EFFECTIVENESS OF ENRICHING DRUG TREATMENT WITH SYSTEMIC OZONE THERAPY IN PATIENTS WITH POST-COVID ASTHENIC SYNDROME

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Post-COVID asthenic syndrome (PCAS) is still the subject of active study. The study was aimed to assess the effects of systemic ozone therapy used to complement drug therapy on plasma levels of TNFα, IL1β, IL6 and parameters of mental status in patients with PCAS. Two randomized groups of patients with PCAS (n = 140, age 18–45) were assessed and treated: patients of the index group (n = 70) received systemic ozone therapy in addition to drug therapy; patients of the comparison group (n = 70) received drug therapy without systemic ozone therapy. Plasma levels of TNFα, IL1β, IL6 were measured and the patients' mental status was assessed using the MFI-20, MoCa, ISI, HARS, and CGI-S scores before and after treatment. After the end of therapy (on day 30) the TNFα, IL1β, IL6 levels reported for the index group showed no significant differences from the values reported for the control group ($p > 0.05$) and were lover, than the values of the comparison group by 39% ($p = 0.003$), 33.3% ($p = 0.022$), and 36.1% ($p = 0.012$), respectively. The changes in mental status were also more pronounced in the index group, than in the comparison group: the average final MFI-20 score was lower by 36.7% ($p = 0.001$), ISI by 50.5% ($p < 0.001$), HARS score by 45.8% ($p = 0.001$), while MoCa score was higher by 10.9% ($p = 0.046$), respectively. In the index group, the number of patients with "no disease" based on CGI-S was 94.2%, while in the comparison group it was 62.9% ($p = 0.001$). In our study adding systemic ozone therapy to drug therapy in patients with PCAS allowed us to achieve normalization of the TNFα, IL1β, IL6 levels and complete reduction of PCAS clinical manifestations in 94.2% of cases. Thus, the use of systemic ozone therapy can be considered as one of the effective and pathogenetically substantiated strategies for combination treatment of patients with PCAS in outpatient settings.

Keywords: post-COVID asthenic syndrome, systemic ozone therapy, TNFα, IL1β, IL6

Author contribution: Soldatenko AA — study concept and design, data acquisition; Gumenyuk LN — data analysis and interpretation, manuscript writing; Berdieva DM — data acquisition; Ponomarchuk EI — data analysis and interpretation.

Compliance with ethical standards: the study was approved by the Ethics Committee of the S.I. Georgievsky Medical Academy, V.I. Vernadsky Crimean Federal University (protocol No. 10 dated 16 October 2021), planned and conducted in accordance with the Declaration of Helsinki. The informed consent was submitted by all individuals included in the study.

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ЭФФЕКТИВНОСТЬ ДОБАВЛЕНИЯ СИСТЕМНОЙ ОЗОНОТЕРАПИИ К ФАРМАКОЛОГИЧЕСКОМУ ЛЕЧЕНИЮ У ПАЦИЕНТОВ С ПОСТКОВИДНЫМ АСТЕНИЧЕСКИМ СИНДРОМОМ

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Постковидный астенический синдром (ПКАС) остается предметом активного изучения. Целью исследования было оценить влияние применения системной озонотерапии в дополнение к фармакотерапии на показатели в плазме крови TNFα, IL1β, IL6 и параметры психического статуса у пациентов с ПКАС. Обследовано и пролечено две рандомизированных группы пациентов с ПКАС (n = 140, возраст 18–45): пациентам основной группы (n = 70) дополнительно к фармакотерапии проводили системную озонотерапию; пациентам группы сравнения (n = 70) проводили только фармакотерапию. До и после лечения в плазме крови измеряли уровни TNFα, IL1β, IL6 и оценивали психический статус пациентов по шкалам MFI-20, MoCa, ISI, HARS и CGI-S. По завершении терапии (на 30-й день) в основной группе уровни TNFα, IL1β, IL6 не имели статистически значимых различий с показателями в контрольной группе ($p > 0,05$) и были ниже значений группы сравнения на 39% ($p = 0,003$), 33,3% ($p = 0,022$) и 36,1% ($p = 0,012$) соответственно. Изменения показателей психического статуса также более выражены в основной группе, чем в группе сравнения: средние итоговые баллы MFI-20 ниже на 36,7% ($p = 0,001$), ISI — на 50,5% ($p < 0,001$), HARS — на 45,8% ($p = 0,001$), MoCa — выше на 10,9% ($p = 0,046$) соответственно. В основной группе число пациентов с «отсутствием заболевания» по CGI-S — 94,2%, в группе сравнения — 62,9% ($p = 0,001$). В выполненном нами исследовании добавление системной озонотерапии к фармакотерапии у пациентов с ПКАС позволило добиться нормализации уровней TNFα, IL1β, IL6 и полной редукции клинических проявлений ПКАС в 94,2% случаев. Таким образом, применение системной озонотерапии можно рассматривать в качестве одной из эффективных и патогенетически обоснованных стратегий комплексного лечения пациентов с ПКАС в амбулаторных условиях.

Ключевые слова: постковидный астенический синдром, системная озонотерапия, TNFα, IL1β, IL6

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Post-COVID asthenic syndrome (PCAS), the leading positions in the structure of which are occupied by chronic fatigue, cognitive dysfunction, sleep disorders, and anxiety [1], is still the subject of active study.

According to the literature data, 40–70% of patients, regardless of their age and past COVID-19 severity, suffer from PCAS [2, 3], which is strongly associated with the significant decline in daily functioning (by 64%), professional and social activity (by 70%) [4], and quality of life (by 92.4%) [5]; up to 20% of patients are unable to return to work a year after the acute phase of the infection [6]. Such statistics show high social significance of PCAS.

It has been proven that PCAS is a multifactorial disorder with the complex and poorly understood pathogenesis. In the light of current knowledge, systemic inflammation, the key role in the development of which is played by aberrant cytokine expression, is one of the major links of the disease process associated with PCAS [7]. Tumor necrosis factor (TNFα) and pro-inflammatory interleukins IL1β, IL6 are considered to be the most important. Plasma levels of these mediators that are increased in patients with PCAS are associated with the severity of clinical manifestations [8, 9]. These data make it possible to consider TNFα, IL1β, and IL6 as potential targets for PCAS therapy and the dynamic changes in these indicators as markers of treatment efficacy [10, 11].

Drug therapy involving the use of drugs of various classes (including antidepressants, tranquilizers, nootropic, neurovascular, and neurometabolic agents, vitamin and mineral supplements, adaptogens) is considered to be a decisive treatment strategy in patients with PCAS [12–14]. Unfortunately, it is currently obvious that these are not always effective both against PCAS symptoms and for the control over the patient's condition and quality of life when used as monotherapy [15]. That is why combinations of several drug classes are often used [16]. It should be emphasized that combination therapy shows higher clinical efficacy [15], however, the use of combination therapy is limited due to potential risk of multiple organ dysfunction and other severe adverse events (such as vertigo, nausea, sleep disorders) and the decrease in patient's adherence to treatment. That is why the use of physical treatment methods capable of enriching monopharmacotherapy of PCAS, increasing treatment efficacy, and preventing polypharmacy becomes relevant [17].

Considering the main mechanisms and clinical manifestations of PCAS, the use of systemic ozone therapy having a potent, broad spectrum of anti-inflammatory effects can be of great interest [18–20]. Furthermore, systemic ozone therapy has multimodal immunomodulatory, antioxidant, metabolic, neuroprotective, and anxiolytic effects [21–24], which is also important for treatment of patients with PCAS. Systemic ozone therapy is well tolerated; there is credible evidence of its efficacy in patients with COVID-19 [25–27]. Thus, in elderly patients (over 60) admitted to the intensive care using due to severe COVID-19, the decrease in C-reactive protein (CRP) levels by 48.2% and IL6 levels by 86.2% was revealed as early as after nine days of using systemic ozone therapy involving five procedures performed daily as part of standard treatment [25]. Some small-scale studies conducted in the specialized hospital settings or under conditions of health resort treatment report the efficacy of ozone therapy as part of the combination treatment regimen for such post-COVID syndrome manifestations, as bilateral polysegmental pneumonia [28], decreased exercise tolerance, sleep disorder, and chronic fatigue [29]. Important results of the studies were are as follows: significantly decreased C-reactive protein (CRP) levels [28, 29], IL6 levels back to normal, restoration of functional status and quality of life (QOL) in 94.6% of patients [29]. These data suggest potential efficacy of systemic ozone therapy as part of PCAS combination treatment. However, to date there is no evidence obtained in randomized controlled trials that would show the effectiveness of complementing drug treatment of PCAS, associated with chronic fatigue, cognitive dysfunction, sleep disorders, and anxiety, with systemic ozone therapy, in outpatient settings.

The study was aimed to assess the effects of systemic ozone therapy used to complement drug therapy on plasma levels of TNFα, IL1β, IL6 and parameters of mental status in patients with PCAS.

METHODS

Assessment and outpatient treatment of 140 patients (77 females and 63 males) aged 18–45 years (average age 34.2 [32.3; 36.2] years) with asthenic syndrome within the framework of the condition meeting the criteria for U 09.9 Post COVID-19 condition, unspecified (ICD-10) was performed at the Simferopol City Clinical Hospital No. 7 in 2022–2023.

Inclusion criteria: age 18–45 years; body mass index 18.5–24.9 kg/m^2; history of serologically verified COVID-19; emergence or noticeable progression of the asthenic syndrome symptoms (chronic fatigue, cognitive dysfunction, sleep disorders, anxiety) persisting for 3–12 months after having COVID-19, which cannot be explained by the fact of having another disorder (other than past coronavirus infection); final MFI-20 score ≥ 30, MoCa score ≤ 26, ISI ≥ 8, ESS score ≥ 11, HARS score ≥ 8; no contraindications to systemic ozone therapy.

Exclusion criteria: body mass index < 18.5 and ≥ 25 kg/m^2; history of mental disorders, including cognitive dysfunction, sleep disorders, mood disorders, and taking psychotropic drugs; history of disorders associated with the use of alcohol or other psychoactive substances; focal neurologic signs (based on the neurological assessment data); structural disorders of the brain (based on magnetic resonance imaging data); increased intracranial pressure; chronic infectious, inflammatory, endocrine, autoimmune, thrombophilic disorders, cancer; taking antibiotics, antiviral, vascular, metabolic, nootropic, anabolic, diuretic, or antioxidant agents, oral contraceptives within 3 months before the beginning of the study; previous drug therapy, psychotherapy or rehabilitation due to PCAS; smoking; refusal to take part in the study.

Patients were randomized into two groups based on the treatment method. The index group consisted of 70 patients (43 females and 27 males, average age 34.3 [32.5; 36.3] years), who received systemic ozone therapy in addition to drug therapy. The comparison group included 70 patients (44 females and 26 males, average age 33.7 [31.9; 35.9] years), who received drug therapy without systemic ozone therapy.

The drug based on the succinic acid complex with trimethylhydrazinium (Biokhimik JSC; Russia) was recommended to patients of both groups as a model of monopharmacotherapy to be used in accordance with the scheme proposed by the manufacturer: orally, 2 capsules twice a day (daily dose 2000 mg) for 30 days. The drug was selected based on the officially approved indications for use in PCAS associated with the increased fatigue, sleep disorders, emotional lability, and cognitive dysfunction [30], as well as on the fact that the drug was effective against PCAS [31]. All patients agreed to pay for the prescribed drug. Patients of the index group received extra systemic ozone therapy in the form of intravenous administration of 200 mL of the ozonized 0.9%

Table 1. Characteristics of patients with PCAS

Indicator	Index group (n = 70)	Comparison group (n = 70)	p_{1-2}
	1	2	
Average age, years (median [25%; 75%])	34.3 [32.5; 36.3]	33.7 [31.9; 35.9]	0.781
Females/males	43 (61.4) / 27 (38.6)	44 (62.9) / 26 (37.1)	0.771
Body mass index, kg/m^2 (median [25%; 75%])	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
Severe COVID-19 (n, %)	7 (10.0)	6 (8.6)	0.075
Time prior to the onset of PCAS symptoms after the acute COVID-19, months (median [25%; 75%])	4.9 [3.0; 5.7]	4.4 [3.3; 5.1]	0.893
MFI-20 AFS (median [25%; 75%])	81.9 [77.7; 84.9]	81.3 [78.1; 83.9]	0.801
MoCa AFS (median [25%; 75%])	24.2 [24.0; 25.7]	24.1 [24.0; 25.4]	0.881
ISI AFS (median [25%; 75%])	18.2 [16.3; 19.2]	17.7 [16.0; 18.8]	0.867
HARS AFS (median [25%; 75%])	21.3 [19.2; 22.9]	20.9 [18.7; 22.7]	0.891
CGI-S severe disorder (n, %)	41 (58.6)	39 (55.7)	0.072
CGI-S moderate disorder (n, %)	29 (41.4)	31(44.3)	0.07

Note: AFS — average final score.

sodium chloride solution (daily, course of 10 procedures, the first three procedures involving the ozone concentration of 2.0 mg/L, with subsequent ozone concentration increase to 3.0–4.0 mg/L) since the first day of prescribed drug therapy. All patients of the index group and comparison group successfully completed the trial.

A total of 50 generally healthy volunteers, unvaccinated and having no history of COVID-19, were assessed as controls. This group included both females and males aged 18–45 years with the body mass index of 18.5–24.9 kg/m^2, final MFI-20 scores <30, MoCa scores >26, ISI <8, and HARS scores <8. The non-inclusion criteria for the control group were identical to that for the group of patients with PCAS. The control group was matched by gender (32 females and 18 males), age (33.9 [32.3; 36.6] years, and body mass index (19.3 [18.8; 23.4] kg/m^2) to the group of patients with PCAS.

All the patients underwent clinical and psychopathological assessment that included collecting patient complaints, assessment of life history and history of the disorder, and assessment of mental status, including using the following methods: Multidimensional Fatigue Inventory (MFI-20) [32], Montreal Cognitive Assessment (MoCa) [33], Insomnia Severity Index (ISI) [34], Hamilton Anxiety Rating Scale (HARS) [35], Clinical Global Impression Scale (CGI), specifically the CGI-S Improvement subscale [36] (before treatment and after 30 days of treatment). Furthermore, any possible adverse event (AE) was recorded.

Assessment of plasma TNFα, IL1β, and IL6 levels by enzyme-linked immunoassay (ELISA) using the tests systems by Vector-Best (Russia) was performed in all healthy volunteers in the control group (once) and patients with PCAS (before treatment and after 30 days of treatment). Blood was collected from the cubital vein in the morning (7.00–9.00) in the fasting state (after the 8–12 h fasting).

Statistical processing of the results was performed using the STATISTICA 8.0 software package (StatSoft.Inc.; USA). Quantitative parameters were presented as the median (Me) with the interquartile range [25th; 75th percentiles (%)], while qualitative parameters were presented as the share and absolute number of values. The Mann–Whitney U test was used for comparative analysis of quantitative parameters, while comparative analysis of qualitative parameters involved the use of the chi-squared test (χ^2). Spearman's rank correlation was used for correlation analysis. The differences were considered significant at $p < 0.05$.

RESULTS

The main characteristics of patients with PCAS are provided in Table 1. The groups of patients were matched by all parameters.

Initially, patients of the index group and comparison group showed a comparable significant increase in plasma levels of TNFα, IL1β, and IL6 relative to the control group. After the end of therapy (on day 30) the TNFα, IL1β, and IL6 levels significantly decreased ($p < 0.05$) in both groups, however, the differences in the decrease were very large. In the index group, the TNFα, IL1β, and IL6 levels showed no significant differences from the values of the control group ($p > 0.05$) and were lower, than the values of the comparison group, by 39% ($p = 0.003$), 33.3% ($p = 0.022$), and 36.1% (p = 0.012), respectively (Table 2).

The dynamic changes in the mental status parameters associated with treatment of patients with PCAS are provided in Table 3. The findings suggest that after the end of therapy (on day 30) the changes were significant ($p < 0.05$) and more pronounced in the index group. The average difference in the changes between the index group and the comparison group based on the average final MFI-20 score was 36.7% ($p = 0.001$), MoCa — 10.9% ($p = 0.046$), ISI — 50.5% ($p < 0.001$), HARS — 45.8% ($p = 0.001$). In the index group, the number of patients with "no disease" based on CGI-S subscale was 66 (94.2%), while in the comparison group it was 44 (62.9%) ($p = 0.001$).

No adverse events were reported for patients of the index group and comparison group during the study.

DISCUSSION

As noted above, the levels of pro-inflammatory cytokines TNFα, IL1β, and IL6 in blood plasma are significantly increased in PCAS [37]; these cytokines cause activation of macrophages and microglial cells after crossing the blood-brain barrier at the damaged sites or sites with increased permeability [38]. This causes cell morphology transformation and initiation of the IBA1 antigen expression. The result is secretion of de novo cytokines (especially IL1β and TNFα) in the brain and neuroinflammation

Table 2. Dynamic changes in the plasma TNFα, IL1β, IL6 levels during treatment of patients with PCAS (median [25%; 75%])

Indicator	Control	Index group (n = 70)		Comparison group (n = 70)		Δ_{3-5} / p_{3-5}
		Before treatment	After treatment	Before treatment	After treatment	
	1	2	3	4	5	
TNFα, pg/mL	5.2 [2.8; 7.6]	14.9 [9.1; 15.2]	6.1 [3.6; 8.2]	15.2 [8.8; 15.7]	10.0 [7.4; 12.3]	39.0% / 0.003
		Δ_{3-2} –59.1%, p_{3-2} < 0.001		Δ_{5-4} –34.2%, p_{5-4} = 0.002		
		p_{2-1} < 0.001, p_{3-1} = 0.074		p_{4-1} < 0.001, p_{5-1} = 0.001		
IL1β, pg/mL	2.9 [1.5; 4.1]	8.1 [6.6; 10.3]	3.6 [2.5; 4.8]	8.0 [6.4; 10.1]	5.4 [4.7; 7.3]	33.3% / 0.022
		Δ_{3-2} –55.6%, p_{3-2} = 0.001		Δ_{5-4} –32.5, p_{5-4} = 0.021		
		p_{2-1} < 0.001, p_{3-1} = 0.079		p_{4-1} < 0.001, p_{5-1} = 0.033		
IL6, pg/mL	3.8 [2.0; 5.6]	9.9 [6.7; 12.4]	3.9 [2.7; 6.2]	9.6 [6.4; 13.0]	6.1 [5.5; 9.3]	36.1% / 0.012
		Δ_{3-2} –60.6%, p_{3-2} < 0.001		Δ_{5-4} –36.5%, p_{5-4} = 0.016		
		p_{2-1} < 0.001, p_{3-1} = 0.082		p_{4-1} < 0.001, p_{5-1} = 0.026		

Note: p — significance of differences between the groups compared, Δ — difference of changes.

[39]. At the same time, neuroinflammation is recognized as the most important mechanism, through which cytokines that change molecular and epigeneric processes eventually cause cell plasticity disturbances, nervous tissue dysfunction [40], and clinical manifestations of PCAS [41, 42]. The broad spectrum of the systemic ozone therapy anti-inflammatory effects is well understood and rather complex. Systemic ozone therapy inhibits transcription activity of the intracellular NF-kB signaling pathway, which results in suppression of the release of a number of inflammatory mediators involved in inflammatory response, such as IL1β, IL6, and TNFα [18]. Furthermore, the activity of the nuclear factor-erythroid-2-related factor 2 (Nrf2) is intensively suppressed [43], which is manifested in the increased activity of the antioxidant enzymes (superoxide dismutase, glutathione peroxidase, catalase) [44, 45] involved in inhibition of inflammation through their influence on the cytokine expression [46]. Moreover, systemic ozone therapy inhibits the p38MAPK and ERK1/ERK2 signaling, thereby reducing TNFα and IL1β production by monocytes [47]. Multiple studies have proven that the systemic ozone therapy effects are consistent, safe, and show high therapeutic potential in many disorders, the common pathogenetic link of which is inflammation, including COVID-19 [25–27]. To date, only three studies have been published (one observational and two randomized controlled trials (RCT)), the authors of which assessed efficacy and safety of systemic ozone therapy used as monotherapy or as part of combination treatment in patients with post-COVID syndrome.

Thus, in one observational study 100 patients (average age 55.2 ± 12.72 years) with PCAS symptoms received systemic ozone therapy: 2–3 procedures per week (course of 6–9 procedure) [48]. Complete reduction of asthenic symptoms and significant (60%) decrease in the symptom severity (based on the Fatigue Severity Scale scores) was reported in 40% of patients by the end of the follow-up period.

In another study, efficacy and safety of the systemic ozone therapy inclusion in the combination treatment (rehabilitation exercises, physical therapy: low-frequency magnetic therapy and iontophoresis with KI, CaCl2) of the hospitalized patients aged 29–78 years with post-COVID bilateral polysegmental pneumonia in the second phase of rehabilitation were assessed in the RCT [28]. Significantly better results based on CRP, D-dimer, overall assessment of clinical status, and achieved improvement of the quality of life were reported for the group of patients, who received systemic ozone therapy.

Efficacy of systemic ozone therapy added to the standard resort treatment complex was also assessed in the RCT involving examination of 140 patients (males — 44.3%, females — 55.7%, average age 49.2 [46.5; 52.3] years) with post-COVID syndrome, who still experienced chronic fatigue, depressed mood, shortness of breath, exercise intolerance 7 months after having the novel coronavirus infection [29]. Patients were randomized into two groups: group 1 (n = 70) underwent systemic ozone therapy in the form of intravenous drop infusions of the ozonized saline solution with the ozone concentration of 2.0 mg/L, course of 10 procedures, one procedure per day + resort treatment complex (climate therapy, rehabilitation exercises, full body massage with the focus on the chest, pelotherapy with the Saki Lake mud, hyaluronic acid inhalations); patients of group 2 (n = 70) received the same resort treatment complex without systemic ozone therapy. The resort treatment duration was 14 days. The study showed that inclusion of systemic ozone therapy ensured a significant decrease in the malondialdehyde levels (3.3-fold, p < 0.001), increase in the glutathione peroxidase activity (1.7-fold, p = 0.003), normalization of the IL6 levels, which were accompanied by significant improvement of the clinical status and quality of life in 94.6% of patients (vs. 62.3% in group 2). It is important to note that the results of the above studies show both efficacy and good safety profile of systemic ozone therapy in patients with post-COVID syndrome [29, 49].

In this study, the results of using systemic ozone therapy to complement drug therapy in patients with PCAS, associated with chronic fatigue, cognitive dysfunction, sleep disorders, and anxiety, in outpatient settings are presented.

Reliance on the systemic ozone therapy efficacy was considered to be associated with the broad spectrum of its anti-inflammatory effects, which was fully confirmed in our study. After the end of therapy (on day 30) the levels of TNFα decreased by 59.1% — from 14.9 [9.1; 15.2] pg/mL to 6.1 [3.6; 8.2] pg/mL, IL1β by 55.6% — from 8.1 [6.6; 10.3] pg/mL to 3.6 [2.5; 4.8] pg/mL, i.e. were completely back to normal and more than 30% lower compared to the comparison group (p = 0.003 and p = 0.022, respectively). The IL6 levels were also significantly lower (by 36.5%), than in the comparison group (p = 0.012). Apparently, this can be considered as one manifestation of the systemic ozone therapy anti-inflammatory effect in patients with PCAS. Our findings demonstrate the anti-inflammatory effect and benefits of adding systemic ozone therapy to drug therapy for adjustment of the TNFα, IL1β, and IL6 level changes in patients with PCAS. Since elevated levels of these cytokines are strongly associated with the PCAS clinical manifestation severity and outcome [8, 9], the above data make it possible to consider adding systemic ozone therapy not only effective, but also maximally pathogenetically substantiated.

Table 3. Dynamic changes in the mental status parameters during treatment of patients with PCAS

Indicator	Index group (n = 70)		Comparison group (n = 70)		Δ_{4-2} / p_{4-2}
	Before treatment	After treatment	Before treatment	After treatment	
	1	2	3	4	
MFI-20 AFS (median [25%; 75%])	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 AFS (median [25%; 75%])	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		Δ4–3 +4.9%, p_{4-3} = 0.072		
ISI AFS (median [25%; 75%])	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		
HARS AFS (median [25%; 75%])	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 no disease (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 disorder (n, %)	0	4 (5.8)	0	19 (27.1)	78.6% / 0.001
	p_{2-1} = 0.072		p_{4-3} < 0.001		
CGI-S severe disorder (n, %)	41 (58.6)	0	39 (55.7)	0	5.0% / 0.893
	p_{2-1} = 0.001		p_{4-3} = 0.001		
CGI-S moderate disorder (n, %)	29 (41.4)	0	31 (44.3)	0	7.0% / 0.887
	p_{2-1} = 0.001		p_{2-1} = 0.001		

Note: AFS — average final score, Δ — difference of changes.

As a result, after the end of therapy (on day 30) we managed to achieve significant clinical status improvement in the form of significant steady decrease in the severity of all PCAS symptoms in patients with PCAS from the ozone therapy group, which was confirmed by the dynamic changes in the MFI-20, MoCa, ISI, and HARS scores. In particular, after the end of therapy (on day 30) the total MFI-20 chronic fatigue severity score significantly decreased (by 74.7%) from the median score of 81.9 "severe" to 20.5 "no symptoms of chronic fatigue"; ISI — from the median score of 18.2 "moderate insomnia" to 5.0 "normal" (the average decrease in the indicator was 81.3%); HARS — from the median score of 21.3 "moderate anxiety severity" to 5.8 "no anxiety" (the average decrease in the indicator was 72.8%). The total MoCa cognitive status score increased by 17.1% from the median score of 24.2 "cognitive impairment" to 28.5 "normal cognition". All the indicators achieved were significantly superior to the indicators of the comparison group (p = 0.001).

Finally, after the end of therapy (on day 30) the number of patients with "no disease", i.e. complete reduction of PCAS clinical manifestations based on CGI, in the systemic ozone therapy group was 94.2%, and there were much more such patients, than in the comparison group (p = 0.001). These data are consistent with the results of assessing other efficacy parameters and confirm clinical benefits of adding systemic ozone therapy to drug therapy in patients with PCAS.

In our study, tolerability of the systemic ozone therapy added to drug treatment in patients with PCAS was good (no adverse events were reported), which was in line with the data of the earlier studies [28, 29].

Thus, according to the findings, the use of systemic ozone therapy to complement drug therapy is highly effective and safe in patients with PCAS.

CONCLUSIONS

In our study, adding systemic ozone therapy to drug treatment in patients with PCAS made it possible to achieve normalization of the TNFα, IL1β, and IL6 levels and complete reduction of PCAS clinical manifestations in 94.2% of cases. The use of systemic ozone therapy can be considered as one effective and pathogenetically substantiated strategy for combination treatment of patients with PCAS in outpatient settings.

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