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Article

Myalgic Encephalomyelitis/Chronic Fatigue Syndrome and Post-COVID Syndrome: A Common Neuroimmune Ground?

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Abstract: A Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is a debilitating chronic disease of unknown aetiology under growing interest now in view of the increasingly recognized post-COVID syndrome as a new entity with similar clinical presentation. We performed the first cross-sectional study of ME/CFS in community population in Russia and then described and compared some clinical and pathophysiological characteristics of ME/CFS and post-COVID syndrome as neuroimmune disorders. Of the cohort of 76 individuals who suggested themselves suffering from ME/CFS 56 subsequently were confirmed as having CFS/ME according to ≥1 of the 4 most commonly used case definition. Of the cohort of 14 individuals with post-COVID-19 syndrome 14 met diagnostic criteria for ME/CFS. The prevalence of clinically expressed and subclinical anxiety and depression in ME / CFS and post-COVID ME/CFS did not differ significantly from that in healthy individuals. Severity of anxiety / depressive symptoms did not correlate with the severity of fatigue neigther in ME / CFS nor in post-COVID ME/CFS, but the positive correlation was found between the severity of fatigue and 20 other symptoms of ME / CFS related to the domains of “post-exertional exhaustion”, “immune dysfunction”, “sleep disturbances”, "dysfunction of the autonomic nervous system", "neurological sensory / motor disorders" and "pain syndromes". Immunological abnormalities were identified in 12/12 patients with ME / CFS according to the results of laboratory testing. The prevalence of postural orthostatic tachycardia assessed by the active standing test was 37.5% in ME / CFS and 75.0% in post-COVID ME/CFS (the latter was higher than in healthy controls, $p = 0.02$) There was a more pronounced increase in heart rate starting from the 6th minute of the test in post-COVID ME/CFS compared with the control group. Assessment of the functional characteristics of microcirculation by laser doppler flowmetry revealed obvious and very similar changes in ME/CFS and post-COVID ME/CFS compared to the healthy controls. The identified pattern corresponded to the hyperemic form of microcirculation disorders, usually observed in acute inflammatory processes or in deficiency of systemic vasoconstriction influences.

Keywords: chronic fatigue syndrome; post-COVID syndrome; postural orthostatic tachycardia; microcirculation; immune system

1. Introduction

Myalgic encephalomyelitis/chronic fatigue syndrome (ME / CFS) is a chronic acquired disease characterized by pathological fatigue, with worsening of fatigue and other symptoms after physical or mental exertion, which often lasts more than 24 hours (so-called post-exertional malaise), unrefreshing sleep, cognitive impairment, pain syndromes, neuroendocrine and immune dysfunctions of and the disorder of autonomic

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nervous regulation (dysautonomia)[1]. The prevalence of ME/CFS according to the recent systemic review is 0.89%, and the disorder is characterized by an approximately 1.5-fold predominance of women[2]. A Norwegian population-based study revealed two age peaks for incidence rate: ϕges 10–19 and 30–39 years[3]. It was noted that in some countries (e.g., Poland), ME/CFS is diagnosed very rarely, which could be related to lack of knowledge and understanding of ME/CFS among general practitioners and the lack of detailed and uniform guidelines allowing an unambiguous diagnosis and initiation of effective treatment in ME/CFS[4,5]. To date ME/CFS in Russia has neither been reported in English-language medical literature, nor its prevalence estimated. There are currently no ME/CFS clinical services in Russia. Given the global prevalence of ME/CFS, it could be suggested that more than 2.5 millions of Russian citizens may suffer from ME/CFS remaining undiagnosed.

ME/CFS is a topic of growing interest now in view of the post-COVID syndrome as an increasingly recognized new clinical entity[6–10]. Early studies which described a constellation of symptoms experienced by 10-30% of people after resolution of acute COVID-19 suggested many overlaps with clinical presentation of ME/CFS[7,8]. Emerging longitudinal studies tracking post-COVID-19 patients' symptoms over 6 months or more (as this is one of the criteria for ME/CFS) confirm increasing number of links between post-COVID syndrome and ME/CFS[8]

2. Materials and Methods

Participants

We performed the first pilot cross-sectional study of ME/CFS in a community population in Russia and then described and compared some clinical and pathophysiological characteristics of ME/CFS and post-COVID syndrome as neuroimmune disorders. Following approval from The Ethics Committee of St Petersburg University, individuals with ME/CFS and post-COVID syndrome as well as healthy volunteers were recruited as part of a study of autoimmune dysautonomia. Requests for participation were made through messages to online support groups and social media. Post-COVID syndrome was defined as the presence of symptoms and / or signs of damage to various organ systems that develop during or after a previous infection with COVID-19, persisting for more than 12 weeks and cannot be explained by an alternative diagnosis [11].

Three cohorts were formed:

1. A cohort of patients from 18 to 75 years old who met ≥1 of the 4 most commonly used ME/CFS case definitions (the Fukuda et al. (1994) CFS criteria [12], the Canadian ME/CFS criteria [13], the Myalgic Encephalomyelitis International Consensus Criteria (ME-ICC) [14], and the Institute of Medicine criteria), in whom the onset of the disease was not associated with COVID-19;
2. A cohort of patients from 18 to 75 years old who met ≥1 of the 4 most broadly used ME / CFS case definitions and those symptoms developed following acute COVID-19;
3. Control group (healthy volunteers from 18 to 75 years old).

The exclusion criteria were:

For the first group: the presence of any of the diseases/conditions from the Table 1 unless complete remission was achieved

Table 1 Exclusion criteria for the 1 cohort (exclusion criteria for diagnosis of ME/CFS)[15]

- endocrine diseases / metabolic disorders: primary adrenal cortex insufficiency, Cushing's syndrome, hyper- and hypothyroidism, diabetes mellitus, hypercalcemia;
- rheumatological diseases: systemic lupus erythematosus, rheumatoid arthritis, polymyositis;
- hematological diseases: iron deficiency anemia, hemochromatosis, idiopathic thrombocytopenic purpura;
- infectious diseases: HIV infection, hepatitis B, hepatitis C, tuberculosis, Lyme disease, giardiasis, helminthiasis, syphilis;
- neurological diseases: multiple sclerosis, narcolepsy, obstructive sleep apnea, restless legs syndrome, Parkinson's disease, myasthenia gravis, vitamin B12 deficiency, cervical spine injuries, epilepsy;
- psychiatric illnesses: bipolar disorder, substance dependence, generalized anxiety disorder, schizophrenia, major depressive disorder;
- gastrointestinal diseases: celiac disease, Crohn's disease, ulcerative colitis
- cardiovascular diseases with congestive heart failure;
- chronic intoxication with heavy metals (lead, mercury);
- the development of the patient's symptoms as side effects of any drugs;
- respiratory diseases (chronic obstructive pulmonary disease, bronchial asthma) with the development of chronic respiratory failure;
- overwork (work more than 50 hours a week), overtraining syndrome;
- body mass index over 40;

For the second cohort:

- the presence of any symptoms (including chronic fatigue) before acute COVID-19.

For the third group:

- complaints of chronic fatigue
- in case of previous viral infections, including COVID-19 <4 weeks after recovery to the moment of enrollment in the study.

Symptom assessment tools

The ME / CFS Symptom Questionnaire DePaul Symptoms Qustionnaire-2

(DSQ-2) is a revised version of DSQ-1, a standardized self-report that assesses the symptomatology of ME / CFS, as well as medical, psychiatric, and social history data[16]. DSQ has demonstrated high reliability and validity, as well as the ability to accurately differentiate ME / CFS patients with other chronic diseases and healthy people from the control group[17]. In DSQ-2, participants rated the frequency and severity of each symptom over the past 6 months on a five-point Likert scale (frequency scale: 0 = never during this time; 1 = rarely (up to 1-2 times a week); 2 = often (3-4 times a week); 3 = very often (almost every day); 4 = every day; severity scale: 0 = no such symptom; 1 = mildly disturbing symptom; 2 = moderately disturbing symptom; 3 = symptom quite disturbed; 4 = very disturbed symptom). Further processing of the results was carried out by correlating the answers of the subject with a key that allows determining the participant's compliance with the four most common sets of diagnostic criteria for ME / CFS in the world clinical and research practice: Fukuda et al. (1994); Canadian ME / CFS (Carruthers et al., 2003); ME-ICC (Carruthers et al., 2011); Institute of Medicine (IOM, 2015). Composite scores were also calculated for each symptom by averaging the scores for the frequency and severity of the symptom and multiplying by 25 to obtain scores from 0 to 100 (higher scores indicate more pronounced symptom manifestation)[17].

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The Hospital Anxiety and Depression Scale (HADS)[18] was used for the assessment of anxiety and depression in this study. It has been shown that HADS is a reliable scale for identifying and assessing the severity of symptoms of anxiety disorders and depression, both among patients with somatic diseases and among patients with mental disorders; both in primary care patients and in the general population[19]. The participants filled in the questionnaire by themselves. Evaluation of the results was carried out for each subscale (anxiety and depression) in accordance with the key: 0-7 points - the norm; 8-10 points "doubtful case of anxiety / depression"; 11 points or more - "probable case of anxiety / depression"

Anamnesis

In order to exclude diseases indicated as the exclusion criteria for ME/CFS, we took the history of the present disease and personal medical history. We analyzed results of the laboratory tests, instrumental evaluation, and medical reports from the specialists in the first group. Results of the immunological testing were analyzed separately for those patients who got tested before.

Active orthostatic test

We perform the active orthostatic test according to the protocol of F. Schellong[20]. The participants were informed about the need to refrain from consuming caffeine- and alcohol-containing beverages and smoking on the day of the test. The last meal was to be light, no later than 3 hours before the test. These recommendations were made in order to minimize the influence of external factors on hemodynamic parameters. We performed the test no later than 4 p.m.. During the test, we placed a compression cuff for measuring blood on the shoulder of the participant and did not remove it until the end of the study. We also placea pulse oximeter on the index finger of the other hand in order to continuously measure heart rate. The participant was asked to lie quietly on the couch for 10 minutes. After that we measured blood pressure and heart rate. We took these values of blood pressure and heart rate as the baseline, and the patient was asked to stand up calmly, spread his legs shoulder-width apart and stand relaxed for 10 minutes. Immediately after getting up, the heart rate was determined, and then at the end of each subsequent minute, the blood pressure and heart rate were measured, and the person's subjective feelings were also assessed. The syndrome of postural orthostatic tachycardia was diagnosed with an increase in heart rate by 30 or more per 1 minute in the standing position during 30 seconds or more and the absence of orthostatic hypotension (drop in systolic blood pressure> 20 mm Hg)[21]. This increase in heart rate had to be stable - that is, to manifest at least in two consecutive heart rate measurements[21].

Assessment of microcirculation

We assessed microcirculation by amplitude– frequency wavelet analysis of blood flow oscillations with laser Doppler flowmetry (LDF) [22]. Because of proven parallelism between microcirculatory changes in the skin and inner organs, we investigated forearm blood flow in the participants with «LASMA MC-1» peripheral blood and lymph flow laser diagnostic complex (LASMA LLC, Russia). Participants were examined once, with the diagnostic probe placed on external surface of the right forearm for 2 minutes. The protocol for the study of microcirculation with LDF included: 1. Determination of the average value of tissue perfusion with blood, M; 2. Determination of the mean square deviation of M oscillations in a given time interval, σ; 3. Determination of the oscillation index, IFM. 4. Spectral analysis of biorhythms of tissue blood flow oscillations with determination of oscillation amplitudes in given frequency ranges: low frequency (LF) 0.05-0.2 Hz, high frequency (HF) 0.2-0.4 Hz, pulse frequency (PF) 0.8-1.6 Hz, as well

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as determining the contribution of individual frequency ranges to the total power of the spectrum of biorhythms. 5. Determination of microvascular tone and vascular resistance. Amplitude–frequency wavelet analysis of blood flow oscillations was performed. Time-averaged vasomotion amplitude was assessed using maximum values (Amax) in the corresponding frequency band (Fmax). The whole technique was detailed elsewhere [23]. The following indices were calculated: the contribution of slow oscillations (vALF) (0.05-0.2 Hz); contribution of fast (venular) oscillations (vAHF) (0.2-0.4 Hz); contribution of pulse oscillations (vACF) (0.8-1.6 Hz); IFM, vascular resistance and microvascular tone. The contribution of the corresponding frequency range (v: vALF, vAHF, vACF) was determined as the percentage of the squared amplitude of a given range (A) to the total spectrum power (M), which is the sum of the squared amplitudes over 3 ranges.

$$M=A^2LF+A^2HF+A^2\ CF \quad (1)$$

$$v=A^2/M*100\% \quad (2)$$

The oscillation index (IFM) is an indicator of the ratio of the mechanisms of active and passive modulation of tissue blood flow and is determined by the ratio of the average amplitudes of oscillations:

$$IFM = ALF / (AHF + ACF) \quad (3)$$

This index characterizes the overall efficiency of microcirculation regulation. Vascular resistance was calculated as the ratio of the sum of the amplitudes of fast and pulse oscillations and the mean square deviation of M:

$$R = (AHF + ACF) / \sigma \quad (4)$$

Normalization of the amplitude of low-frequency oscillations (ALF) relative to the mean square deviation of M allows us to assess the microvascular tone, which was calculated as:

$$CT = \sigma / ALF \quad (5)$$

Perfusion value (M), standard deviation (σ) and amplitudes of blood flow modulation sections were assessed in arbitrary perfusion units.

Statistical analyses

Statistical analyses were conducted with GraphPad Prism v. 9.1.1. (USA) with the assumed level of statistical significance of $\alpha<0.05$. The normality of continuous variables distribution was evaluated with the Shapiro–Wilk test. Statistical characteristics of measured values were presented for the normal variables as arithmetic means ±SD and for non-normal variables as median and interquartile range [25%; 75%]. Depending on the number of independent samples, the Mann–Whitney U test or the Kruskal-Wallis were used to evaluate significance of differences between measured values obtained in the cohorts. For categorical variables, the Pearson χ-square test and Fisher's exact test were performed. Pearson correlation tests was used to assess the association between composite score for “Fatigue / Extreme tiredness” (question 13 in DSQ-2) and: 1) composite score for every other of the 90 symptoms from the DePaul Symptom Questionnaire; 2) HADS-A and HADS-D subscales scores.

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3. Results

3.1. Participants

Among the members of the community of patients who suspected they had a diagnosis of ME / CFS, 76 people completed the questionnaire DSQ-2. Of these, 56 people who met all inclusion and exclusion criteria were selected into the first cohort. 5 people were excluded from the study because they did not meet any of the four ME/CFS clinical definition. 15 people were excluded from the study because they met the exclusion criteria (i.e. suffered from other medical conditions that could potentially cause chronic fatigue).

Among the members of the community of patients with post-COVID syndrome, 15 people completed the DSQ-2 questionnaire. Of these, 14 people were selected for the second cohort meeting all the inclusion and exclusion criteria. 1 person was excluded because met the exclusion criteria (over 75 years of age).

The control group of healthy individuals consisted of 9 volunteers who met all the inclusion and exclusion criteria.

The demographic of the sample population is summarized in Table 2. Participants from three groups were comparable regarding their age and sex.

Table 2. Characteristics of the participants in ME/CFS, post-COVID ME/CFS and controls cohorts.

Parameters	**1st cohort (ME/CFS)**	**2nd cohort (post-COVID ME/CFS)**	**3rd cohort (healthy controls)**	**p value**
Number of participants	n = 56	n = 14	n = 9	
Age	39,3 [31,4;45,9]	34,9 [29,8;40,2]	31,7 [22,2;45,1]	0,28
Sex, male/female.	18/38	4/10	4/5	0,71

3.2. Clinical and anamnestic characteristics of ME/CFS as neuroimmune disorder

We obtain information about possible triggers for the development of ME / CFS from the answers of the participants of the 1st cohort to the 110th question of DSQ-2 ("Did your fatigue/energy related illness start after you experienced any of the following? Check one or more and please specify"). 35 people (62.5%) chose the response option "an infectious illness", 23 people (41.1%) chose the response option "severe stress s (bad or unhappy event(s))". At the same time, both these answer options were chosen by 13 people (23.2%).

Analysis of the relationship between composite score of fatigue and every other of the 90 symptoms from DSQ-2 revealed a statistically significant positive correlation between the severity of fatigue and other 20 symptoms listed in the Table 3.

Seven of these symptoms belonged to the "post-expression malaise" domain, which is one of the key manifestations of ME / CFS[1]. 4 symptoms belonged to the domain of "immune dysfunction", 4 symptoms - to the domain of "sleep disorders", 2 symptoms to the domain "neurological sensory / motor disorders", 2 symptoms to the domain "dysfunction of the autonomic nervous system", and 1 to the domain of "pain syndromes" . The data obtained are consistent with the concept of ME / CFS as a neuroimmune disease.

Table 3 Symptoms that correlate with the severity of fatigue according to the composite symptoms scores from the DSQ-2 questionnaire

Question from DSQ-2, (number of question in DSQ-2)	Symptom's domain	r; p value
Dead, heavy feeling after starting to exercise (14)	PEM	0,62; <0,0001
Next-day soreness or fatigue after non-strenuous, everyday activities (15)	PEM	0,63; <0,0001

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Mentally tired after the slightest effort (16)	PEM	0,31; 0,02
Minimum exercise makes you physically tired (17)	PEM	0,60; <0,0001
Physically drained or sick after mild activity (18)	PEM	0,71; <0,0001
Feeling unrefreshed after you wake up in the morning (19)	Sleep disorder	0,47; 0,0003
Needing to nap daily (20)	Sleep disorder	0,29; 0,03
Sleeping all day and staying awake all night (24)	Sleep disorder	0,37; 0,01
Pain or aching in your muscles (25)	Pain syndromes	0,38; 0,004
Muscle weakness (33)	Neurological sensory / motor symptoms	0,44; 0,0007
Nausea (48)	Dysautonomia	0,31; 0,02
Feeling unsteady on your feet, like you might fall (49)	Neurological sensory / motor symptoms	0,26; 0,05
Sore throat (64)	Immune dysfunction	0,28; 0,04
Tender / Sore lymph nodes (65)	Immune dysfunction	0,27; 0,045
Flu-like symptoms[1] (67)	Immune dysfunction	0,30; 0,02
Muscle fatigue after mild physical activity (75)	PEM	0,31; 0,02
Worsening of symptoms after mild mental activity (77)	PEM	0,32; 0,01
Daytime drowsiness (84)	Sleep disorder	0,32; 0,02
Sinus infections[2] (87)	Immune dysfunction	0,27; 0,04
Urinary urgency (88)	Dysautonomia	0,39; 0,01

1 A combination of several of the following symptoms: high body temperature, headaches and muscle aches, cough, sore throat, severe fatigue, nasal congestion or runny nose, chills, nausea or vomiting

2 Symptoms of sinusitis (discomfort in the cheekbones, bridge of the nose or above the eyes, often accompanied by persistent headache, nasal congestion, persistent nasal discharge).

3.3. Mental health screening in patients with ME / CFS and assessment of the relationship between mental health and severity of fatigue

The HADS questionnaire was completed by 46 patients from the first group, 14 people from the second group and 9 people from the third group. The characteristics of the groups according to the presence and severity of depressive symptoms according to the HADS-D subscale are presented in Table 4

Table 4 Presence and severity of depressive symptoms in the cohorts according to HADS-D subscale

	1st cohort (ME/CFS)	**2nd cohort** (post-COVID ME/CFS)	**3rd cohort** (healthy controls)	**p value**		
				1st vs 3rd	2nd vs 3rd	1st vs 2nd
Probable case of depression n, %	13 (28,3%)	5 (35,7%)	0 (0%)	0,10	0,12	>0,99
Doubtful case of depression n, %	19 (41,3%)	5 (35,7%)	3 (33,3%)	0,73	>0,99	>0,99
Absence of depression n, %	14 (30,4%)	4 (28,6%)	6 (66,7%)	0,06	0,10	>0,99

When assessing intergroup differences, it was found that the differences in the incidence of depression according to HADS-D between the groups were not statistically significant.

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At the same time, as can be seen from Table 5, the median values of the severity of depression according to HADS-D among the studied groups are higher in the groups of patients compared with healthy controls.

Table 5 Medians of the severity of depressive symptoms in the cohorts according to HADS-D subscale scores

	1st cohort (ME/CFS)	**2nd cohort** (post-COVID ME/CFS)	**3rd cohort** (healthy controls)	**p value**	
				1st vs. 3rd	2nd vs 3rd
Severity of depressive symptoms, HADS-D score	8,0 [6,0; 11,0]	8,5 [4,75; 12,0]	2,0 [1,0; 8,0]	0,002	0,002

The characteristics of groups according to the presence and severity of anxiety symptoms according to the HADS-A subscale are presented in Table 6

Table 6 Presence and severity of anxiety symptoms in the cohorts according to HADS-A subscale

	1st cohort (ME/CFS)	**2nd cohort** (post-COVID ME/CFS)	**3rd cohort** (healthy controls)	**p value**		
				1st vs 3rd	2nd vs 3rd	1st vs 2nd
Probable case of anxiety disorder n, %	9 (19,6%)	7 (50,0%)	2 (22,2%)	>0,99	0,27	0,07
Doubtful case of anxiety disorder n, %	22 (47,8%)	2 (14,3%)	1 (11,1%)	0,06	>0,99	0,03
Absence of anxiety symptoms n, %	15 (32,6%)	5 (35,7%)	6 (66,7%)	0,07	0,21	>0,99

When assessing intergroup differences, it was found that the incidence of anxiety according to HADS in the 2nd group was higher than in the 1st group, but not in the 1st or 2nd group compared to healthy controls.

At the same time, there was no statistically significant difference between the median values of the severity of anxiety according to HADS among the studied groups of patients (Table 7).

Table 7 Medians of the severity of anxiety symptoms in the cohorts according to HADS-A subscale scores

	1st cohort (ME/CFS)	**2nd cohort** (post-COVID ME/CFS)	**3rd cohort** (healthy controls)	**p value**	
				1st vs. 3rd	2nd vs 3rd
Severity of anxiety symptoms, HADS-A score	8,0 [5,8; 10,0]	9,5 [5,3; 15,3]	6,0 [1,5; 9,5]	0,41	0,13

In order to test the hypothesis about the relationship between fatigue and mental health in the first group, a correlation analysis was performed, which did not reveal a significant relationship between severity of the depressive or anxiety symptoms and the severity of fatigue (Table 8).

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Table 8 Results of the correlation analysis between severity of the depressive or anxiety symptoms and the severity of fatigue

		r; p
Severity of fatigue (the composite score of the 13th question of the DSQ-2 questionnaire)	Severity of depressive symptoms (HADS-D score)	0,11; 0,44
Severity of fatigue (the composite score of the 13th question of the DSQ-2 questionnaire)	Severity of anxiety (HADS-A score)	-0,18; 0,22

3.4. Assessment of the immune status in patients with ME / CFS

12 patients from the 1st cohort had results of a previous a primary immunological examination, which consisted of a comprehensive assessment of the lymphocytes subpopulations and, in some cases, was supplemented by an assessment of the level of circulating immune complexes, C3-, C4-components of complement, the study of interferon status and indicators of the function of the granulocytes and monocytes. Despite the fact that 5/12 patients had an increase in the relative number of CD3 + cells, an increase in the absolute number of these cells was found only in one patient. The same patient had an increase in the number of CD3 + CD4+ cells. Another patient had a decrease in the number of CD3+CD4+. Despite the fact that a decrease in the absolute number of CD3 + CD8 + cells was detected in the single patient, 5 patients had an increase in the immunoregulatory index (CD4+/CD8+) of more than 2.0. At the same time, two patients had a decrease in the immunoregulatory index of less than 1.0. In 5 people, laboratory analysis included the determination of the absolute content of double positive CD4 + CD8+ cells. In 3/5 patients the number of these cells was lower than 25% percentile of the reference values.. The relative number of NK cells, determined in all 12 patients, was reduced in three patients according to the latest data on reference values in the Russian population[24]. One patient had an increase in the number of these cells. However, in 8/12 patients number of NK cells was lower than 25% of the reference values. Most of the patients had normal B lymphocyte counts. One person showed a decrease and one - an increase in the number of these cells. An additional analysis of subpopulations of B lymphocytes carried out in two people revealed that in the patient with an increase in the total number of B lymphocytes, it occurred mainly due to B2 lymphocytes and B memory cells. The level of C3 component of complement was determined in 5 people - in 2 of them an increase in its level was recorded, and in 1 person - a decrease. The serum levels of interferones (IFNs), basal and induced levels of the IFNs secretion by leukocytes was analyzed in 4 people. All of them had an increase in serum IFNα levels. Moreover, the induced production of IFNα by leukocytes was reduced in 3/4 people. The induced secretion of IL-1b, measured in 3 people, was markedly increased in all patients. Assessment of the functional properties of granulocytes and monocytes was carried out in three people, however, due to the difference in the methods used (inhibition of leukocyte migration, Nitroblue Tetrazolium Test, killing coefficient, phagocytic index, phagocytic number), it was not possible to compare the data between the participants.

3.5. Identification of autonomic dysfunction during an active orthostatic test

An active orthostatic test was performed in some participants from all three cohorts. 6/16 people in cohort 1, 6/8 people in cohort 2 and 1/6 healthy controls met the criteria for postural orthostatic tachycardia syndrome (POTS). As can be seen from Table 9, POTS was statistically significantly more frequent in the ME / CFS group that developed after COVID-19 than in the control group of healthy individuals.

Table 9 Prevalence of POTS in the participants with the ME / CFS developed outside the context of COVID-19, ME / CFS that developed after COVID-19, and healthy controls

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	1st cohort (ME/CFS)	**2nd cohort** (post-COVID ME/CFS)	**3rd cohort** (healthy controls)	**p value**	
				1st vs. 3rd	2nd vs. 3rd
Prevalence of POTS	6 (37,5%)	6 (75,0%)	1 (11,1%)	0,35	0,02

An alternative form of orthostatic intolerance, i.e. orthostatic hypotension was detected only in 1 person in cohort 1, 1 person in cohort 3 and none of the people in cohort 2.

To test the hypothesis that hemodynamic disorder of the POTS type is one of the characteristics of the ME / CFS that developed after COVID-19, and there exists a causal association between these two condition rather than a coincidence, we calculated the average increase in heart rate relative to the basal values in all three study cohorts for each minute of the test. Pairwise comparison of these values between cohorts at each minute of the test revealed that the ME/CFS developing after COVID-19 was characterized by a statistically more pronounced increase in heart rate at the 6th, 7th, 8th, 9th and the 10th minute of the test compared with the control group and at the 8th and 9th minutes of the test compared with the ME / CFS developed outside the context of COVID-19. These results support our hypothesis.

3.6. Assessment of the dynamic characteristics of microcirculation with laser doppler flowmetry

A non-invasive study of blood microcirculation parameters using the LDF method was carried out in 11 participants from cohort 1, 7 participants from cohort 2, and 7 healthy individuals. When processing results, one person from cohort 1 was excluded from the analysis, because due to the short period of signal registration, no peak was registered in the amplitude-frequency spectrum in the low-frequency range. The parameters of skin microcirculation in the cohorts are presented in Table 10.

Table 10 Characteristics of microcirculation assessed with laser doppler flowmetry in the participants with the ME / CFS developed outside the context of COVID-19, in the participants with ME / CFS that developed after COVID-19, and healthy controls. M - average value of tissue perfusion with blood, σ - mean square deviation of M oscillations in a given time interval, vALF - contribution of low frequency oscillations (0.05-0.2 Hz) to the total power of the spectrum of biorhythms; vAHF - contribution of high frequency oscillations (0.2-0.4 Hz) to the total power of the spectrum of biorhythms; vACF - contribution of pulse frequency oscillations (0.8-1.6 Hz) to the total power of the spectrum of biorhythms; IFM – the oscillation index; R - vascular resistance; CT - microvascular tone

Parameters	**1st cohort** (ME/CFS)	**2nd cohort** (post-COVID ME/CFS)	**3rd cohort** (healthy controls)	**p value**		
				1st vs 3rd	2nd vs 3rd	2nd vs 3rd
M, perfusion units	4,86 [4,55; 6,88]	5,84 [4,13; 6,74]	3,08 [2,19; 4,71]	0,02	0,01	0,72
σ, perfusion units	0,59 [0,46; 0,91]	0,70 [0,51; 0,83]	0,69 [0,52; 0,95]	0,65	0,95	0,52
vALF, perfusion units	16,96 [8,43; 24,36]	15,94 [10,17; 21,57]	38,08 [22,28; 43,82]	0,05	0,02	0,98
vAHF, perfusion units	16,97 [5,42; 27,31]	4,11 [3,35; 29,88]	5,42 [2,81; 14,65]	0,25	0,94	0,32
vACF, perfusion units	68,09 [48,26; 76,42]	76,52 [59,96; 81,22]	54,87 [45,20; 67,23]	0,35	0,30	0,35
IFM,	0,33 [0,23; 0,47]	0,36 [0,24; 0,42]	0,60 [0,41; 0,70]	0,04	0,04	0,94
R	1,01	0,82	0,83	0,09	0,94	0,04

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	[0,86; 1,16]	[0,66; 0,96]	[0,70; 0,92]			
CT	2,77 [2,28; 3,71]	3,32 [3,00; 6,20]	2,77 [2,14; 3,80]	0,94	0,18	0,15

As follows from the Table 10, there was a statistically significant increase in average value of tissue perfusion with blood and a decrease in IFM (oscillation index) in participants with ME/CFS, including ME/CFS developed following COVID-19, compared with the control group. There was a statistically significant decrease in the contribution of low frequency oscillations (0.05-0.2 Hz) to the total power of the spectrum of biorhythms, detected in the cohort of ME/CFS that developed after COVID-19. In the case of ME/CFS of a different origin, this decrease was at the border of statistical significance. At the same time, statistically significant differences between cohorts 1 and 2 were revealed only in terms of vascular resistance, which was higher in the first cohort.

4. Discussion

In this study for the first time in Russian population a validated tool (DSQ-2) was used for identifying those cases meeting four most common ME and CFS case definitions. We tested members of the Internet community of patients who suspected they had ME / CFS and members of the Internet community of patients with symptoms persisting for more than 12 weeks after COVID-19. The results of filling out the questionnaire showed the validity of the assumption of the most members of the Internet community of patients who suspected they had ME / CFS about their disease - 56/76 people (73.7%) met at least one of the case definitions for ME/CFS. 15 people who met the inclusion criteria according to the DSQ-2 questionnaire were excluded from the study because they met the exclusion criterion (the presence of other diseases that can potentially cause chronic fatigue) This data indicated the key role of thorough examination of people with complaints of chronic fatigue in clinical practice for the purpose of differential diagnosis. The median age of patients in the first group was 39.3 [31.4; 45.9] years old, and male/female ratio was 2:1, which corresponds to the data of epidemiological studies conducted in other countries where ME/CFS is more recognized[2].

In 2021, against the backdrop of the COVID-19 pandemic, the ME/CFS problem acquired additional urgency. The fact is that at least some of the patients after the acute phase of COVID-19 (often experienced just in a mild form) acquired some prolonged symptoms, among them fatigue was the most common[25]. Despite negative nasal-swab PCR, these individuals continue to suffer from various symptoms that persist for more than 12 weeks after the onset of the disease or appear after the acute phase of COVID-19. There is an increasing number of publications which point to a pronounced similarity between the clinical manifestations of the post-COVID syndrome and ME/CFS. Notably, that ME/CFS in 70% of cases also develops following an infectious disease [26].

In order to test the hypothesis of the relationship between ME / CFS and post-COVID syndrome, the DSQ-2 questionnaire was sent to the members of the Internet community of patients with symptoms persisting for more than 12 weeks after COVID-19. The fact that all individuals who completed the questionnaire met at least one of the ME/CFS case definitions confirms the presence of a close relationship between post-COVID syndrome and ME/CFS.

In the first group of patients (in whom the development of ME / CFS was not associated with COVID-19), the infectious disease was indicated as a probable trigger for the development of ME / CFS by 62.5% of patients, which is similar to the data of other authors (70%) [26] and indirectly indicates the involvement of the immune system in the pathogenesis of the disease.

The key symptom of ME/CFS is severe fatigue that does not go away even after adequate rest and persists for more than 6 months. Some information about the possible mechanisms of chronic fatigue in ME / CFS can be obtained from the correlation analysis between the severity of this symptom and the presence and severity of other symptoms

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of ME/CFS, divided into several domains, remembering, however, that the identification of a positive correlation does not yet indicate the presence causal relationship. The statistically significant positive association between fatigue that does not go away after adequate rest and 20 other symptoms of ME / CFS allow us to draw the following conclusions. Firstly, the fact that 7 out of 20 symptoms belong to the domain of "post-expression malaise" additionally indicates the key role of this phenomenon in ME / CFS. There is evidence that the two-day cardiopulmonary exercise test objectively measuring PEM makes it possible to distinguish ME / CFS patients from healthy individuals[27], which is of great importance in the absence of reliable laboratory biomarkers of ME/CFS. Deterioration of the patient's condition after physical activity should serve as a warning for medical doctors against recommending ME/CFS patients to increase the level of physical activity without careful supervision by a rehabilitation specialist. Secondly, the fact that 4 out of 20 symptoms belong to the domain of "immune dysfunction" (this domain contains 7 questions in total), despite the non-obviousness of the pathophysiological connection of pathological fatigue, for example, with soreness of the lymph nodes or symptoms of sinusitis, indicates the important role of immune dysfunction in the development of symptoms of the disease and justify the usage of the immunomodulatory drugs to alleviate the main symptom (fatigue). Thirdly, the belonging of four symptoms to the domain of "sleep disturbance" and one symptom to the domain of "pain syndromes" confirms the validity of the treatment approach to ME/CFS, described in the recent guidelines from the European Network on Myalgic Encephalomyelitis/ Chronic Fatigue Syndrome (EUROMENE)[28]. According to this approach, in order to reduce the severity of fatigue patients are provided first of all with symptomatic help aimed at normalizing sleep and combating pain. Finally, the belonging of four symptoms to the domains associated with disorders of the nervous system (autonomic, sensory and motor functions) confirms classification of ME/CFS in ICD-10, where it belongs to the chapter G – "Diseases of the nervous system".

Our data, therefore, not only agree with the concept of ME/CFS as a disease with neuroimmune pathogenesis, but also allow us to make assumptions about approaches to diagnosis and treatment of this disease, as well as the most effective organization the patient care in ME / CFS, which optimally should be provided by neurologists.

In the past, ME/CFS has often been misdiagnosed as a psychiatric disorder of the affective spectrum, leading to mismanagement and deterioration of the patients health[29]. Today, it is believed that anxiety and depressive symptoms, which are common in ME/CFS[30,31], should not always be considered as a sign of an alternative diagnosis. The prevalence of clinically significant and subclinical anxiety and depression in ME/CFS, determined based on the Hospital Anxiety and Depression Scale (HADS) in our study, is consistent with the literature[32]. At the same time, it should be remembered that recent research data on this topic suggests that depression and anxiety in ME/CFS are associated with neuroinflammation process, pain syndromes, psychological distress due to the inability to return to work and reduced physical functioning, social isolation, as well as insufficient knowledge of medical specialists about the disease and, as a consequence, their deontologically vulnerable skeptical attitude towards the patient's problems[29]. In addition, the absence of a statistically significant difference between the prevalence of anxiety and depressive symptoms between the group of patients with ME / CFS and healthy individuals, as well lack of correlation between the severity of fatigue and psychological distress (anxiety and depression) in the group of patients with ME / CFS call to question the potential effectiveness of the treatment of depressive and anxiety symptoms in order to reduce the severity of fatigue. This is consistent with our experience with patients suffered from ME/CFS: just very few of them reported positive effect of antidepressants on the severity of the their main complaint – fatigue.

Deviations from the reference values for several parameters of the screening immunologic tests were observed in 12/12 patients (100%) who underwent this laboratory evaluation. The changes concerned lymphocyte subset profile, level of circulating immune

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complexes, C3 and C4 components of the peripheral blood complement, interferons levels and secraetion and indicators of the function of the granulocytes and monocytes. However, apparently, the clinical heterogeneity and polyaetiological nature of ME/CFS, as well as the rapid dynamics of the indicators of the immunological status were the reasons for the difference in the patterns of immune dysfunction in patients. A follow-up study should be performed to assess the relationship between the CD4+/CD8+ ratio and clinical features, course and outcome of the disease. In our study we noticed that according to this ratio patients slip into three, almost equal groups (decrease in CD4 + / CD8 + <1.0, increase in CD + 4 / CD8 +> 2.5, normal value). Analysis of small cell populations such as double-positive T-lymphocytes, which is rarely performed in routine clinical practice, may also be of interest for the further research. A decrease in the absolute count of NK cells is often mentioned in the literature as an important sign of ME/CFS. Contrary to the expectations, the absolute count of NK cells was reduced (according to the latest data on reference values in the adult Russian population) only in 3/12 patients. However, it is important to acknowledge, that the normal level of NK cells does not exclude their functional insufficiency. Even though phagocyte activity was reduced in all (3/3) patients who underwent corresponding laboratory evaluation, the unification of research methods in this area is needed to compare the results obtained in different laboratories.

Several DSQ-2 questions are related to orthostatic intolerance, because it is a frequent finding in ME/CFS[4]. In particular, 11 to 50% of patients with ME / CFS may suffer from postural orthostatic tachycardia syndrome (POTS). Interestingly, POTS itself often develops following infectious diseases, and, at least in some cases, may be associated with the production of autoantibodies against adrenergic and cholinergic receptors[33]. We found that according to the results of active orthostatic test 37.5% of patients with ME / CFS met diagnostic criteria for POTS. The diagnosis of POTS in this group is of clinical value because there are pharmacological and non-pharmacological methods that allow to cope with the symptoms of this condition, which can additionally affect the quality of life of patients with ME / CFS. However, even more interesting is the extremely high prevalence of POTS among patients with post-COVID syndrome (6/8 (75.0%) versus 1/9 (11.1%) in the control group, $p = 0.02$). It is important to note that among the control group, 6/9 people (66.7%) were infected with COVID-19 and recovered >4 weeks ago at the time of enrollment in the study, however POTS was detected in only 1 of them. Pairwise comparison of the average increase in heart rate relative to basal values between the cohorts at each minute of the active standing test showed that in ME / CFS that developed after COVID-19, there is a more pronounced increase in heart rate starting from the 6th minute of the test compared to the control group, which allowed us to assume that POTS is one of the key characteristics for ME / CFS of post-COVID genesis. It is important to note that 4/13 people who met POTS criteria in our study, the required increase in heart rate was achieved only at the 8-10th minute of the active orthostatic test, which confirms the practice of carrying out the test in its complete (within 10 minutes), and not abridged (5 minutes) version.

The rhythmic characteristics of oscillatory processes in the microcirculation system are useful for diagnosis of many diseases related to the changes in microcirculation[34]. The LDF method allows non-invasive assessment of disturbances in the human blood microcirculation system. In this work LDF was applied to assess the dynamic characteristics of microcirculation in ME/CFS including the cases of post-COVID genesis (in the latter subgroup – it was done for the first time, to the best of our knowledge). A change in the microcirculation index (increase or decrease) characterizes, respectively, an increase or decrease in perfusion. Its increase can be associated both with a lower tonus of the arterioles, which leads to an arterial hyperemia, or with the phenomena of congestion of blood in the venules and venous hyperemia. Regarding regulation of microcirculation, there are "active" and "passive" mechanisms. The "passive" mechanisms includes external factors that act outside the microcirculatory bed: a pulse wave and the suction action of the "res-

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piratory pump" from the veins. "Active" factors directly affect the vessels of the microvasculature by periodically changing the resistance of blood vessels to blood flow through vasomotions and create transverse fluctuations in blood flow. These active factors are sympathetic nerve fibers, smooth muscle cells of the vascular wall and endothelium-derived regulatory molecules. When carrying out spectral analysis, the active factors correspond to the low frequency oscillations. [35]. There are several forms of microcirculation disorders: arterial hyperemia, venous hyperemia, combined hyperemia, ischemia and stasis[36]. The changes identified in this study in ME / CFS, including ME/CFS of post-COVID-19 nature, correspond to the hyperemic form of microcirculation disorders, which is characterized by increased blood flow into the microcirculatory bed. It is distinguished by a significant increase in the number of functioning capillaries, an increase in tortuosity, vasodilation, and an increase in the permeability of the vascular wall. This form of microcirculation disorders is usually observed in acute inflammatory processes or other conditions of decreased systemic vasocontriction[23]. The state of microcirculation in ME / CFS which developed before COVID-19 pandemic and ME/CFS of post-COVID-19 nature differed only in terms of intravascular resistance. Bond et al. showed that chronic oxidative stress can contribute significantly to the development of ME / CFS symptoms due to the development of endothelial dysfunction[37]. The relationship between chronic inflammatory processes and increased arterial stiffness is well known[38]. An increase in vascular resistance in cohort 1 compared to cohort 2 may reflect the contribution of chronic inflammatory process of a long course to microcirculation disorders and suggests the existence of long-term consequences of ME / CFS, in particular, an increased risk of cardiovascular diseases.

5. Conclusions

1. Among the patients who suspected they had ME / CFS and took part in the study, 73.7% met the diagnostic criteria for this disease. 19.7% had symptoms consistent with ME/CFS, but were excluded from the study due to the presence of other diseases that could potentially cause chronic fatigue. This data confirmed the key role of a thorough examination of patients with complaints of chronic fatigue in the clinical practice in order to perform differential diagnostics. Among patients with symptoms persisting for more than 12 weeks after recovery from acute COVID-19, 100% of individuals met the diagnostic criteria for ME / CFS, which confirms the presence of a close relationship between post-COVID syndrome and ME / CFS.

2. We found a statistically significant positive relationship between fatigue that does not get better with adequate rest and 20 other symptoms of ME / CFS related to the domains of “post-exertional exhaustion” (7 symptoms), “immune dysfunction” (4 symptoms), “sleep disturbances” (4 symptoms), “dysfunction of the autonomic nervous system” (2 symptoms), “neurological sensory / motor disorders” (2 symptoms), “pain syndromes” (1 symptom). This data not only agree with the concept of ME / CFS as a disease with neuroimmune pathogenesis, but also allowed us to make assumptions about the approaches to the diagnosis, treatment and organization of care for patients with ME / CFS.

3. The prevalence of clinically expressed and subclinical anxiety and depression in ME/CFS, including ME/CFS of post-COVID-19 nature, was in line with the results of other authors and does not differ significantly from that in healthy individuals. There was no correlation between anxiety/depressive symptoms and the severity of fatigue in ME / CFS.

4. Immune dysfunction was detected in 12/12 patients with ME / CFS (100%) based on the analysis of the results of the laboratory screening immunological evaluation.

5. The prevalence of POTS in patients with ME / CFS was 37.5%, while in patients with ME / CFS of post-COVID-19 nature it was 75.0%, which is higher than in healthy controls (11.1%, $p = 0.02$). Comparison of the average increase in heart rate relative to basal values between cohorts at each minute of the test showed that in ME/CFS that developed after COVID-19, there is a more pronounced increase in heart rate starting from the 6th

minute of the test compared with the control group, which allowed us to assume that POTS is one of the key characteristics of ME/CFS of post-COVID-19 nature.

6. Changes in microcirculation identified in ME / CFS, including ME/CFS of post COVID-19 nature with the LDF method, correspond to the hyperemic form of microcirculation disorders which is generally observed in acute inflammatory processes or other conditions of reduced systemic vasoconstrictor activity. An increase in intravascular resistance in ME/CFS which developed before COVID-19 pandemic compared to ME / CFS of post-COVID-19 nature may reflect the contribution of chronic inflammatory process of a long course to microcirculation disorders in the first cohort and suggests the existence of long-term consequences of ME / CFS, in particular, an increase in the risk of developing cardiovascular diseases.

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