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RESEARCH ARTICLE

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Ozone as adjuvant support in the treatment of COVID-19: A preliminary report of probiozovid trial

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Abstract

The evaluation of new therapeutic resources against coronavirus disease 2019 (COVID-19) represents a priority in clinical research considering the minimal options currently available. To evaluate the adjuvant use of systemic oxygen-ozone administration in the early control of disease progression in patients with COVID-19 pneumonia. PROBIOZOVID is an ongoing, interventional, randomized, prospective, and double-arm trial enrolling patient with COVID-19 pneumonia. From a total of 85 patients screened, 28 were recruited. Patients were randomly divided into ozone-autohemotherapy group (14) and control group (14). The procedure consisted in a daily double-treatment with systemic Oxygen–ozone administration for 7 days. All patients were treated with ad interim best available therapy. The primary outcome was delta in the number of patients requiring orotracheal-intubation despite treatment. Secondary outcome was the difference of mortality between the two groups. Moreover, hematological parameters were compared before and after treatment. No differences in the characteristics between groups were observed at baseline. As a preliminary report we have observed that one patient for each group needed intubation and was transferred to ITU. No deaths were observed at 7–14 days of follow up. Thirty-day mortality was 8.3% for ozone group and 10% for controls. Ozone therapy did not significantly influence inflammation markers, hematology profile, and lymphocyte subpopulations of patients treated. Ozone therapy had an impact on the need for the ventilatory support, although did not reach statistical significance. Finally, no adverse events related to the use of ozone-autohemotherapy were reported. Preliminary results, although not showing statistically significant benefits of ozone on COVID-19, did not report any toxicity.

KEYWORDS

autohemotherapy, ozone, respiratory insufficiency

1 | INTRODUCTION

Italy was the first European country affected by a severe outbreak of the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) epidemic emerged from Wuhan region (China), with high morbidity and mortality associated with the disease.[1]

The lung damage has initially characterized coronavirus disease 2019 (COVID-19) disease. Later considerable amounts of patients with a multiorgan involvement, principally characterized by coagulopathy and cardiovascular disorders, were diagnosed as COVID-19.[2–13]

COVID-19 is currently a challenge for clinicians in light of the minimal therapeutic options available. Therefore, the evaluation of new resources, designed in the first instance for other pathologies but potentially active against COVID-19, represents a priority in clinical research.[13,14]

Systemic medical Ozone, a complementary medical procedure mainly performed in Europe, has proved to help in several chronic obstructive pulmonary disease and chronic inflammation processes.[15] Moreover, some authors highlighted that Ozone could exhibit an inhibiting activity on viral replication associated with (but not only) anti-oxidizing and anti-inflammatory action, arousing considerable interest in the possibility of adopting this nonpharmacological adjuvant in the treatment of COVID-19.[15–22]

The PROBIOZOVID trial was designed to evaluate the adjuvant use of oxygen-ozone therapy and probiotics in the early control of disease progression in patients with COVID-19. This ongoing study enrolls subjects hospitalized in infectious disease wards. It evaluates the effectiveness of an ozone therapy-based intervention (accompanied by ad interim best available therapy [BAT]) in containing the progression of COVID-19 and in preventing the need for hospitalization in intensive care units (ICUs). Here we present a preliminary snapshot analysis of the initial data of the trial.

2 | METHODS

2.1 | Design of the study and setting

This is an interventional, nonpharmacological, open, randomized, prospective, double arms. non-profit study. The trial was designed to enroll a total of 152 COVID-19 patients shared equally between experimental (formally designed as "ozone group" and treated with BAT plus ozone therapy and supplemented with a multistrain probiotic mixture) and "control group" (treated only with BAT). The flow chart of the study was reported in Figure 1.

Primary outcome is delta (Δ) in the number of patients requiring orotracheal intubation despite treatment. Several secondary outcomes were considered in the planning of the trial: in this snapshot analysis we specifically evaluated the comparison between the two groups for Δ of crude mortality (at Day 7–14–30). Moreover, inflammation markers, D-dimer, hematology profile with lymphocyte count, kidney, and liver functions were compared in the two groups before (T0) and after (T7) treatment.

Considering as a primary endpoint the reduction of at least 15% of the number of COVID-19 positive patients who undergo a clinical deterioration requiring transfer to ICU, a sample of 152 total patients was estimated necessary for the study, 76 for each group (alpha = 0.0500, power = 0.8000, delta = −0.1500).

This single-center study is enrolling COVID-19 patients hospitalized in the infectious disease wards of Azienda Universitaria-Ospedaliera (AUO) Umberto I in Rome, one of the largest teaching hospitals of Italy, from April 2020.

The trial was registered on Clinicaltrials. gov website with official title "Oxygen–Ozone as Adjuvant Treatment in Early Control of COVID-19 Progression and Modulation of the Gut Microbial Flora" and identifier code NCT04366089.

2.2 | Population enrolled

Only participants who meet the eligibility criteria are included in the study. Subjects must meet the following inclusion criteria to be enrolled in the trial: (1) Age greater than 18 years, (2) nasopharyngeal swab positive for COVID-19, (3) COVID-19 stages III, and (4) Hospitalization in the infectious disease wards.

Patients are excluded from enrollment, if any of the following criteria are present: (1) COVID-19 Stages IV–VI, (2) hospitalization in ICUs, (3) pregnancy, (4) glucose-6-phosphate dehydrogenase (G6PD) deficiency, (5) patients who deny consent to the proposed treatment, (6) Inability to provide informed consent, (7) contraindications to performing oxygen–ozone therapy (hyperhomocysteinemia, favism or thyroiditis, coagulopathies, neurodegenerative diseases).

COVID-19 stages are compliant with indications published by Italian Society of Anesthesia, Analgesia Resuscitation and Intensive Care and are defined in the following way: sick disease–mild COVID-19 (I stage), light pneumonia–mild COVID-19 (II stage), serious pneumonia–severe COVID-19 (III stage), acute respiratory distress syndrome–critical COVID-19 (IV stage), sepsis–critical COVID-19 (V stage), septic shock–critical COVID-19 (VI stage). [23]

A serial number is assigned to each patient enrolled to randomly assign participants to one of the two groups using dedicated software (SPSS version 20.0; SPSS Inc.)

2.3 | Pharmacological treatment

Treatment options were based on the interim guidelines of the Italian Society of Infectious and Tropical Diseases.[24] Antiviral treatments with lopinavir/ritonavir 200/50 mg (2 tablets bid) or azithromycin 500 mg/daily plus hydroxychloroquine 200 mg/bid were available. Tocilizumab 8 mg/kg iv (up to a maximum of 800 mg per dose) twice with an interval of 12 h was administered in case of

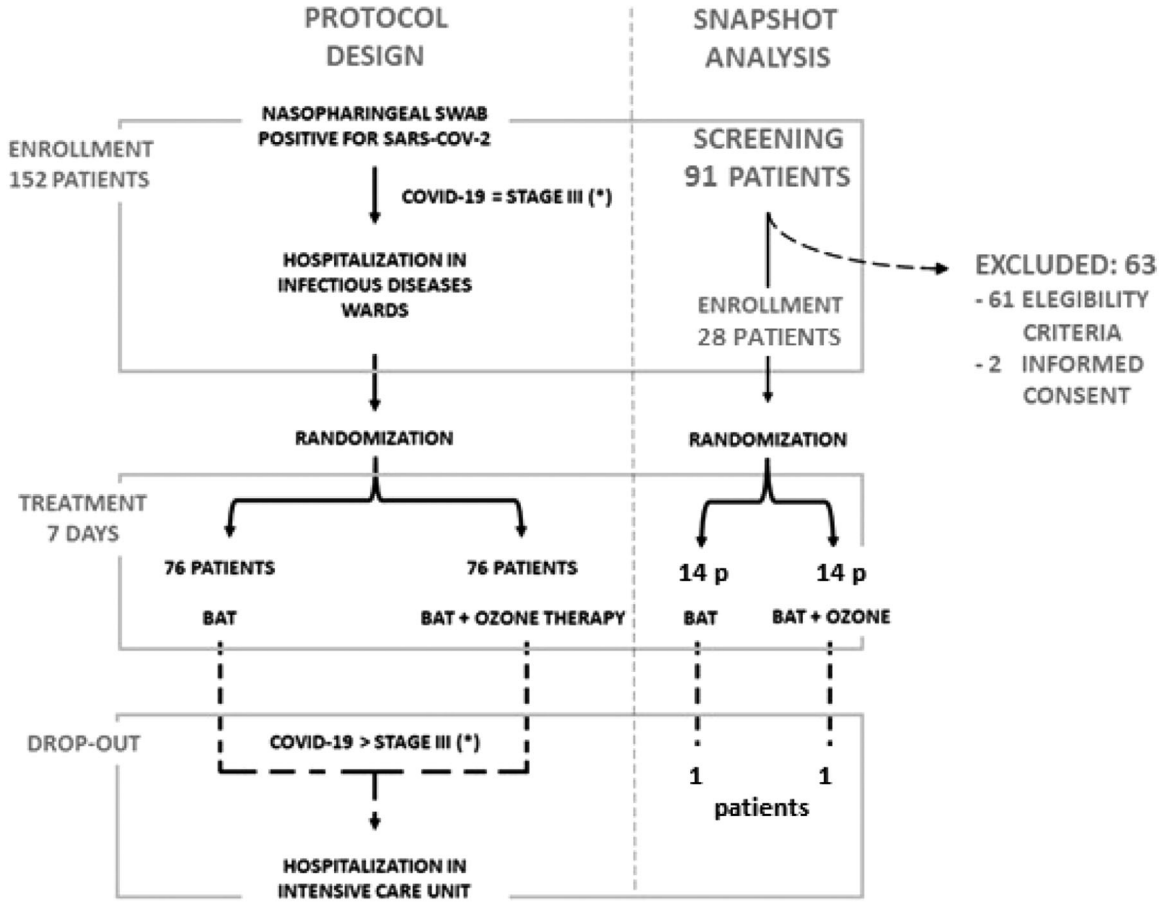

FIGURE 1 Flow chart of the PROBIOZOVID trial. (*) Italian Society of Anesthesiology, Analgesia, Resuscitation, and Intensive Care (SIAARTI) COVID-19 classification SIAARTI. Care pathway for the patient with COVID-19. Section 2: Recommendations for the local management of the critically ill patient-version 01, posted on March 14, 2020 and available at http://www.siaarti.it/SiteAssets/News/COVID19%20-%20documenti%20SIAARTI/Percorso%20COVID19%20-%20Sezione%202%20-%20Raccomandazioni%20per%20la%20gestione%20locale.pdf. Last accessed June 19, 2020. BAT, best available therapy

high serum levels of Interleukin (IL)-6 or worsening of respiratory function. An empirical broad-spectrum antibiotic treatment was considered when appropriate.

2.4 | Systemic oxygen–ozone (O2/O3) administration

A total volume of 250 ml peripheral venous blood was collected through an antecubital vein access (18 Ga) into a disposable sterile ozone dedicated bag via a "Y" connector previously added with 25 ml of Sodium Citrate 38 mg/ml. The same amount of O2/O3 gas mixture (ratio 1:1250 ml) titrated at 30 mcg/ml ozone concentration, was then added using an antibacterial filter and continuously gently mixed on a mechanical scale. After 5 min the ozonized blood was reinfused through the closed circuit by with a filtered dripping device. The procedure consisted in a daily double treatment with systemic Oxygen–ozone administration for seven days. A total of 15×10^3 mcg of ozone was the daily dose.

2.5 | Ventilatory support

Patients, both spontaneously breathing in ambient air (AA) and supported with venturi mask (VMK) or high flow nasal cannula, or continuous positive airway pressure (CPAP), were considered eligible. Patients breathing in AA were deemed to be enrollable in case of progressive deterioration of the blood gas analytical parameters concomitant with COVID-19 related bilateral pneumonia.

2.6 | Probiotic supplementation

A probiotic supplementation with a commercial product composed of *Streptococcus thermophilus*, DSM322245, *Bifidobacterium lactis* DSM 32246, *Bifidobacterium lactis* DSM 32247, *Lactobacillus*, *acidophilus* DSM 32241, *Lactobacillus helveticus* DSM 32242, *Lactobacillus paracasei* DSM 32243, *Lactobacillus plantarum* DSM 32244, *Lactobacillus brevis* DSM 27961 (SivoMixx) was coadministered. The dosage used was one sachets every 12 h for 7 days.

2.7 | Laboratory tests

Blood tests were performed in all patients at the enrollment (T0) and after seven days (T7), corresponding to the end of the ozone treatment and probiotic supplementation for the experimental group. They included hematology profile with lymphocyte count (a marker of severity of the disease) and multiparameter analysis of human lymphocyte subpopulations using flow cytometry, kidney and liver function, inflammation markers, martial status, coagulation, D-dimer, IL-6, and vitamin D.

2.8 | Statistical analysis

All data were analyzed using SPSS 19.0 (IBM Corporation) and GraphPad Prism 5.0 (GraphPad Software, Inc.).

Data are presented as mean and *SD* or median and range (interquartile range: 25%–75%). Paired sample *t* test or Wilcoxon signed-rank test were applied to evaluate paired samples between T0 and T7. Kolmogorov–Smirnov test was used to verify the normal distribution of values of the considered variables and, based on the evidence obtained, independent sample *t* test was subsequently applied to analyze variables that showed a normal distribution, while the Wilcoxon signed-rank test was applied in the case that the variables did not show a normal distribution. Variation in the modality of ventilation support are presented in the form of histogram plots.

2.9 | Ethics

This study was approved by the Ethical Committee of Sapienza University and AUO Umberto I of Rome, Italy (Rif. 5966, Prot. 110/2020). All patients signed written, informed consent to participate. All procedures performed in studies involving human participants were following the ethical standards of the institutional committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

3 | RESULTS

3.1 | Patients enrolled and characteristics of the cohort at baseline

From a total of 91 patients affected by COVID-19 hospitalized in infectious diseases wards and screened, 28 with severe lung involvement were recruited from April 2020 to May 2020 (Figure 1). Patients were randomly divided into the ozone autohemotherapy group (14 subjects) and the control group (14 subjects).

Baseline anamnestic and clinical characteristics of the cohort were showed in Table 1. No statistically significant differences in the characteristics between groups were observed at baseline ($p > .05$). No differences were also reported for pharmacological therapy. All patients were treated with ad interim BAT based on azithromycin 500 mg/daily plus hydroxychloroquine 200 mg/bid) and supported with Tocilizumab 8 mg/kg twice with a time lapse of 12 h, upon admission.

3.2 | Ozone therapy does not seem to impact on the progression of COVID-19 and related mortality

Two patients, one for each group, needed resuscitation support, and were transferred to an ICU. No deaths were observed among enrolled patients at 7 and 14 days of follow up. Thirty-day mortality was 7.1% ($n = 1$) for ozone group and 7.1% ($n = 1$) for controls.

3.3 | Influence of ozone-therapy on inflammation markers and hematology profile

Weekly modifications of the inflammation markers, D-dimer, hematology profile with lymphocyte count, kidney, and liver function were showed in Table 1. No statistically significant changes between T0 and T7 were observed for most of the parameters evaluated.

Interestingly in pre–post intervention analysis, lymphocyte blood count was significantly improved at T7 in comparison with T0 in the ozone group. C reactive protein decreased in the two groups

TABLE 1 Characteristics of population enrolled

	Ozone						Control						
Parameter	Mean	*SD*	Median	IQR 25%	IQR 75%	N° (%)	Mean	*SD*	Median	IQR 25%	IQR 75%	N° (%)	*p* Value
Age	63.3	12.1	59.2	50.1	68		60.1	14.4	64.7	52.5	69		.478
Female sex	-	-	-	-	-	5 (36)	-	-	-	-	-	7 (50)	.988
BMI	28.2	4.6	27.4	25.4	29.2		28.9	3.2	29.3	25	31		.377
Charlson index score	2.2	2.3	1.5	0	4		2.6	1.6	2	0	3		.701

Abbreviations: BMI, body mass index; IQR, interquartile range.

but the decrease reached statistical significance only in the ozone group. Despite this, the comparison between the group of patients treated with ozone and controls did not reach statistical significance for both variables.

3.4 | Ozone therapy does not significantly modify human *lymphocyte subpopulations*

We observed no statistically significant changes in the profile of lymphocyte subpopulations in terms of T-lymphocyte (CD3+ CD45+, CD3+, CD3+ CD4+ CD45+, CD3+ CD4+, CD3+ CD8+ CD45+, CD3+ CD8+), natural killer cells (CD3-/CD16+ CD56+ CD45+, CD3-/CD16+ CD56+), B-lymphocyte (CD19+, CD19+, CD45+) and CD4+/CD8+ ratio, between T0 and T7.

3.5 | Ozone therapy moderately reduces the need for the ventilatory support

There were no significant differences in respiratory support modalities between the two groups at T0. An overlapping progressive reduction of the need for CPAP support between T0 and T7 was observed in both the control group and the ozone group, however, the proportion of cases managed in AA or with VMK at T7 was higher in the "ozone group" compared with the T0, although not reaching statistical significance. The daily history of changes in the need for different types of ventilatory support for the two groups was reported in Figure 2.

3.6 | Safety

No adverse events or side effects related to the use of ozone autohemotherapy were reported until a current median follow-up of 21 days. Moreover, we did not observe ozone-related gut alterations o intestinal side effects due to antibiotic and antiviral treatments in any of the patients enrolled in subjects treated with ozone therapy plus probiotic supplementation. Otherwise, 30% of the control group presented gastrointestinal symptoms, such as diarrhea.

4 | DISCUSSION

Several studies analyzed the mechanisms by which ozone therapy could combat viral infections. In particular, (1) the improvement of the release of oxygen in the peripheral tissues, (2) the anti-inflammatory action, and (3) a virucidal activity have been described. Because of these possible properties, some international clinical trials exploring the potential activity of ozone therapy against COVID-19 are currently ongoing (Table 2).[15–22]

PROBIOZOVID trial offers a "real-life" view on the role of ozone autohemotherapy as an adjuvant nonpharmacological tool in the treatment of severe cases of COVID-19. In this trial, the results

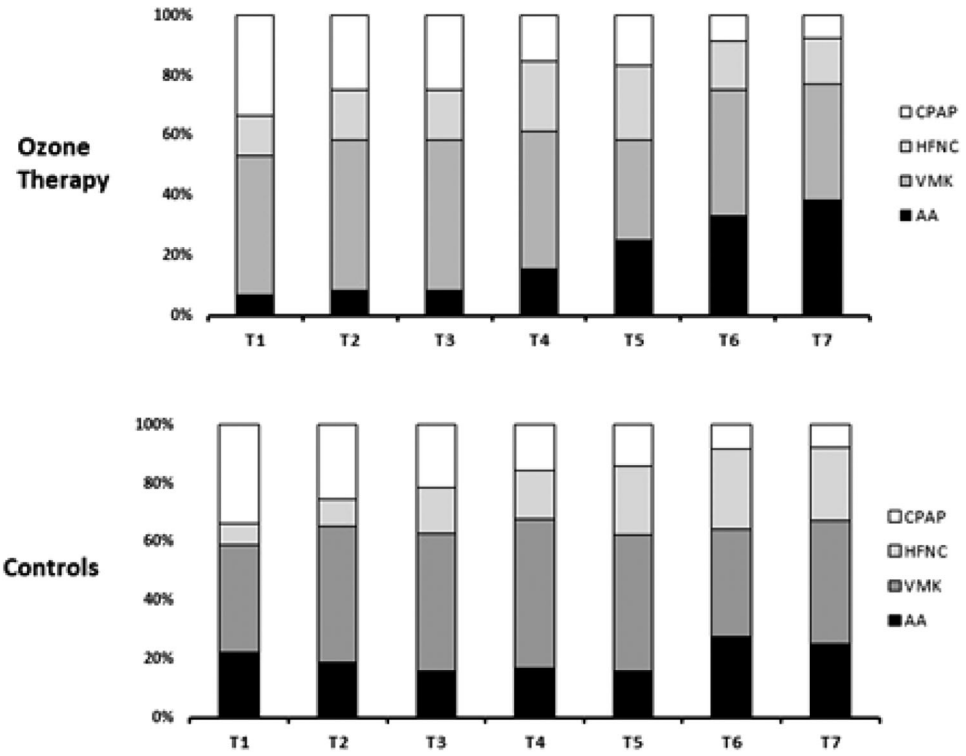

FIGURE 2 Changes (expressed in % of patients) in ventilatory support system used in the treatment of patient enrolled during the 7 days of treatment. AA, ambient air; CPAP, continuous positive airway pressure; HFNC, high flow nasal cannula; VMK, venturi mask

TABLE 2 Blood analysis results at enrollment (T0) and at Day 7 (T7)

		Ozone					Control					Ozone versus control t0	Ozone	Control
Parameter	Time	Mean	*SD*	Median	IQR 25%	IQR 75%	Mean	*SD*	Median	IQR 25%	IQR 75%	*p* Value	T0 vs T7	T0 vs T7
Creatinin	T0	0.84	0.28	0.80	0.62	0.95	0.97	0.28	1.00	0.72	1.17	0.21	0.92	0.20
Albumin	T0	39.09	6.27	38.00	34.50	41.50	39.77	5.63	40.00	35.00	45.00	0.78	0.85	0.99
AST	T0	41.93	23.83	38.00	28.50	53.75	29.71	11.33	28.50	21.00	37.50	0.10	0.81	0.08
ALT	T0	56.07	46.04	48.50	23.25	61.00	36.64	33.18	22.50	16.00	40.00	0.21	0.14	**0.03**
Total Bilirubin	T0	0.57	0.19	0.56	0.38	0.71	0.49	0.31	0.39	0.29	0.49	0.43	0.09	0.57
Sideremia	T0	14.33	5.37	16.70	9.63	18.43	9.16	10.07	5.25	4.38	7.88	0.23	0.12	0.31
Ferritin	T0	1337	2112	641.0	308.0	1294	766.7	552.9	833.5	259.7	996.0	0.36	0.86	0.31
Transferrin	T0	2.28	0.46	2.16	2.03	2.31	1.91	0.46	1.99	1.67	2.29	0.20	0.56	0.31
CRP	T0	34407	40453	12850	1250	57475	55236	83051	20550	9875.	58950	0.41	**0.02**	0.12
HB	T0	13.01	1.39	13.35	12.08	13.60	12.81	1.65	12.70	11.75	13.83	0.73	0.85	0.21
WBC	T0	7.29	3.70	5.90	4.86	7.96	7.87	3.93	6.57	5.50	9.58	0.69	0.72	0.81
Lymphocyte	**T0**	**1.17**	**0.47**	**1.04**	**0.85**	**1.54**	**1.37**	**0.80**	**1.09**	**0.87**	**1.5**	0.89	**0.00**	0.55
Plateled	T0	232.7	93.23	218.0	187.0	303.5	275.1	86.3	260.5	213.2	306.7	0.22	0.25	0.20
MPV	T0	9.44	1.02	9.35	8.98	9.75	8.78	1.19	8.55	8.13	9.50	0.10	0.68	0.89
Fibrinogen	T0	**4.42**	**1.10**	**4.49**	**3.61**	**5.07**	**5.50**	**1.90**	**5.59**	**4.14**	**5.78**	0.08	**0.00**	**0.01**
D-Dimer	T0	1192	1122	786.0	365.0	1369	865.6	492.8	824.0	396.5	1181	0.33	0.50	0.28
ATIII	T0	104.8	11.4	109.8	96.39	112.1	104.9	8.59	108.9	105.0	110.4	0.98	0.11	0.59
PT%	T0	116.8	5.54	117.0	113.0	120.0	113.4	9.11	116.0	107.5	120.5	0.24	0.39	0.42
PT sec	T0	11.46	2.44	10.77	10.62	11.00	11.08	0.53	11.20	10.61	11.33	0.59	0.51	0.78
INR	**T0**	**0.95**	**0.03**	**0.95**	**0.93**	**0.96**	**1.11**	**0.18**	**1.03**	**0.99**	**1.32**	**0.01**	0.33	**0.02**
PTT sec	T0	28.56	4.45	28.10	24.60	32.40	27.66	3.09	27.25	25.95	28.35	0.55	0.16	0.26
PTT ratio	**T0**	**0.95**	**0.15**	**0.94**	**0.82**	**1.08**	**1.09**	**0.18**	**1.10**	**0.94**	**1.26**	**0.03**	0.15	**0.00**
VIT-D3	T0	17.38	6.93	19.35	13.24	21.52	17.81	10.29	14.84	11.77	24.16	0.90	0.87	0.94
IL-6	T0	71.31	130.0	29.1	21.86	49.62	245.8	640.9	32.89	24.86	36.51	0.44	0.61	0.61
PCT	T0	0.11	0.07	0.11	0.06	0.16	0.24	0.50	0.07	0.03	0.10	0.51	0.06	0.69
Creatinin	T7	0.83	0.18	0.83	0.75	0.95	0.85	0.22	0.76	0.68	0.99	0.78	-	-

TABLE 2 (Continued)

		Ozone					Control					Ozone versus control t0	Ozone	Control
Parameter	**Time**	**Mean**	***SD***	**Median**	**IQR 25%**	**IQR 75%**	**Mean**	***SD***	**Median**	**IQR 25%**	**IQR 75%**	***p* Value**	**T0 vs T7**	**T0 vs T7**
Albumin	T7	39.63	5.88	39.50	35.25	44.50	39.80	6.18	41.50	34.25	45.00	0.95	-	-
AST	T7	39.45	25.48	34.00	26.00	42.50	65.29	68.38	45.00	26.50	68.00	0.21	-	-
ALT	T7	101.9	88.7	88.0	50.0	112.0	130.7	142.9	105.5	42.5	157.7	0.54	-	-
Total bilirubin	T7	**0.84**	**0.46**	**0.79**	**0.52**	**1.08**	**0.44**	**0.10**	**0.44**	**0.40**	**0.49**	**0.02**	-	-
Sideremia	T7	23.00	8.00	23.50	19.15	27.35	13.61	6.22	14.30	9.60	18.33	0.10	-	-
Ferritin	T7	1223	945.9	1215	558.0	1598	571.6	427.5	538.0	134.0	907.2	0.06	-	-
Transferrin	T7	2.05	0.54	2.30	1.87	2.36	2.17	0.45	2.13	2.01	2.41	0.75	-	-
CRP	T7	4640	9585	950.0	600.0	2350	16454	31646	3200	700.0	4100	0.22	-	-
HB	T7	13.11	1.28	13.40	12.35	14.00	11.91	2.03	11.80	11.20	13.48	0.09	-	-
WBC	T7	6.83	2.53	7.48	4.79	9.02	7.41	5.97	4.99	3.68	7.98	0.75	-	-
Lymphocyte	T7	1.94	0.67	2.10	1.43	2.19	1.61	1.29	1.33	0.93	1.65	0.41	-	-
Plateled	T7	280.9	106.9	255.0	217.0	357.5	315.2	73.54	318.5	261.0	386.2	0.38	-	-
MPV	T7	9.28	0.83	9.10	8.80	9.35	8.72	0.85	8.40	8.05	9.40	0.11	-	-
Fibrinogen	T7	3.03	0.82	3.27	2.39	3.48	3.70	1.39	3.39	2.94	4.06	0.16	-	-
D-Dimer	T7	914.8	791	681.0	402.0	1079	1187	955.4	816.0	579.2	1252	0.47	-	-
ATIII	T7	91.4	9.25	95.61	88.20	96.70	101	15.68	102.9	95.9	107.0	0.29	-	-
PT%	T7	113.7	10.13	114.0	110.0	117.5	110.8	7.45	112.0	107.2	116.0	0.45	-	-
PT sec	T7	10.99	0.58	10.96	10.75	11.19	11.14	0.45	11.04	10.81	11.32	0.50	-	-
INR	T7	0.97	0.06	0.97	0.94	0.99	0.98	0.05	0.98	0.95	1.00	0.51	-	-
PTT sec	T7	26.36	2.70	27.10	25.33	27.63	26.56	1.80	26.40	25.20	27.30	0.84	-	-
PTT ratio	T7	0.88	0.09	0.90	0.85	0.92	0.89	0.06	0.88	0.84	0.91	0.81	-	-
VIT-D3	T7	17.92	7.02	18.84	16.78	22.06	18.12	9.22	14.96	13.01	25.98	0.96	-	-
IL-6	T7	44.57	68.41	19.58	5.17	39.35	704.5	936.9	704.5	373.3	1036	0.50	-	-
PCT	T7	0.04	0.01	0.04	0.03	0.05	0.17	0.05	0.17	0.15	0.18	0.17	-	-

Abbreviations: ALT, alanine aminotransferase; AST, aspartate transaminase; CRP, C-reactive protein; HB, hemoglobin; IL-6, interlwukin-6; INR, international normalised ratio; IQR, interqaurtile range; MPV, mean platelet volume; PCT, procalcitonin; PTT, partial thromboplastin time; WBC, white blood cell.

obtained in patients treated with this intervention were compared with a homogeneous control group, in compliance with a strict methodology based on a prospective randomized enrollment (Table 3).

In this preliminary snapshot analysis, we observed a high level of safety of the procedure, since no adverse effects were reported in any of the cases studied. On the other hand, we observed that treated and untreated groups had an overlapping clinical trend with no significant differences relating to the changes in blood tests and the modalities of ventilatory support in the seven days of treatment.

Based on the preliminary data available, the primary objective of the study was not achieved. The adjuvant use of ozone therapy did not seem to significantly impact the early control of COVID-19 progression, failing the secondary outcomes taken into consideration in this analysis.

Hospitalization, dietary changes, antibiotics, and systemic inflammation related to COVID-19 are all variables that contribute to changes in the intestinal and lung microbiota with significant repercussions on the outcomes of the disease.[6,25-37] Furthermore, in case of use of topic ozone therapy, rectal insufflation could also lead to a modification of the microbial flora [30,34] Probiotic supplementation can help to correct these issues. Previously it has been reported that bacteriotherapy with the same multistrain probiotic supplementation could help to improve the prognosis in patients affected by COVID-19.[6,29] Anyway, we did not observe statistically-significant differences in clinical outcomes between the treated and

TABLE 3 Clinical trials on the topic of ozone therapy currently ongoing in patient affected by COVID-19; data from ClinicalTrials. gov (https://clinicaltrials.gov/ct2/home) and Chinese clinical trial registry (http://www.chictr.org.cn/enIndex.aspx), accessed on June 21, 2020

Title of clinical trial	Intervention	Proponent of trial /references
Ozone therapy in the prevention of COVID-19 infection ClinicalTrials. gov Identifier: NCT04400006	To be completed at least ten sessions of ozone therapy applied by the method of major autohemotherapy in the last six months from the time the first COVID-19 case of Turkey reported (March 11, 2020).	Marmara University, Istanbul, Turkey https://clinicaltrials.gov/ct2/show/NCT04400006?term=ozone&cond=COVID&draw=2&rank=5
Randomized clinical trial to evaluate efficacy and safety of systemic indirect endovenous ozone therapy (SIEVOT) as adjuvant treatment in COVID19 non-intubated patients ClinicalTrials. gov Identifier: NCT04359303	Systemic indirect endovenous ozone therapy: 200 ml at 40 mcg/ml of medical ozone /oxygen in 200 ml of patient blood mixed in an homologated device for the procedure. Duration of treatment not reported	Universidad Católica San Antonio de Murcia https://clinicaltrials.gov/ct2/show/record/NCT04359303?term=Ozone&cond=COVID&draw=1&rank=1
A trial of ozone auto-hemotherapy in adults hospitalized with COVID-19 pneumonia ClinicalTrials. gov Identifier: NCT04370223	Treatment mixing 100–200 ml of blood with Ozone at a concentration of 40 μg/ml with a gas volume of 200 ml. Treatment will occur every 12 h during 5 days.	Institut d'Investigació Biomèdica de Girona https://clinicaltrials.gov/ct2/show/record/NCT04370223?term=ozone&cond=COVID&draw=1&rank-=2
Blood ozonization in patients with SARS-CoV-2 respiratory failure (CORMOR) ClinicalTrials. gov Identifier: NCT04388514	The autologous blood was mixed with a gas mixture of a 200 cc composed of 96% of Oxygen and 4% of ozone with a therapeutic O3 range of 40 μg/ml of gas per ml of blood. The duration of ozone treatment lasted for 3 consecutive days.	Azienda Sanitaria-Universitaria Integrata di Udine https://clinicaltrials.gov/ct2/show/record/NCT04388514?term=Ozone&cond=COVID&draw=1&rank=4
Clinical study for ozonated autohemotherapy in the treatment of novel coronavirus pneumonia (COVID-19) ChiCTR2000030165.	Ozonated autohemotherapy (no further data is available)	Academy of Medical Engineering and Translational Medicine, Tianjin University. http://www.chictr.org.cn/showproj.aspx?proj=49947
A randomized controlled trial for the efficacy of ozonated autohemotherapy in the treatment of novel coronavirus pneumonia (COVID-19). ChiCTR2000030006.	Ozonated autohemotherapy (no further data is available)	Union Hospital, Tongji Medical College, Huazhong University of Science and Technology. http://www.chictr.org.cn/showproj.aspx?proj=49737
A multicenter randomized controlled trial for ozone autohemotherapy in the treatment of novel coronavirus pneumonia (COVID-19). ChiCTR2000030102.	Ozonated autohemotherapy (no further data is available)	Tianjin Huanhu Hospita. http://www.chictr.org.cn/showproj.aspx?proj=49747

Abbreviations: COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

untreated groups in PROBIOZOVID trial. We hypothesize that the treatment with the probiotic in this specific setting was able to restore some microbiome functions, but too short and/or at an inadequate dosage to observe an effect on COVID-19 related damage.

We did not observe ozone-related gut alterations o intestinal side effects due to antibiotics, and antiviral treatments in any of the patients enrolled in the ozone group, probably as a result of the repair effect on the microbiome achieved by supplementation with the probiotic. On the other hand, 30% of patients in the control group presented gastrointestinal symptoms, such as diarrhea. The safety of ozone treatment in COVID-19 patients represents the most significant evidence of this study and is a promising premise for continuing the research on the topic.

This study has many limitations, including the small sample size, the short follow up and the impossibility of discriminating specific effects of different drugs used. Not less important, our observations were limited to patients not requiring mechanical ventilation. Moreover, the method of preparation of the blood bag treated with Ozone can influence the ozone autohemotherapy's physical properties. Therefore, the results of this study should only be reproducible under the same experimental conditions and are not necessarily similar to those carried out under different circumstances. Finally, the change of epidemiology of COVID-19 in Italy, with the progressive decrease of new cases, and the improvement of diagnostic and therapeutic resources, with the reduction of severity and fatality rates, seriously impact the possibility of trial completion. These new conditions limit the possibility of new enrollments and change the pattern of potentially eligible patients, who appear clinically less compromised.

Nevertheless, this is one of the first randomized trials on the use of ozone therapy in subjects affected by COVID-19. The snapshot analysis was performed on patients enrolled in the first phase of the epidemic in Italy when the diseases appeared more aggressive. Moreover, even with all the limitations mentioned above, the rigorous study design offers a guarantee on the results obtained, which cannot always be traced in some previous studies on ozone therapy.

At the moment, only 4 papers including case series of patients affected by COVID-19 and treated with ozone therapy are present in the indexed and peer reviewed scientific literature. In particular Fernández-Cuadros ME et al.[38] reported a prospective quasi-experimental before-and-after study on four severe COVID-19 patients treated by rectal ozone administration, Hernández et al.[39] described three patients with COVID-19 pneumonia treated by 1–4 sessions of oxygen–ozone (O_2–O_3) therapy, Zheng et al.[40] portrayed two severe cases with COVID-19 received ozone therapy. Finally, Franzini et al. reported a larger study on 50 SARS-COV2 positive elderly patients suffering from acute respiratory disease syndrome treated with four cycles of O_2–O_3. Anyway this was not a randomized study but a case series report based on a pre-post intervention evaluation and no control group was considered.[41] One of the main methodological difficulties to be faced in the studies on COVID-19 is related to the fact that SARS-CoV2 causes an acute pathology with a clinical evolution concentrated in a short chronological period: therefore in the absence of a control group, the main bias of the studies on a specific therapeutic intervention is the fact that the patient could present an improvement or worsening variation of the clinical status regardless of the therapeutic intervention analyzed.

Despite all limitations previously listed for our ad interim analysis, this is currently the only prospective randomized study on this topic. We believe that in the absence of certainly effective therapeutic resources, it is important to share all available data, especially if obtained by randomized trials.

5 | CONCLUSION

This report does not aim to establish the effectiveness of the analyzed approach. However, in the absence of rigorous RCT on ozone effects on COVID-19 we consider important sharing preliminary data. Our results clearly state that systemic ozone therapy is not toxic and has no side effects in critically-ill patients affected by COVID-19. Although the efficacy of ozone autohemotherapy in COVID-19 remains unclear, the lack of toxicity is promising and warrants further studies.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

All authors have contributed significantly to the work and have seen and approved the manuscript. Trial supervision: Francesco Pugliese, Franco Ruberto, Roberto Poscia, Claudio Mastroianni. Trial execution: Fabio Araimo, Paolo Tordiglione, Carmela Imperiale, Gabriella d'Ettorre. Data collection: Andrea Calò, Serena Zancla, Gregorio Egidio Recchia, Vera Mauro. Data analysis: Cristian Borrazzo, Letizia Santinelli, Giuseppe Pietro Innocenti, and Claudia Pinacchio. Paper writing: Giancarlo Ceccarelli, Federico Bilotta, Francesco Alessandri.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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