus [157, 270]. However, in our study, the levels of AAb to GABA receptors in ME/CFS patients were low, supporting the concept of secondary production of these AAb in response to the nervous tissue damage or changes in the GABA receptor expression (as opposed to primary autoaggression against the receptor, leading to its blockade, which is the primary pathogenic mechanism in autoimmune encephalitis). In ME/CFS the production of these AAb is likely a manifestation of regulatory, rather than pathogenic autoimmunity [73, 159]. The literature analysis revealed cases of AAb to GABA receptors in bipolar affective disorder combined

with autoimmune thyroiditis (titer not specified) [248], in schizophrenia (in 5 out of 57 patients (8.6%), with two of them having high titers) [111], and in depression (in 4 out of 106 patients (3.8%), all in low titers) [234].

Evaluation of the presence and levels of AAb to the GABA receptor in sera of ME/CFS patients has previously been conducted in only one study: Danilenko et al. [109] used the same method for determining natural AAb as was used in our study (ELISA-based ELI-Viscero-Test-24 and ELI-Neuro-Test-12 kits). In the group of patients with ME/CFS, presumably of post-viral origin (associated with infections caused by herpesviruses types 1, 2, 4, 5, 6), the authors found increased levels of AAb to 10 out of 12 autoantigens from the ELI-Neuro-Test-12, (NF200, GFAP, S100, MBP, V-Ca-Channel, N-Ach-R, Glu-R, GABA-R, Dopa-R, 5HT-R) and to 4 out of 24 autoantigenes from the ELI-Viscero-Test-24 (three non-organ-specific autoantigens – dsDNA, β2-GP, Collagen, and only one organ-specific autoantigen – AdrM-D/C)

Comparing these data with our findings, it is important to note that the ME/CFS group in the study of Danilenko et al. differed from ours. In that study all patients had ME/CFS of post-viral origin and clinically significant depression (which was defined as a score of ≥11 on the depression subscale of HADS), whereas our study included patients with various disease triggers, and 11 out of 26 (42.3%) patients had a HADS depression score of <11. However, even considering the differences between the patient samples, there is no contradiction in the results of two studies – patients with post-viral ME/CFS in the study of Danilenko et al. had elevated levels of AAb to the GABA receptor (as did ME/CFS patients in our study), as well as to the membrane antigen of adrenal medullary cells (similar to the PCS patients in our study). Notably, this was the only organ-specific autoantigen to which AAb levels were elevated in the group of post-viral ME/CFS, although the study of Danilenko et al. was conducted before the COVID-19 pandemic. This finding may suggest a connection between different viral infections, post-viral fatigue syndrome, and dysautonomia as one of its underlying mechanisms. Moreover, seasonal low-pathogenic human coronaviruses circulated in the population before the COVID-19 pandemic, and it cannot be entirely ruled out that they may provoke

autoimmunity and virus-associated ME/CFS, as molecular mimicry have been identified also between their antigens and human ones [250].

Returning to AAb to the GABA receptor and their potential role in ME/CFS, it is worth mentioning that experimental studies have demonstrated two pathogenic effects of these AAb from patients with autoimmune encephalitis (where they are present in high titers): 1) a reduction in the concentration of the corresponding receptors on the postsynaptic membrane caused by AAb binding to the receptors and their internalization; 2) direct blockade of the signal transmission without reduced receptor density on the cell membrane [123]. Both mechanisms result in the decreased effectiveness of GABAergic transmission (which is the major mechanism of inhibition in human CNS), which predispose individuals among other manifestations also to seizures.

Literature provides evidence of GABAergic transmission dysfunction in patients with ME/CFS. MR spectroscopy showed that GABA levels in the anterior cingulate cortex were elevated in ME/CFS patients, and the authors hypothesized that these neurochemical abnormalities might be linked to glial cell dysfunction in ME/CFS, specifically reactive astrogliosis [225]. Although GABAergic transmission is most commonly associated with inhibitory interneurons, astrocytes also have GABA receptors that modulate the secretion of other neurotransmitters [71]. Additionally, reactive astrocytes themselves can synthesize and release GABA, sometimes serving as a significant source of increased GABA levels in various brain regions. In animal models of Alzheimer's disease, hepatic encephalopathy, and ischemic stroke, increased brain GABA level has been associated with neuroinflammation, hypometabolic state of the nerve cells, impaired neuroplasticity, and cognitive dysfunction [71]. It is also noteworthy that central GABAergic mechanisms play a critical role in the physiological recovery from stress and in preventing distress-associated pathology [20].

In light of the hypothesis linking GABAergic transmission dysfunction in ME/CFS to reactive astrogliosis, it is important to note the significant increased level of AAb to GFAP (a key marker of the astrocyte injury and activation [277]) in the PCS patient group compared to the ME/CFS group. This result was expected, as chronic fatigue in all PCS

patients was of a post-viral origin (in contrast to the ME/CFS group, where patients reported various potential triggers of their disease onset), and the duration of the illness in the PCS group was significantly shorter than in the ME/CFS group (1.46 years versus 7 years). Reactive astrogliosis is often associated with neurotropic viral infections [277]. Previously, Danilenko et al. [43] showed that ME/CFS flares are associated with the abnormal level of AAb to GFAP in this group compared to healthy donors (notably, that bidirectional deviations of the AAb levels may be registered).

Autopsy studies of patients who died from COVID-19 revealed signs of reactive astrogliosis in deceased individuals (specifically elevated GFAP levels in the brain's white matter) and showed that astrocytes were a primary target of direct SARS-CoV-2 virus impact on CNS [281]. According to *in vivo* studies, elevated GFAP levels compared to healthy individuals were found both in blood and cerebrospinal fluid in hospitalized patients with moderate to severe disease (regardless of the presence of neurological symptoms during the acute phase of COVID-19), as well as in the blood of patients with mild COVID-19 in a week after negative SARS-CoV-2 PCR tests were obtained [100, 226].

The blood level of GFAP during the acute phase of COVID-19 correlated with the severity of the disease [100, 226]. The data regarding GFAP and AAb to this antigen are consistent with the principle of immunological clearance, formulated by the Franco-Russian immunologist Pierre Grabar and the Russian immunochemist I.E. Kovalev [18, 139]. According to the main tenet of this concept, the production of natural AAb is under negative feedback regulation based on the quantity/availability of the corresponding antigen molecules. In the development of acute or chronic diseases, there is cell death through apoptosis or necrosis, or abnormalities in the expression, secretion, and/or utilization of specific antigens. A persistent increase in the extracellular content of any endogenous antigen will inevitably be accompanied by quantitative shifts in the levels of the AAb of corresponding specificity.

Contradictory results have been obtained regarding the association of elevated GFAP levels in biological fluids with neurological symptoms – while most studies have not

found such a link [230], Spanos et al. [122] demonstrated that elevated GFAP levels in the blood of patients during the acute phase of COVID-19 were associated with the presence of neurological symptoms one year after recovery. Regarding the dynamics of GFAP levels in biological fluids in the remote period, the results of numerous studies also remain largely contradictory. Most authors have shown normalization of GFAP levels in the blood of patients within 3-6 months; however, two studies found that GFAP levels in PCS patients decreased over time, but remained elevated compared to healthy individuals in 11 months after the acute infection [152]. It was also true for a group of patients who had mild COVID-19 in 7 months after infection, regardless of whether they had symptoms in the long-term period or not [226]. According to Bark et al. [88] patients treated for COVID-19 in the intensive care unit who showed moderate cognitive decline (MoCA <26 points) in 3-6 months after discharge had higher GFAP levels in the blood than those with normal cognitive performance. However, patients with significant fatigue (determined as >26 points on MFI-20 scale) had lower GFAP blood levels compared to those with <26 points on MFI-20.

It is important to note that GFAP concentration in the blood increases within an hour after brain injury, peaks within 20-24 hours, and then decreases over 72 hours ($T_{1/2}$ 24-48 hours) [226]. Thus, elevated GFAP levels in the blood should indicate persistent reactive astrogliosis at the time of examination. It can be assumed that anti-GFAP AAb levels, which remain elevated for a longer period than the levels of GFAP, may provide information about the intensity and duration of preceding astrocyte injury/activation.

At the same time, it has been suggested that AAb themselves in PCS may damage nervous tissue leading to reactive astrogliosis. Chen et al. [311] demonstrated that injecting mice with IgG from PCS patients who had elevated serum levels of neurofilament light chains and GFAP (as markers of astrogliosis) induced increased mechanical and thermal sensitivity in the animals within 3 to 15 days.

It's important to note that in our study, deviations in the levels of AAb against GFAP were found in 26.3% of PCS patients, 15% of healthy individuals, and were absent in the ME/CFS group, making the differences significant only between the PCS and ME/CFS

groups. Additionally, abnormal levels of AAb against the GABA receptors were significantly more common in the ME/CFS group compared to the PCS group. These changes in the network of natural AAb, which is considered as the ‘mirror’ of the organism's physiological state [246], may reflect a natural history of post-viral ME/CFS. At first, activation of astrocytes in response to damaging factors leads to increased GFAP expression, which then triggers the production of anti-GFAP AAb; increased production of GABA by reactive astrocytes (resulting in higher levels of anti-GABA-R AAb) may result in excessive tonic inhibition (defined as the reduction of neuronal background activity due to GABA’s action on extrasynaptic neuronal receptors). This, in turn, is known to limit neuroplasticity and is associated with poorer functional recovery after stroke and progression of memory impairment in Alzheimer's disease [265, 310].

In our study, analysis of ME/CFS subgroups with either abnormal or normal levels of AAb against the GABA receptors revealed that patients in the former subgroup had significantly more pronounced physical fatigue and depression, further supporting the involvement of the GABAergic system in the pathogenesis of ME/CFS and the close relationship between the nervous and immune regulatory systems of the human body.

Thus, our findings indicate some differences in the pathogenesis of ME/CFS and PCS that are clinically similar. While there is evidence suggesting a key role of GABAergic neurotransmitter system dysfunction in ME/CFS (which is also characteristic of fatigue syndrome in multiple sclerosis [61]), in case of PCS a more significant contribution may come from the activation or dysfunction of the sympathoadrenal system (as reflected in the increased prevalence of abnormal levels of AAb against the membrane antigen of adrenal medullary cells) and possibly ongoing astrocyte activation (astrogliosis). However, given the results regarding AAb to GFAP levels in PCS, ME/CFS, and healthy individuals, as well as the non-specific nature of reactive astrogliosis, further research is needed to determine the specificity and sensitivity of GFAP and anti-GFAP AAb in the diagnosis of ME/CFS and PCS.

When discussing the regulatory function of the immune system, it is impossible to overlook the state of the microbiome in ME/CFS and PCS. The microbiota of various

human body loci plays an immunogenic role by stimulating local immunity and the development of the mucosa-associated lymphoid tissue (MALT), which includes tonsils, appendix, Peyer's patches, and solitary lymphoid follicles of the mucous membranes. The circulation of immune cells through these structures links the microbiota to systemic immunity. The gastrointestinal tract contains the highest number and diversity of microorganisms in the human body. The gut microbiota can be fundamentally divided into wall-adherent and luminal fractions. The former is more concentrated and differs in composition from the latter [29]. Faecal microbiota, which is most commonly studied in clinical practice, primarily reflects the state of the luminal microbiota. At the same time, it appears that the wall-adherent microbiota has a closer connection to the immune system. Osipov et al. [17, 237] developed a method for assessing the wall-adherent gut microbiota based on the evaluation of microbial markers (components of bacterial cell walls) in the blood, where they enter during the natural processes of microbial cell replication and death, followed by phagocytosis (which already implies the involvement of the immune system). The choice of this method to assess the microbiota composition in our study was driven by our primary focus not on the microbiota itself, but on its potential links to the immune and nervous systems in ME/CFS and PCS.

The increase in markers of facultatively anaerobic cocci in the blood, particularly Streptococcus mutans (which is well-known as a representative of oral microbiota and a key player in the development of dental caries), observed in our study in both patient groups compared to healthy individuals, is considered a microbiological criterion for small intestine bacterial overgrowth (SIBO) and the development of chronic duodenal dysfunction [3, 21]. Chronic fatigue is widespread in these conditions and is associated with malabsorption and the development of iron, vitamin B12, and vitamin D deficiencies, as well as with chronic endogenous intoxication [3]. Thus, targeted screening for SIBO in patients with ME/CFS and PCS appears to be reasonable.

On the other hand, an increase in Streptococcus mutans markers in the blood may be related to its invasion into the circulation from the oral cavity which occurs both in case of the progression of caries involving pulp damage and in periodontitis through micro-

damages to periodontal tissues [103]. It has been established that when Streptococcus mutans translocates into the bloodstream, strains expressing the collagen-binding protein Cnm have the ability to invade endothelial cells and persist intracellularly which is associated with the development of endocarditis, atherosclerosis, and small vessel damage in the brain, leading to cerebral microbleeds – a common cause of cognitive impairment [236, 284]. Finally, antigenic mimicry between the primary pathogenic factor of Streptococcus mutans (adhesion factor antigen I/II) and human peptides associated with cardiovascular diseases has been identified [195]. Therefore, thorough oral hygiene, as well as the treatment of not only caries but also gingivitis and periodontitis, particularly in carriers of Streptococcus mutans strains expressing the collagen-binding protein Cnm, may potentially prevent additional endothelial damage in patients with ME/CFS and PCS, who, as shown above, are characterized by signs of endothelial dysfunction.

Gas chromatography-mass spectrometry of microbial markers had not previously been used in the examination of microbiome in individuals with ME/CFS and PCS. In the only study where this method was used for the assessment of microbiome in patients with COVID-19 during the acute phase of the infection, significant changes compared to the control group were observed regarding many representatives. The increase in the level of Streptococcus mutans markers that we identified in PCS patients was also present in this group of patients [25].

The detailed consideration of the potential damaging effects of Streptococcus mutans on the health of ME/CFS and PCS patients was undertaken due to the correlation that we found between the levels of Streptococcus mutans microbial markers in the blood of ME/CFS patients and the severity of fatigue in this group.

It is also necessary to focus on the increase in markers of Alcaligenes spp. in ME/CFS patients compared to healthy individuals (which was also noted in PCS, though it did not reach statistical significance in this group). The significance of this finding is primarily due to the unique role of these microorganisms in the mucosal immune system: Alcaligenes spp. are major colonizers of gut-associated lymphoid tissue (GALT), including Peyer’s patches and lymphoid follicles [167]. However, there is reason to

consider these microorganisms as cosmopolitan species closely associated with lymphoid compartments of mucosa-associated lymphoid tissue in different parts of the gastrointestinal tract. For example, the content of Alcaligenes spp. in swabs from the oral cavity habitats of the microbiota from healthy children was twice as high as it was in saliva [26].

It is known that LPS of these bacteria stimulate dendritic cells which are in close contact with Alcaligenes spp. in the Peyer's patches. However, LPS of Alcaligenes spp. activate dendritic cells weaker than the LPS of pathogenic bacteria (due to relatively weak agonistic activity towards Toll-like receptor 4), thus ensuring the activation of antigen-presenting cells and enhancing the antigen-specific immune response without the excessive inflammatory reaction characteristic of pathogenic bacterial LPS [309]. The elevated levels of Alcaligenes spp. markers in ME/CFS patients compared to healthy individuals may reflect the involvement of the MALT in the pathogenesis of ME/CFS. This is MALT which is responsible for the immune activation in response to exogenous stimuli such as viruses or opportunistic pathogens like Streptococcus mutans and Enterococcus spp., whose markers were also elevated in the blood of patients with ME/CFS in our study. In the early stages of the disease, such activation of the immune response may be predominantly beneficial – this may explain why fatigue severity in PCS was negatively correlated with the increased levels of Alcaligenes spp. markers. However, if the immune activation persist, the negative impact of chronically excessive systemic action of pro-inflammatory cytokines (also known by the established, albeit not entirely accurate in general pathophysiology, term "low-grade systemic inflammation") on the functions of the nervous system may manifest. This can explain a positive correlation between the overall level of the immune system activity (as reflected by AIR, see section 3.7) and the severity of depression in the ME/CFS group. According to this hypothesis, the level of LPS in the blood of ME/CFS patients in our study was higher compared to healthy individuals (results of borderline statistical significance, $p=0.05$). From the pathophysiological perspective, these findings could be interpreted as a conflict between the local immune reaction (inflammation) tending to act on a systemic level, and

systemic neuroendocrine protective programs. This conflict may underlie failure of adaptation as the general basis for the development of various pathological conditions [44].

In conclusion, it should be noted that different aspects of this controversy between the immune and neuroendocrine components of the human body's immune-neuroendocrine regulatory network were identified in our study by various methods both in ME/CFS and PCS.

CONCLUSIONS

1. 45.7% of patients with chronic fatigue associated with PCS met all three of the most widely used clinical diagnostic criteria for ME/CFS, which highlights the importance of educational efforts among physicians to increase awareness of ME/CFS, modern approaches to its diagnosis and treatment.
2. Both patients with chronic fatigue associated with PCS and those with ME/CFS not related to COVID-19 exhibit a decrease in HRV, an increase in BPV, and a reduction in baroreflex sensitivity, that correlated with fatigue severity. This indicates a similar pattern of dysautonomia in PCS and ME/CFS.
3. Microcirculatory dysfunction in patients with chronic fatigue associated with PCS is characterized by a specific feature – reduced signal in laser doppler flowmetry during arterial occlusion compared to healthy controls, which may indicate microcirculatory stasis. In contrast, microcirculatory disturbances in ME/CFS patients correspond to the classic picture of endothelial dysfunction, which manifests as a decrease in peak flow during post-occlusive reactive hyperemia response.
4. Reduced CAR was observed in the group of ME/CFS not related to COVID-19 but not in the group of chronic fatigue associated with PCS, reflecting the role of HPA axis dysfunction in ME/CFS, which does not appear to be a significant factor in the pathogenesis of chronic fatigue in PCS.
5. Signs of polyclonal immune system activation were observed in both groups of patients. However, the analysis of natural AAb profiles, which reflect the expression of the corresponding autoantigens and the regulatory function of the immune system, revealed specific features of immune reactivity in PCS and ME/CFS. These include increased immune reactivity to GABA receptors in ME/CFS and to adrenal medulla, thyroglobulin, and GFAP in PCS. The changes observed in natural AAb profiles may indicate dysfunction in GABAergic transmission in ME/CFS and reactive astrogliosis combined with dysfunction of the sympathoadrenal system and a potential increased risk of autoimmune thyroid pathology in PCS.

6. Both patient groups showed elevated blood levels of Streptococcus mutans markers, which correlated with the fatigue severity in ME/CFS not related to COVID-19. According to the literature, elevation of these markers may serve as a microbiological sign of dysbiosis in the small intestine, leading to malabsorption and chronic endogenous intoxication. It may also result from the invasion of this microorganism from the oral cavity into the systemic circulation, which is associated with the development of endothelial dysfunction. Additionally, ME/CFS patients had elevated blood levels of Alcaligenes spp. markers, which are major colonizers of gut-associated lymphoid tissue. This finding may indicate mucosal immune dysfunction in ME/CFS and reflect chronic immune activation in response to exogenous stimuli, such as viruses or opportunistic pathogens like Streptococcus mutans.

7. A comparison of the function of the human body's regulatory systems (nervous, endocrine, and immune ones) and microcirculation between patients with chronic fatigue associated with PCS and those with ME/CFS not related to COVID-19 revealed significant differences from the healthy controls in both patient groups, suggesting some similarities in the pathogenesis of these conditions. However, it also highlighted specific features unique to each condition.

PRACTICAL RECOMMENDATIONS

1. It is advisable to conduct targeted screening for ME/CFS in patients with PCS using the IOM/NAM diagnostic criteria (2015) at the primary care level. In complex cases implementation of the 2003 CCC criteria, which have greater specificity for diagnosing ME/CFS, may be recommended in order to confirm a positive screening result.
2. Efforts should be made to raise awareness among physicians (especially general practitioners and internists) about ME/CFS, modern approaches to its diagnostics and therapy as reflected in the 2021 international consensus recommendations on ME/CFS. This is particularly important given the relatively high prevalence of ME/CFS in population which has been significantly increased due to cases of ME/CFS related to PCS.
3. Patients with ME/CFS and PCS should undergo HRV (and, if possible, BPV) assessment with spectral analysis during spontaneous breathing and paced breathing at 12 breaths/minute. This will allow to identify dysautonomia (as a pathogenic mechanism and objective biomarker of ME/CFS and PCS) and to evaluate separately dysfunction of sympathetic and parasympathetic nervous system. The detection of dysautonomia should prompt targeted identification of its most common clinical manifestation in ME/CFS and PCS – postural orthostatic tachycardia syndrome (POTS) through an orthostatic test. If dysautonomia is detected, it can serve as one of the therapeutic targets in ME/CFS and PCS: for example, patients may be offered biofeedback therapy based on HRV, which, thanks to modern fitness trackers, is accessible for self-application by patients at home. As part of biofeedback therapy, techniques of slow rhythmic diaphragmatic breathing at 6 breaths/min can be recommended to increase parasympathetic activity. Additionally, to correct the characteristic pattern of dysautonomia in ME/CFS and PCS (reduced parasympathetic nervous system activity and, in a subgroup of PCS, increased sympathetic nervous system activity), it is recommended to continue study of several methods that have shown good clinical effects in observational research works (non-invasive vagus nerve stimulation, the use of cholinesterase inhibitors (pyridostigmine),

acetylcholine receptor agonists (nicotine in the form of transdermal therapeutic systems) for reduced parasympathetic activity; stellate ganglion blockade for increased sympathetic activity) [118, 134].

4. It is recommended that ME/CFS and PCS patients undergo functional assessment of microcirculation due to the high prevalence of its dysfunction. For this purpose, LDF-based endothelial-dependent vasodilation assessment during an arterial occlusion test can be used; in the future, studying the potential of other methods for the evaluation of microcirculation (finger plethysmography, optical tissue oximetry, vital computer capillaroscopy, etc.) is promising. Considering the identified patterns of microcirculation disorders in ME/CFS and PCS, one of the directions of the pathogenetic therapy in these conditions should be the correction of endothelial dysfunction and blood rheology. To this end, hyperbaric oxygen therapy, extracorporeal hemocorrection technologies, and some drugs such as pentoxifylline, sulodexide, and pyridostigmine, which have already demonstrated positive effects, are being studied [134].

5. Laboratory assessment of CAR can be used as a screening tool for the HPA axis dysfunction in ME/CFS. The absence of CAR can serve as a guideline for selecting patients for clinical trials of the pathogenetic therapy targeting this aspect (physiological doses of hydrocortisone as replacement therapy [59]). However, the high prevalence of reduced CAR in healthy individuals (30%) necessitates further research to find the optimal method for the evaluation of the HPA axis function in ME/CFS (e. g. determination of dehydroepiandrosterone and cortisol in saliva at multiple points throughout the day).

6. Evaluation of the spectrum of natural AAb cannot currently be recommended on an individual level in clinical practice due to the high prevalence of various deviations from the reference values among healthy individuals and the uncertainty of the clinical significance of the identified changes. However, statistically significant differences found in patient groups compared to healthy individuals suggest that in the development of pathogenetic therapy treatment options with potential impact on GABAergic transmission in ME/CFS and on the process of astrogliosis and the activity of the sympathoadrenal

system in PCS should be considered. Further research on natural autoimmunity is also recommended, with mandatory inclusion of a control group of healthy individuals to confirm the reproducibility of the identified deviations in natural autoimmunity in PCS and ME/CFS.

7. The data obtained on changes in blood microbial markers in ME/CFS and PCS suggest that targeted diagnosis and treatment of small intestinal bacterial overgrowth, as well as careful oral hygiene and treatment of not only caries but also periodontal disease in these groups should be recommended to reduce chronic endogenous intoxication.

LIST OF ABBREVIATIONS

12BR	- paced breathing at a rate of 12 breathing cycles per minute
6BR	- paced breathing at a rate of 6 breathing cycles per minute
AAb	- autoantibodies
AIR	- average individual immunoreactivity
ASIA	- Autoimmune/Autoinflammatory Syndrome Induced by Adjuvants
AUC	- area under the curve
AUC asc	- area under the curve for the ascending part of the post-occlusive reactive hyperaemia curve
AUC 1 min	- area under the curve for the first minute of the post-occlusive reactive hyperaemia curve
AUCg	- total area under the cortisol curve
AUCi	- area under the cortisol curve above the awakening cortisol value
BEI	- baroreflex effectiveness index
BMI	- body mass index
BPV	- blood pressure variability
BRS	- baroreflex sensitivity
BZ	- biological zero
CAR	- cortisol awakening response
CCC 2003	- Canadian Consensus Criteria 2003
CDC	- Centers for Disease Control and Prevention
CDC 1994	- Fukuda criteria 1994
CNS	- central nervous system
COVID-19	- Coronavirus disease 2019
DBP	- diastolic blood pressure
DBPV	- diastolic blood pressure variability
DCS	- diurnal cortisol slope
DD	- disease duration
DSQ-SF	- DePaul Symptom Questionnaire Short Form
EUROMENE	- European Network on Myalgic Encephalomyelitis/Chronic

Fatigue Syndrome

FR - flow reserve

GABA - gamma-aminobutyric acid

GF - general fatigue

GFAP - glial fibrillary acidic protein

HADS - Hospital Anxiety and Depression Scale

HC - healthy controls

HF - high frequency

HLA - human leukocyte antigens

HPA - hypothalamic-pituitary-adrenal

HRV - heart rate variability

ICD - International Classification of Diseases

IOM/NAM - Institute of Medicine/US National Academy of Medicine criteria 2015

2015

IPAQ - International Physical Activity Questionnaire (Short Form)

LDF - laser doppler flowmetry

LF - low frequency

LPS - lipopolysaccharide

Me - median

ME/CFS - myalgic encephalomyelitis/chronic fatigue syndrome

MET - metabolic equivalent of task

MF - mental fatigue

MFI-20 - Multidimensional Fatigue Inventory

PA - physical activity

PEM - post-exertional malaise

PCS - post-COVID-19 syndrome

PF - peak flow

PhF - physical fatigue

PORH - post-occlusive reactive hyperemia

P.U. - perfusion units

RA - reduced activity

RM - reduced motivation

RF - rest flow
RPF - reperfusion flow
RSA - respiratory sinus arrhythmia
SBP - systolic blood pressure
SBPV - systolic blood pressure variability
T1/2 - half recovery time
TP - total power
T_{PF} - time to peak flow
VAS - visual analogue scale
VLF - very low frequency
Vmax - rate of achieving peak flow
WHO - World Health Organization

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ANNEX A

DePaul Symptom Questionnaire - Short Form, DSQ-SF

For each symptom below, please circle **one number for frequency and one number for severity**:

Please complete the chart from left to right.

Frequency:	***Severity:***
Throughout the **past 6 months**, **how often** have you had this symptom? For each symptom listed below, circle a number from: **0 = none of the time** **1 = a little of the time** **2 = about half the time** **3 = most of the time** **4 = all of the time**	Throughout the **past 6 months,** **how much** has this symptom bothered you? For each symptom listed below, circle a number from: **0 = symptom not present** **1 = mild** **2 = moderate** **3 = severe** **4 = very severe**

	Frequency:				**Severity:**			
1. Fatigue/extreme tiredness	1	2	3	4	1	2	3	4
2. Next day soreness or fatigue after non-strenuous, everyday activities	1	2	3	4	1	2	3	4
3. Minimum exercise makes you physically tired	1	2	3	4	1	2	3	4
4. Feeling unrefreshed after you wake up in the morning	1	2	3	4	1	2	3	4
5. Pain or aching in your muscles	1	2	3	4	1	2	3	4
6. Bloating	1	2	3	4	1	2	3	4
7. Problems remembering things	1	2	3	4	1	2	3	4
8. Difficulty paying attention for a long period of time	1	2	3	4	1	2	3	4
9. Irritable bowel problems	1	2	3	4	1	2	3	4
10. Feeling unsteady on your feet, like you might fall	1	2	3	4	1	2	3	4
11. Cold limbs (e.g. arms, legs, hands)	1	2	3	4	1	2	3	4
12. Feeling hot or cold for no reason	1	2	3	4	1	2	3	4
13. Flu-like symptoms	1	2	3	4	1	2	3	4
14. Some smells, foods, medications, or chemicals make you feel sick	1	2	3	4	1	2	3	4