ISSN 2304-3415, Russian Open Medical Journal
2025. Volume 14. Issue 1 (March). Article CID e0109
DOI: 10.15275/rusomj.2025.0109

Original article

Dynamics of oxidative stress markers and mental status in patients with post-COVID-19 asthenic syndrome: Effects of adjunctive systemic ozone therapy

Alexander A. Soldatenko [1], Lesya N. Gumenyk [2]

[1] OOO (limited liability company) Rein-LTD Avicenna Clinic, Simferopol, Russia
[2] Medical Academy named after S.I. Georgievsky, V.I. Vernadsky Crimean Federal University, Simferopol, Russia

Received 10 July 2024, Revised 7 October 2024, Accepted 18 December 2024

Abstract: This study *aimed* to evaluate the effect of systemic ozone therapy, when used as an adjunct to pharmacological treatment, on plasma levels of malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx), and measures of mental status in patients with post-COVID-19 asthenic syndrome (PCAS).
Material and Methods — A total of 140 patients, aged 18 to 45 years, with post-COVID-19 asthenic syndrome (PCAS) were examined and treated. These patients were randomly divided into two groups: a main group (n=70) receiving systemic ozone therapy in addition to pharmacological treatment, and a comparison group (n=70) receiving only pharmacological treatment. Pre- and post-treatment outcomes were assessed by measuring plasma levels of MDA, SOD, and GPx, and by employing the MFI-20, MoCA, ISI, HAM-A, and CGI-S scales.
Results — Prior to treatment, patients exhibited elevated levels of insulin-like growth factor 1 (IGF-1) and reduced levels of neurotrophic factors (BDNF and NGF) in blood serum. Those patients treated with resveratrol in addition to standard treatment showed more pronounced changes in plasma IGF-1, BDNF, and NGF levels compared to the comparison group, with their levels approaching those of the control group. Based on the MoCA scale, the main group exhibited a more significant improvement in cognitive function.
Conclusion — Adjunctive systemic ozone therapy, combined with pharmacological treatment, resulted in a reduction of oxidative stress and a significant improvement in mental status among patients with post-COVID-19 asthenic syndrome (PCAS). Notably, 95% of patients in the ozone therapy group experienced a complete or near-complete resolution of PCAS symptoms. These findings suggest that systemic ozone therapy may be an effective and pathogenetically relevant strategy for the comprehensive outpatient management of PCAS.

Keywords: Post-COVID-19 asthenic syndrome, systemic ozone therapy, malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx).

Cite as Soldatenko AA, Gumenyk LN. Dynamics of oxidative stress markers and mental status in patients with post-COVID-19 asthenic syndrome: Effects of adjunctive systemic ozone therapy. *Russian Open Medical Journal* 2025; 14: e0409.

Correspondence to Lesya N. Gumenyk. Address: 4, Acad. Vernadsky ave, Simferopol, Crimean Republic, 295007, Russia. Email: lesya_gymenyuk@mail.ru.

Introduction

Post-COVID-19 asthenic syndrome (PCAS), characterized primarily by chronic fatigue, cognitive dysfunction, sleep disturbances, and anxiety [1], remains an area of active investigation.

Global statistics indicate that 40% to 70% of patients, regardless of age or initial COVID-19 severity (including those with mild or asymptomatic infections), develop PCAS [2, 3]. This condition is associated with significant impairments in daily functioning (64%), occupational and social functioning (70%) [4], and quality of life (92.4%) [5]. Furthermore, 20% of patients are unable to return to work one year after the acute phase of the infection [6]. These findings underscore the substantial social burden of PCAS.

Post-COVID-19 asthenic syndrome (PCAS) is a multifactorial condition with a complex and incompletely understood pathophysiology. Current understanding suggests that oxidative stress plays a central role in the pathological processes underlying PCAS [7]. Specifically, patients with PCAS exhibit elevated plasma levels of malondialdehyde (MDA) and reduced glutathione peroxidase (GPx) activity, which correlate with the severity of clinical manifestations [8, 9]. These findings indicate that oxidative stress may represent a potential therapeutic target for PCAS, and that changes in oxidative stress markers could serve as indicators of treatment effectiveness.

Currently, specific clinical guidelines for the treatment of post-COVID-19 asthenic syndrome (PCAS) are lacking. However, pharmacotherapy, involving various drug classes (including antidepressants, tranquilizers, nootropics, neurovascular and neurometabolic agents, vitamin-mineral complexes, and adaptogens), is considered the primary therapeutic strategy for patients with PCAS [10-12]. Unfortunately, evidence suggests that these agents, when used as monotherapy, are often insufficient in addressing PCAS symptoms or improving the condition and quality of life of patients [13]. Consequently, a combination of several drug classes is frequently employed [14]. While combination therapy may offer increased clinical efficacy [13], its use is often limited by the potential risk of multi-organ dysfunction and other serious adverse events (e.g., dizziness, nausea, sleep

disturbances), as well as decreased patient adherence. Therefore, physiotherapy modalities, which can complement pharmacotherapy for PCAS, potentially enhance treatment effectiveness, and reduce the risk of polypharmacy, are particularly relevant [15]. Given the underlying mechanisms and clinical manifestations of PCAS, systemic ozone therapy, with its potent antioxidant effects, may be of particular interest. Furthermore, systemic ozone therapy exerts multimodal anti-inflammatory, immunomodulatory, antioxidant, metabolic, neuroprotective, and anxiolytic effects [16-19], which are also beneficial in the treatment of patients with PCAS. Systemic ozone therapy is generally well-tolerated and has demonstrated efficacy in patients with COVID-19 [20]. Several small studies, primarily conducted in specialized hospital or sanatorium settings, have reported the benefits of ozone therapy as part of a comprehensive treatment approach for post-COVID syndrome manifestations, including bilateral multisegmental pneumonia [21], reduced exercise tolerance, sleep disturbances, and chronic fatigue [22]. Notably, these studies indicated a restoration of functional status and improvements in quality of life in a majority of patients [21, 22]. These findings suggest the potential utility of systemic ozone therapy as part of a comprehensive treatment strategy for PCAS. However, to date, there remains a lack of evidence from randomized controlled trials (RCTs) demonstrating the efficacy of adjunctive systemic ozone therapy in the pharmacological treatment of outpatients with PCAS presenting with chronic fatigue, cognitive dysfunction, sleep disturbances, and anxiety. Furthermore, our literature search identified only one study that examined the impact of systemic ozone therapy in a sanatorium setting on oxidative stress markers in patients with PCAS [22], thus highlighting the need for further investigation in this area.

Therefore, the purpose of this study was to evaluate the impact of systemic ozone therapy, when used as an adjunct to pharmacological treatment, on plasma levels of MDA, superoxide dismutase (SOD), GPx, and various measures of mental status in patients with PCAS.

Material and Methods

Study Design

This prospective, controlled, comparative, randomized study was conducted at Simferopol City Clinical Hospital No. 7 in Simferopol, Republic of Crimea, between November 2021 and October 2023.

This study enrolled 140 outpatients (77 women and 63 men) aged 18 to 45 years (mean age 34.2 [32.3; 36.2] years) who were diagnosed with asthenic syndrome according to ICD-10 diagnostic code U09.9, Post COVID-19 condition, unspecified. Based on the assigned treatment regimen, patients with post-COVID-19 asthenic syndrome (PCAS) were randomly allocated into two groups using sealed envelopes. The main group comprised 70 patients (43 women and 27 men, mean age 34.3 [32.5; 36.3] years) who received systemic ozone therapy in addition to pharmacological treatment. The comparison group consisted of 70 patients (44 women and 26 men, mean age 33.7 [31.9; 35.9] years) who received only pharmacological treatment.

As a control, 50 healthy volunteers who had neither been vaccinated against nor contracted COVID-19 were examined. The control group was matched with the PCAS patient groups for sex (32 women and 18 men), age (33.9 [32.3; 36.6] years), and body mass index (BMI) (19.3 [18.8; 23.4] kg/m²).

This study was conducted in accordance with Good Clinical Practice guidelines and the principles of the Declaration of Helsinki, as adopted by the World Medical Association. The study protocol was approved by the Ethics Committee of the Georgievsky Medical Academy of Vernadsky Crimean Federal University. All participants provided written informed consent before enrollment.

Eligibility Criteria

Patients with Post-COVID-19 Asthenic Syndrome (PCAS).

Inclusion criteria: Diagnosis of asthenic syndrome according to ICD-10 code U09.9 (Post COVID-19 condition, unspecified); age 18-45 years; body mass index (BMI) 18.5-24.9 kg/m²; history of prior, serologically confirmed COVID-19 infection; new onset or significant worsening of asthenic syndrome symptoms (chronic fatigue, cognitive dysfunction, sleep disturbances, anxiety) after COVID-19, persisting for 3-12 months, and not attributable to any other underlying condition; total score on the MFI-20 scale >30, MoCA scale <26, ISI index >8, ESS scale >11, and HAM-A scale >8; absence of contraindications to systemic ozone therapy; and provision of written informed consent.

Table 1. Baseline Characteristics of Patients with Post-COVID-19 Asthenic Syndrome

Parameter	*Main Group (n=140)*	*Comparison Group (n=70)*	p_{1-2}
Mean age, years, median [25th%; 75th%]	34.3 [32.5; 36.3]	33.7 [31.9; 35.9]	0.781
Women/Men	43 (61.4) / 27 (38.6)	44 (62.9) / 26 (37.1)	0.771
Body mass index, kg/m² median [25th%; 75th%]	20.3 [18.3; 22.6]	21.0 [18.6; 22.7]	0.874
Mild COVID-19, n (%)	43 (61.4)	44 (62.9)	0.884
Moderate COVID-19, n (%)	20 (28.5)	20 (28.5)	1.000
Severe COVID-19, n (%)	7 (10.0)	6 (8.6)	0.075
Time to PCAS onset post acute COVID-19 (months), Median [25th%; 75th%]	4.9 [3.0; 5.7]	4.4 [3.3; 5.1]	0.893
MFI-20 Total Score, median [25th%; 75th%]	81.9 [77.7; 84.9]	81.3 [78.1; 83.9]	0.801
MoCA Total Score, median [25th%; 75th%]	24.2[24.0; 25.7]	24.1 [24.0; 25.4]	0.881
ISI Total Score, median [25th%; 75th%]	18.2 [16.3; 19.2]	17.7 [16.0; 18.8]	0.867
HAM-A Total Score, median [25th%; 75th%]	21.3 [19.2; 22.9]	20.9 [18.7; 22.7]	0.891
CGI-S Marked Illness (n, %)	41 (58.6)	39 (55.7)	0.072
CGI-S Moderately Ill (n, %)	29 (41.4)	31 (44.3)	0.070

Total Scores represent mean summary scores.

Exclusion criteria: body mass index (BMI)<18.5 kg/m² or >25 kg/m²; pre-existing mental disorders, including cognitive dysfunction, sleep disturbances, affective disorders, or past use of psychotropic medications; history of alcohol or other psychoactive substance use disorders; presence of focal neurological signs or symptoms (based on neurological examination); evidence of structural brain abnormalities (based on magnetic resonance imaging); elevated intracranial pressure; chronic infectious, inflammatory, endocrine, autoimmune, thrombophilic, or oncological diseases; use of antibiotics, antiviral agents, vascular, metabolic, nootropic, anabolic, diuretic, or antioxidant agents, or oral contraceptives within 3 months prior to study enrollment; previous use of pharmacotherapy, psychotherapy, or rehabilitation interventions for PCAS; smoking; or refusal to provide informed consent.

Inclusion criteria: individuals of any sex, 18-45 years of age; body mass index (BMI) 18.5-24.9 kg/m²; total score on the MFI-20 scale <30, MoCA scale ≥26, ISI index<8, and HAM-A scale <8.

Exclusion criteria were the same as those for the patient group with PCAS.

Treatment Protocol

As a model of pharmacological monotherapy, patients in both groups received Brainmax® at a dosage of 2000 mg orally (two capsules twice daily) for 30 days, according to the manufacturer's recommendations. The selection of this drug was based on its officially approved indications for the treatment of post-COVID-19 asthenic syndrome (PCAS), specifically for symptoms such as increased fatigue, sleep disturbances, emotional lability, and cognitive dysfunction [23], as well as evidence of its efficacy in PCAS [24]. Brainmax® (Biokhimik JSC, Russia – marketing authorization from the Ministry of Health of Russia No. LP-007988, dated March 28, 2022) is a combination medication containing meldonium (trimethylhydrazinium propionate) and ethylmethylhydroxypyridine succinate, and is reported to possess antioxidant, antihypoxic, membrane- and stress-protective, anxiolytic, nootropic, and anti-ischemic properties. All patients provided consent to independently obtain the prescribed medication. Starting on the first day of pharmacotherapy, patients in the main group also received systemic ozone therapy, consisting of daily intravenous infusions of 200 mL of ozonated 0.9% sodium chloride solution for a total of 10 procedures. The initial 3 procedures were performed with an ozone concentration of 2.0 mg/L, followed by an increase to 3.0-4.0 mg/L. The ozonated solutions were produced using an automated UOTA-60-01 unit (Medozone LLC, Russia; medical device registration certificate No. 29/06050796/1561-01), designed for producing ozonated solutions with pre-set concentrations. A 200 mL volume of 0.9% sodium chloride solution was saturated with an ozone-oxygen mixture at a concentration of 2.0-4.0 mg/L for 2 minutes at room temperature. Subsequently, intravenous access was established via puncture of the cubital vein, and the resulting solution was administered via intravenous drip infusion over 30 minutes.

Table 2. Plasma Levels of MDA, SOD, and GPx in Patients with Post-COVID-19 Asthenic Syndrome and Healthy Volunteers (Control Group) (Median [25th%; 75th%])

Parameter	*Patients with PCAS (n=140)*	*Control (n=50)*	p_{1-2}
MDA, nmol/mL	4.1 [3.4; 5.4]	1.1 [0.7; 1.7]	<0.001
SOD, U/mL	2.6 [2.1; 3.6]	4.6 [3.9; 5.8]	0.001
GPx, U/mL	2.2 [1.6; 2.4]	3.8 [3.0; 5.6]	<0.001

Table 3. Dynamics of Plasma Levels of MDA, SOD, and GPx During Treatment of Patients with Post-COVID-19 Asthenic Syndrome

Parameter	*Control*	*Main Group (n=70)*		*Comparison Group (n=70)*		Δ_{3-5} / p_{3-5}
		Pre-treatment	*Post-treatment*	*Pre-treatment*	*Post-treatment*	
MDA. nmol/mL	1.1 [0.7; 1.7]	4.2 [3.3; 5.3]	1.3 [0.9; 1.5]	4.1 [3.5; 5.4]	2.5 [1.7; 3.7]	48.0% / 0.002
		Δ_{3-2} -69.1%		Δ_{5-4} -39.0%		
		p_{3-2}<0.001. p_{3-1}=0.078		p_{5-4}=0.012. p_{5-1}=0.003		
SOD. U/mL	4.6 [3.9; 5.8]	2.7 [2.1;3.5]	4.0 [3.3; 5.2]	2.6 [2.2; 3.6]	3.3 [3.0; 4.0]	21.2% / 0.036
		$\Delta 3_{-2}$ +48.2%		Δ_{5-4} +26.9%		
		p_{3-2}=0.002. p_{3-1}=0.079		p_{5-4}=0.021. p_{5-1}=0.036		
GPx. U/mL	3.8 [3.0; 5.6]	2.2 [1.6; 2.4]	3.5 [2.8; 5.4]	2.1 [1.5; 2.3]	2.8 [2.0; 3.1]	25.0% / 0.028
		Δ_{3-2} +52.2%		Δ_{5-4} +33.3%		
		p_{3-2}=0.012. p_{3-1}=0.084		p_{5-4}=0.044. p_{5-1}=0.036		

Δ – delta (difference) of changes.

Table 4. Mental Status Measures in Patients with Post-COVID-19 Asthenic Syndrome and Healthy Controls (Median [25th%; 75th%])

Parameter	Main Group (n=70)		Comparison Group (n=70)		Δ_{4-2} / p_{4-2}
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	
MFI-20 Total Score. Median [25th%; 75th%]	81.9 [77.7; 84.9]	20.5 [18.1; 22.3]	81.6 [78.3; 84.6]	32.4 [29.0; 34.2]	36.7% / 0.001
	Δ_{2-1} -74.7%. p_{2-1}<0.001		Δ_{4-3} -60.3%. p_{4-3}<0.001		
MoCA Total Score. Median [25th%; 75th%]	24.2 [24.0; 25.7]	28.5 [27.3; 29.5]	24.5 [24.2; 25.6]	25.7 [25.1; 26.2]	10.9% / 0.046
	Δ_{2-1} +17.1%. p_{2-1}=0.041		Δ_{4-3} +4.9%. p_{4-3}=0.072		
ISI Total Score. Median [25th%; 75th%]	18.2 [16.3; 19.2]	5.0 [3.7;7.5]	17.7 [16.0; 18.5]	10.1 [9.3; 11.7]	50.5% / <0.001
	Δ_{2-1} -81.3%. p_{2-1}<0.001		Δ_{4-3} -42.9%. p_{4-3}=0.001		
HAM-A Total Score. Median [25th%; 75th%]	21.3 [19.2; 22.9]	5.8 [4.3; 6.8]	20.7 [18.8; 23.1]	10.7 [9.5; 12.1]	45.8 / 0.001
	Δ_{2-1} -72.8%. p_{2-1}<0.001		Δ_{4-3} -48.3%. p_{4-3}<0.001		
CGI-S Absence of Illness (n. %)	0	66 (94.2)	0	44 (62.9)	49.8% / 0.001
	p_{2-1}<0.001		p_{4-3}<0.001		
CGI-S Mild Illness (n. %)	0	4(5.8)	0	19 (27.1)	78.6% / 0.001
	p_{2-1}=0.072		p_{4-3}<0.001		

Total Scores represent mean summary scores, Δ – delta (difference) of changes.

All patients in both the main and comparison groups successfully completed the study protocol.

Examination Methods

Treatment effectiveness was monitored using the following standardized instruments: Multidimensional Fatigue Inventory (MFI-20) [25], Montreal Cognitive Assessment (MoCA) [26], Insomnia Severity Index (ISI) [27], Hamilton Anxiety Rating Scale (HAM-A) [28], and Clinical Global Impression Scale (CGI), specifically item CGI-I, "Global Improvement" [29]. The occurrence of any adverse events (AEs) was also recorded.

In addition, all healthy volunteers in the control group underwent a single examination. Patients with post-COVID-19 asthenic syndrome (PCAS) were examined at baseline and again 30 days after treatment to assess plasma levels of malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GPx) using enzyme-linked immunosorbent assays (ELISA) on a Multiskan FC analyzer (Thermo Fisher Scientific Inc., Finland) with ELISA kits from Immundiagnostik AG, Bender MedSystems GmbH, and Biomedica Medizinprodukte GmbH. Blood samples were collected from the cubital vein between 7:00 AM and 9:00 AM after an 8-12-hour overnight fast.

Statistical Analysis

Statistical analysis of the results was performed using STATISTICA version 8.0 (StatSoft Inc., USA). Quantitative data are presented as median (Me) with interquartile range [25th; 75th percentiles (%)], while qualitative data are presented as proportions and absolute numbers. The Mann–Whitney *U* test was used to compare quantitative data between groups, and the χ^2 (chi-square) test was used for qualitative data. Correlations were assessed using Spearman's rank correlation coefficient. Statistical significance was defined as $p<0.05$.

Results

Baseline characteristics of patients with post-COVID-19 asthenic syndrome are presented in *Table* 1. The patient groups were well-matched with respect to all baseline parameters.

At baseline, patients with post-COVID-19 asthenic syndrome (PCAS) exhibited significantly higher plasma levels of MDA and significantly lower levels of SOD and GPx compared to the healthy controls (*Table* 2). The main and comparison groups were well-balanced with respect to baseline MDA, SOD, and GPx levels.

The dynamics of plasma MDA, SOD, and GPx levels during the treatment of patients with post-COVID-19 asthenic syndrome (PCAS) are shown in *Table* 3. The data indicate that, after completion of therapy (day 30), MDA levels decreased significantly ($p<0.05$) in both groups, while SOD and GPx levels increased significantly ($p<0.05$). The magnitude of change was significantly greater in the main group. The mean difference in change between the main and comparison groups was 48.0% ($p=0.002$) for MDA, 21.2% ($p=0.036$) for SOD, and 25.0% ($p=0.028$) for GPx.

Changes in mental status scores during the treatment of patients with post-COVID-19 asthenic syndrome (PCAS) are presented in *Table* 4. All mental status indicators showed statistically significant changes ($p<0.05$), with improvements being more pronounced in the main group. The mean difference in change between the main and comparison groups was 36.7% ($p=0.001$) for MFI-20 total scores, 10.9% ($p=0.046$) for MoCA scores, 50.5% ($p<0.001$) for ISI scores, and 45.8% ($p=0.001$) for HAM-A scores. According to the Clinical Global Impression-Severity scale (CGI-S), 66 patients (94.2%) in the main group were rated as "Normal, not at all ill" compared to 44 patients (62.9%) in the comparison group ($p=0.001$).

No adverse events were reported in either the main or comparison groups during the study period.

Discussion

The potent antioxidant effects of systemic ozone therapy are well-established and attributed to several mechanisms, including activation of nuclear factor (erythroid-derived 2) – like 2 (Nrf2) [30] and enhanced oxidative carboxylation of pyruvate [31]. These processes lead to the suppression of excessive reactive oxygen species production and lipid peroxidation, increased generation of free glutathione, and increased activity of antioxidant enzymes, such as SOD and GPx [32]. The antioxidant efficacy of systemic ozone therapy has been experimentally demonstrated in *in vitro* models studying conditions involving apoptosis [33], experimental models of neurodegenerative diseases [34], and clinical studies involving patients with diverse pathological conditions linked by oxidative stress [30, 35, 36], including asthenic spectrum disorders [37]. However, to date, only three studies (one observational and two randomized controlled trials) have examined the efficacy and safety of systemic ozone therapy, both as monotherapy and as part of a comprehensive treatment approach for patients with post-COVID syndrome.

For example, in an observational study by Tirelli *et al*. [38], 100 patients (mean age 55.2±12.72 years) experiencing symptoms of post-COVID-19 asthenic syndrome (PCAS) underwent systemic ozone therapy at a frequency of 2-3 sessions per week (total of 6–9 sessions). By the end of the study, 40% of patients achieved complete resolution of asthenic symptoms, and 60% experienced a significant decrease in symptom severity (based on the Fatigue Severity Scale).

Tsvetkova A.V. *et al.* [21] conducted a randomized controlled trial to evaluate the efficacy and safety of adjunctive systemic ozone therapy within a comprehensive treatment approach (including therapeutic exercises and physiotherapy modalities such as low-frequency magnetotherapy and drug electrophoresis with KI and $CaCl_2$) for hospitalized patients aged 29 to 78 years with post-COVID bilateral multisegmental pneumonia during the second phase of rehabilitation. The study demonstrated significantly better outcomes in the systemic ozone therapy group with respect to C-reactive protein levels, D-dimer levels, overall clinical assessment scores, and improvements in quality of life.

Gumenyuk *et al*. [22] also evaluated the efficacy of adjunctive systemic ozone therapy with standard sanatorium-resort therapy in a randomized controlled trial. A total of 140 patients (44.3% men, 55.7% women, mean age 49.2 [46.5; 52.3] years) with post-COVID syndrome, who continued to experience chronic fatigue, low mood, dyspnea, and exercise intolerance seven months after contracting SARS-CoV-2, were enrolled. Patients were randomly assigned to two groups: Group I (n=70) received systemic ozone therapy via intravenous drip infusions of ozonated normal saline (ozone concentration of 2.0 mg/L) for 10 days, in addition to sanatorium-resort therapy (including climatotherapy, therapeutic exercises, general massage focused on the chest, peloid therapy using mud from Lake Saki, and hyaluronic acid inhalation); Group II

(n=70) received the same sanatorium-resort therapy without systemic ozone therapy. The sanatorium-resort therapy lasted for 14 days. The study demonstrated that adjunctive systemic ozone therapy resulted in a statistically significant decrease in malondialdehyde levels (by 3.3-fold, $p<0.001$), a statistically significant increase in glutathione peroxidase activity (by 1.7-fold, $p=0.003$), and normalization of IL-6 levels. These changes were associated with a significant improvement in clinical status and quality of life in 94.6% of patients in Group I compared to 62.3% in Group II. Importantly, these studies highlight both the efficacy and the favorable safety profile of systemic ozone therapy in patients with post-COVID syndrome [21, 22].

This article presents the results of adjunctive systemic ozone therapy with pharmacological treatment in outpatients with post-COVID-19 asthenic syndrome (PCAS) characterized by chronic fatigue, cognitive dysfunction, sleep disturbances, and anxiety. To the best of our knowledge, this is the first randomized controlled trial to report such an experience.

The rationale for the efficacy of adjunctive systemic ozone therapy was attributed to its potent antioxidant effect, which was fully supported by our findings. At the completion of therapy (day 30), MDA levels decreased by 69.1% – from 4.2 [3.3; 5.3] nmol/mL to 1.3 [0.9; 1.5] nmol/mL – demonstrating a return to near-normal levels and a significant reduction compared to the comparison group (approximately 50% lower). SOD and GPx levels increased by 48.2% – from 2.7 [2.1; 3.5] U/mL to 4.0 [3.3; 5.2] U/mL and 52.2% – from 2.2 [1.6; 2.4] U/mL to 3.5 [2.8; 5.4] U/mL, respectively. These increases were significantly higher (by 21.2% and 25%, respectively) than those observed in the comparison group ($p=0.036$ and $p=0.028$, respectively). These findings suggest that the observed changes reflect the antioxidant effects of systemic ozone therapy in patients with PCAS. Our results further support the antioxidant benefits of adjunctive systemic ozone therapy with pharmacological treatment in mitigating oxidative stress in PCAS patients. Furthermore, given the established association between elevated MDA and decreased GPx levels and the severity of PCAS symptoms and prognosis [8, 9], our data suggest that adjunctive systemic ozone therapy is not only effective but also pathogenetically relevant.

Consequently, at the completion of therapy (day 30), patients with post-COVID-19 asthenic syndrome (PCAS) in the systemic ozone therapy group demonstrated a significant improvement in clinical status, characterized by a statistically significant reduction in the severity of the range of PCAS symptoms, as evidenced by changes in MFI-20, MoCA, ISI, and HAM-A scores. Specifically, at the completion of therapy (day 30), the total MFI-20 score, reflecting the severity of chronic fatigue, decreased significantly (by 74.4%) from a median of 81.9 (“pronounced”) to 20.5 (“no symptoms of chronic fatigue”). Similarly, ISI scores decreased from a median of 18.2 (“moderate sleep disturbances”) to 5.0 (“normal”), representing an average decrease of 81.3%. HAM-A scores also decreased from a median of 21.3 (mild to moderate anxiety severity) to 5.8 (“no symptoms of anxiety”), representing an average decrease of 72.8%. The total MoCA score, reflecting cognitive status, increased by 17.1% from a median of 24.2 (“cognitive impairment”) to 28.5 (“no cognitive impairment”). All these changes were significantly different from those observed in the comparison group ($p=0.001$).

Correspondingly, at the completion of therapy (day 30), 94.2% of patients in the systemic ozone therapy group were rated as having “absence of illness,” indicating a complete resolution of PCAS symptoms according to the CGI scale, a significantly higher proportion than in the comparison group ($p=0.001$). These findings are consistent with the other efficacy parameters assessed, and further support the clinical benefits of adjunctive systemic ozone therapy with pharmacological treatment in patients with PCAS.

Systemic ozone therapy was well-tolerated by PCAS patients receiving pharmacological treatment in our study (no adverse events were reported), which aligns with findings from previous studies [21, 22].

Conclusion

In this study, adjunctive systemic ozone therapy with pharmacological treatment for patients with post-COVID-19 asthenic syndrome (PCAS) resulted in the reduction of oxidative stress and improved mental status, with 95% of patients experiencing complete resolution of PCAS symptoms. Therefore, systemic ozone therapy may be considered an effective and pathogenetically relevant strategy for the comprehensive management of PCAS in outpatient settings.

Limitations

Limitations of this study include the reliance on questionnaires for assessing sleep disturbances, without employing objective diagnostic methods such as polysomnography. Additionally, we did not evaluate the effects of adjunctive systemic ozone therapy with other drug classes that may influence the clinical manifestations of post-COVID-19 asthenic syndrome.

Ethical Approval

All procedures involving human participants were conducted in accordance with the ethical standards of the institutional and/or national research committees, and with the 1964 Declaration of Helsinki and its subsequent amendments, or comparable ethical standards.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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Authors:
Alexander A. Soldatenko – psychiatrist, OOO (limited liability company) Rein-LTD Avicenna Clinic, Simferopol, Russia. http://orcid.org/0009-0001-4791-8674.
Lesya N. Gumenyk – MD, DSc, Professor, Faculty of Training of Medical Personnel of the Highest Qualification and Additional Vocational Education, Department of Psychiatry, Narcology, Psychotherapy with the Course of General and Medical Psychology, Medical Academy named after S.I. Georgievsky, V.I. Vernadsky Crimean Federal University, Simferopol, Russia. http://orcid.org/0000-0003-2785-3882.