Compassionate use of rectal Ozone (O₃) in severe COVID-19 pneumonia: a case-control study.

Marcos Edgar Fernández-Cuadros (✉ marcosefc@hotmail.com)

Hospital Universitario Santa Cristina, Madrid <https://orcid.org/0000-0001-6153-9075>

María Jesús Albaladejo-Florín

Hospital Universitario Santa Cristina

Sandra Alava-Rabasa

Hospital Universitario Santa Cristina

Juan Gallego-Galiana

Hospital Universitario Santa Cristina

Gerardo Fabiel Pérez-Cruz

Hospital Universitario Santa Cristina

Isabel Usandizaga-Elio

Hospital Universitario Santa Cristina

Enrique Pacios

Hospital Universitario Santa Cristina

David Torres-García

Hospital Universitario Santa Cristina

Daiana Peña-Lora

Hospital Universitario Santa Cristina

Luz Casique-Bocanegra

Gabinete Medico Zamora

María Jesús López-Muñoz

Hospital Universitario Santa Cristina

Javier Rodríguez-de-Cía

Hospital Universitario Santa Cristina

Olga Susana Pérez-Moro

Hospital Universitario Santa Cristina

Research Article

Keywords: Ozone, Ozone therapy, Pneumonia, COVID-19, SARS-Cov-2, Rectal insufflation.

Posted Date: February 24th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-231696/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Version of Record: A version of this preprint was published at SN Comprehensive Clinical Medicine on March 22nd, 2021. See the published version at <https://doi.org/10.1007/s42399-021-00849-9>.

Abstract

Objectives: To evaluate effect of rectal Ozone in severe COVID-19 pneumonia and to compare to Standard-of-care (SOC).

Material and Methods: In a case-control study, 14 patients with severe bilateral COVID-19 pneumonia (positive RT-PCR), treated with SOC and rectal Ozone, were evaluated before-and-after treatment and compared with SOC (14 patients) in a 10 day follow-up period. Ozone-protocol consisted of 8 sessions (1 session/day) of intra-rectal Ozone, (150mL volume, 35mg/ml concentration [5.25mg total dose]). The SOC-protocol included O₂-supply, antivirals (Remdesivir), corticosteroids (Dexamethasone/Metilprednisolone), monoclonal antibodies (Anakinra/Tocilizumab), antibiotics (Azytromicine), anticoagulants (Enoxaparine) and hyperimmune serum (if necessary). Primary outcome variables: a) clinical (O₂-saturation and O₂-supply); b) biochemical (Lymphocyte count, Fibrinogen, D-Dimer, Urea, Ferritin, LDH, IL-6 and CRP); c) radiological Taylor Scale. Secondary outcome variables: a) hospitalization length-of-stay, b) mortality-rate.

Results: At baseline, Ozone/SOC-groups were not different on age, comorbidities, O₂-saturation and O₂-supply. Patients in Ozone-Group improved O₂-saturation and decrease O₂-supply. SOC maintained O₂-saturation and required more O₂-supply. Lymphocyte-count improved only in Ozone-group and with statistical difference ($p<0.05$). Biomarkers of inflammation (Fibrinogen, D-Dimer, Urea, LDH, CRP and IL-6) decreased in both groups, but only significantly in favor of Ozone-group ($p<0.05$). Ferritin showed a significant decrease in the Ozone-group but an increase on the SOC-Group. Radiological pneumonitis decreased on both groups but the decrease was only significant in the Ozone-Group ($p<0.0001$). Mortality and length-of-stay, although not significant, were inferior in Ozone-Group.

Conclusion: Compassionate use of Rectal Ozone improved O₂-saturation, reduced O₂-supply, decreased inflammation biomarkers and improved Taylor's radiological scale significantly when compared to SOC-Group. Mortality and length-of-stay was inferior in the Ozone-group, but this difference was not significant.

Introduction

After the discovery of a new coronavirus in December 2019 on Wuhan, province of China, by the 3rd of March 2020, the World Health Organization (WHO) has declared an exceptional situation of pandemic due to the new SARS-CoV-2 or COVID-19 virus ¹.

Nowadays, there is no effective treatment for the management of SARS-CoV-2 infection or COVID-19 disease. The Spanish Ministry of Health, literally states that at the moment there is no evidence from controlled-clinical-trials to recommend a specific treatment for the SARS-CoV-2 infection in patients with suspected/confirmed COVID-19. However, there are several ongoing clinical trials, which could modify this situation on the short-mid-term ².

In Spain, the pandemic situation constitutes a real risk of saturation of the Health System, and there may be a need to reorganize Material and Human Resources because of the shortage of such professionals and materials ³.

Currently, since there is no definite cure for COVID ⁴, there are 8 clinical trials (CT) that postulate the potential use of Ozonized Autohemotherapy on the management of COVID-19 disease (1 CT from Turkey, 2 CT from Italy, 2 CT from Spain and 3 CT from China) but the results, except the CT from Italy, are still unreported ⁵. As far as we know, there is only one CT from Cuba which considers rectal Ozone as an alternative for the management of COVID-19 infection, but the study is still in phase of recruiting ⁶.

The actual Standard-of-care for COVID-19 is supportive, and respiratory failure is the main cause of mortality secondary to acute respiratory distress syndrome (ARDS). A small percentage of patients (15%) with severe COVID-19 could develop a "cytokine storm" or hyper inflammation syndrome ⁷. Early identification and treatment of hyper inflammation will result in a decrease of mortality rate; therefore, any therapies with acceptable safety profiles and capable of decreasing/modulating inflammation (as corticosteroids, monoclonal antibodies and Ozone, to date some) are indicated at this Stage ⁸.

There is growing Medicine-Based-Evidence that comes from countries such as Cuba, Italy, Germany, Russia and Spain, that states that Ozone (O₃) is capable of modulating pain and inflammation; and recognized bactericidal, fungicidal, virucidal and anti-parasitic properties are attributed to Ozone ^{9,10}. The germicidal effect of Ozone is such, that many of the water purification plants worldwide use Ozone with great results ⁹. Fernández-Cuadros et al have postulated Ozone as an alternative therapy for the management of the present SARS-CoV-2 pandemic ¹¹. Virucidal, immunomodulatory and vasodilator properties that favor O₂ transport to hypoxemic tissues are the main features to postulate Ozone as a promising alternative in COVID-19 ¹¹.

Three evolutionary stages are recognized in the SARS-CoV-2 infection: a) Stage 1 (early infection); b) Stage 2a and b (normoxic and hypoxic lung phase); and c) Stage 3 (systemic hyper inflammation or "cytokine storm") ¹¹. In this scenario, Fernández-Cuadros et al consider 4 biological properties of Ozone (O₃) that could act on the different phases of SARS-CoV-2 infection. A) Ozone could inactivate the virus by direct (O₃) or indirect oxidation (ROS [reactive oxygen species] and LOPs [lipid oxidative products]). B) Ozone could stimulate the humoral and cellular immune system. Properties A and B could be useful on early COVID-19 infection phase (Stage 1 and 2a). C) Ozone reduces inflammation, and modulates the antioxidant system, making it useful in the hyper inflammation or "cytokine storm" phase. D) Ozone improves gas exchange. Properties C and D make Ozone useful in the hypoxemia and / or multi-organ failure phase (Stage 2b and Stage 3) ¹¹.

Recently, our study Group has presented the preliminary results of 4 mild-severe COVID-19 patients treated by rectal Ozone, with very promising results on the management of SARS-Cov-2 infection ¹².

The objective of this article is to show the updated results of the effectiveness on the compassionate use of rectal Ozone (O₃) in a series of COVID-19 patients with severe bilateral pneumonia, and compare them with a series of patients treated by Standard-of-care, in terms of clinical, biochemical and radiological variables (primary outcomes). Mortality and Hospitalization length-of-stay were also compared between groups (secondary outcomes).

Materials And Methods

A prospective, before-and-after, non-profit, case-control study was performed. The study included 28 severe COVID-19 patients admitted at Hospital Universitario Santa Cristina, with clinical symptoms and RT-PCR (reverse transcriptase polymerase chain reaction) positive for SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2). The study run from August to November 2020 and the Health Care Ethics Committee (Report 15/4/2020) of Santa Cristina's Hospital and the Ethics Committee for Medical Investigation of La Princesa's Hospital (ACTA CEIm 12/20, 28/5/20) authorized the study and ozone treatment for compassionate use.

Inclusion criteria: 1) Man or woman, 18 years and older; 2) positive result on new coronavirus nucleic acid test (RT-PCR SARS-CoV-2); 3) moderate-severe pneumonia ($\text{SpO}_2 < 93\%$ or $\text{PaO}_2/\text{FiO}_2 < 300\text{mmHg}$, fever or moderate/severe respiratory symptoms; 4) bilateral "ground glass image" (compatible with lung lesions) on chest X-ray (according to Taylor's scale)¹³; 5) Hospitalized patients due to moderate or severe respiratory symptoms; 6) O_2 supply with non-mechanical ventilation; 7) The patient/legal representative must be willing to be given informed consent to participate in the trial.

Exclusion criteria: 1) Pregnancy or breast feeding; 2) Glucose 6-phosphate dehydrogenase (G6PD) deficiency (favism); 3) Patients enrolled in other clinical studies.

In the initial evaluation, after Standard-of-care was completed in all patients, and the treating physician considered that Ozone could be prescribed as last alternative and for compassionate use in COVID-19 treatment, because of stability/ceiling effect or even deterioration after SOC; the procedure/indications/contraindications were explained to the patients and/or legal representative, the initial biochemical evaluation (leucocyte and lymphocyte count, Ferritin, Dimer-D, Fibrinogen, CRP [C-reactive protein] and IL-6) and the initial radiography of the chest were performed, and informed consent was signed (Figure 1).

The Standard-of-care protocol included O_2 -supply, antivirals (Remdesivir [200mg/1d, the first day and 100mg/d for 4d]), corticosteroids [Dexamethasone 6mg/d for 7d] or Methylprednisolone [40mg/d for 7 d]), antibiotics (Azytromicine [500mg/d per 5d]) and anticoagulants (Enoxaparine [40mg SC/d, all hospitalization period], anti-IL-6 (Tocilizumab 8mg/kg IV twice with an interval of 12h, and up to a maximum of 800mg per dose]) or anti-IL-1 (Anakinra 100mg, single dose) (**Figure 1**).

In the Ozone-Group (after Standard-of-care was administered) the proposed technique, according to the Madrid International Ozone Therapy Declaration, was to administer intra rectally a dose of 5,25mg of Ozone (insufflation of a volume of 150 mL at a concentration of 35 mg/mL for 5 to 10 days), according to the severity of the patients (**Figure 1**).

The supplies needed to perform the technique were: a) Ozonosan α -Plus ® [Ozone Generator]; b) Rectal probe; and c) three silicone syringes of 50 mL capacity.

For administration, the patient was placed in the supine (for sedated patients) or lateral decubitus position (for collaborative patients) with the lower limbs flexed. Three 50-mL silicone syringes of Ozone were loaded with the corresponding concentration (35 mg/mL), and were slowly injected rectally through a 14 French rectal probe, after lubrication with medical gel-type solution. The insufflation time will be a few minutes, at an administration rate of 1 mL / second.

After 10 sessions of the Ozone protocol (O₃), the final evaluation was performed, clinical and biochemical analysis and chest radiographies were performed and evaluated, and adverse effects (if any) were recorded. The same analysis was performed on Standard-of-care Group (including best and worst value of variables during hospitalization period), in a 10 day follow-up period. Mortality and Hospitalization length-of-stay were compared between both Groups, at 20-30 days (before discharge) (**Figure 1**).

Chest Radiography was used to confirm diagnosis and to grade severity. Taylor has proposed a Severity Scale for (SARS) Severe Acute Respiratory Infection, ranging from 1 to 5 degrees. Grade 1 is considered normal. Grade 2 shows patchy atelectasis or hyper inflammation or thickening of the bronchial wall. Grade 3 includes focal alveolar consolidation but without involving more than one segment or lobe. Grade 4 shows multifocal consolidation and grade 5 includes diffuse alveolar consolidation ¹³.

Statistical analysis was performed using SPSS ® (Statistical Package for Social Sciences, Illinois, USA) version 20.0. Frequencies and percentages were used to evaluate qualitative variables; while for the evaluation of quantitative variables, means and standard deviation were used. The Student T-test was the statistical tool used to evaluate a change before-and-after treatment in quantitative variables. The level of significance was 95% (p <0.05).

Results

We present the results of a series of severe COVID-19 pneumonia patients, confirmed with (+) RT-PCR for SARS-CoV-2, and with clinical and radiological signs of bilateral pneumonitis, who received Standard-of-care, (Azithromycin [500mg/d for 5d), corticotherapy in descending regimen, Dexamethasone [6mg/d for 7 days] or Methylprednisolone [40mg/d for 7d], monoclonal antibodies such as Anakinra, [anti IL-1, 100mg, single dose] or Tocilizumab [anti IL-6, 800mg c/12h, up to 2 doses]); who despite this, persisted with dyspnea, requiring high flow O₂ supply.

At this point, and by decision of the treatment physician, based on stability/ceiling effect or even deterioration after SOC, one Group of patients were asked to be treated by rectal Ozone (Ozone-Group), as compassionate use, while the other patients continued with O₂-supply as needed (Standard-of-care Group) (**Figure 1**).

Baseline characteristics

Mean age of Ozone-Group was 84.35 ± 9.52 years (range from 57 to 98) years). The male to female ratio was 3:1. The mean number of sessions was 7.83 ± 2.4 (range from 5 to 10 sessions) [**Table 1**]. Mean age of Standard-of-care Group was 83 ± 12.55 years (range from 60 to 104) years). The male to female ratio was 1.7:1. No difference in age was observed between both groups (p=0.7566).

Charlson Index, O₂ Saturation and O₂ supply were similar between both Groups, making Groups comparable (**Table 1**). No difference in comorbidities (p=0.4431), O₂-saturation (p=0.1129) and O₂-supply (p=0.2192) was observed between both groups.

Table 1. Baseline Characteristics of Ozone-Group and Standard-of-care Group.

Variable	Ozone	Standard of care	p
Age, years	84,35 ± 9.52	83 ± 12.55	0,7566
Charlson Index (comorbidities)	4 ± 1.7	4,42 ± 1.57	0,4431
O ₂ Saturation %	94,3 ± 0.94	92,96 ± 4.2	0,1129
O ₂ supply L/min	7,1 ± 6.31	4,4 ± 5.15	0,2192
Hospitalization length-of-stay, days	28, 58± 16.97	35,67 ± 21.04	0,1736
Male, %	85.7	50	NR

NR, not referenced. P, statistical T-student Test, significant if p<0.05.

PRIMARY OUTCOMES (Clinical, biochemical and radiological variables)

Clinical Variables

In Ozone-Group, clinical variables (O₂-saturation and O₂-supply) improved in all patients. Ozone saturation improved from 94.3% to 94.5% (p=0.6682), and O₂ supply decreased from 7.1 L/min to 3.5 L/min (p=0.09).

(Table 2, Figure 2).

In Standard-of-care Group, clinical variables (O₂-saturation and O₂-supply) did not improved at all. Oxygen saturation slightly changed from 92.96 % to 92.9 % (p=0.9389), and O₂ supply worsened from 4.4 L/min to 5.04 L/min (p=0.7920). (Table 2, Figure 2).

Table 2. Clinical, biochemical and radiological variables before-and-after treatment in Ozone-Group and Standard-of-care Group.

VARIABLES	Ozone before (n=14)	Ozone after (n=14)	P	Standard of care before (n=14)	Standard of care after (n=14)	P
Clinical variables						
O ₂ Saturation %	94.3 ± 0.94	94.5 ± 2.09	0.6682	92.96 ± 0.42	92.9 ± 0.12	0.9389
O ₂ Supply L/min	7.1 ± 6.31	3.5 ± 2.3	0.0930	4.4 ± 5.1	5.04 ± 6.1	0.7920
Biochemical variables						
Leucocytes cells/mL	8602 ± 3676	7823 ± 2568	0,4165	6791 ± 3252	6058 ± 1950	0,5478
Lymphocytes cells/mL	985 ± 484	1278 ± 583	*0,0403	1616 ± 2350	1158 ± 503	0,5104
Fibrinogen mg/dL	713 ± 112	572 ± 163	*0,0107	602 ± 160	528 ± 149	0,3659
D-Dimer ng/mL	3240 ± 2484	1343 ± 1320	*0,0110	1153 ± 595	853 ± 330	*0,0251
Urea mg/dL	67 ± 41	55 ± 24	0,1089	73 ± 47	69 ± 41	0,5997
Ferritin ng/mL	989 ± 799	840 ± 1060	0,6043	861 ± 806	1028 ± 1219	0,3379
LDH U/L	329 ± 111	241 ± 89	*0,0209	262 ± 128	242 ± 84	0,0570
CRP mg/mL	8.9 ± 6.14	2,46 ± 3.78	*0,0040	6,5 ± 6.6	1.91 ± 2.3	0,0525
IL-6 pg/mL	85.07 ± 50.5	30.48 ± 38.1	*0,0048	44,2 ± 23.2	23,3 ± 17.3	0,2365
Radiological variables						
Taylor Scale	4,78 ± 0.42	3 ± 0.78	*0,0000	4.25 ± 0.75	3,75 ± 0.96	0,3145

P, statistical Student T-test. L/Min, liters per minute. *, p<0.05. LDH, lactate dehydrogenase. CRP, C-reactive protein.

Biochemical Variables

In Ozone-Group, biochemical variables of inflammation, leucocyte and lymphocyte count improved significantly in an overall view. Except from Leucocyte count and Urea, all changes were significant ($p < 0.05$). Leucocytes decreased from $8.6 \times 10^3/\text{ml}$ to $7.82 \times 10^3/\text{ml}$ ($p = 0.4165$); Lymphocytes improved from 0.98×10^3 to $1.27 \times 10^3/\text{mL}$ ($p = 0.0403$). Fibrinogen ameliorated from 713 mg/dL to 572 mg/dL ($p = 0.0107$). D-Dimer lowered from 3240 ng/mL to 1343 ng/mL ($p = 0.0060$). Urea improved slightly its value from 67 to 55 mg/dL ($p = 0.1089$). Ferritin decreased its value from 989 to 840 ng/mL ($p = 0.6043$). LDH ameliorated from 329 to 241 U/L ($p = 0.0209$). CRP diminished values from 8.9 to 2.46 mg/mL ($p = 0.0040$). IL-6 improved from 85.07 to 30.48 pg/mL ($p = 0.0048$) (**Table 2, Figure 3**).

In Standard-of-care Group, except for D-Dimer, all biochemical variables of inflammation, leucocyte and lymphocyte count also improved in an overall view, but in a non-significant manner ($p > 0.05$). Ferritin was the only variable that actually worsened after Standard-of-care. All the changes in the variables analyzed were not significant ($p > 0.05$). Leucocytes decreased from $6.79 \times 10^3/\text{ml}$ to $6.05 \times 10^3/\text{ml}$ ($p = 0.5478$); Lymphocytes decreased from 1.61×10^3 to $1.15 \times 10^3/\text{mL}$ ($p = 0.5104$). Fibrinogen ameliorated from 602 mg/dL to 528 mg/dL ($p = 0.3659$). D-Dimer lowered from 1153 ng/mL to 853 ng/mL ($p = 0.0251$). Urea improved slightly its value from 73 to 69 mg/dL ($p = 0.5997$). Ferritin worsened its value from 861 to 1028 ng/mL ($p = 0.3379$). LDH ameliorated from 262 to 242 U/L ($p = 0.0570$). CRP diminished its value from 6.5 to 1.91 mg/mL ($p = 0.0525$). IL-6 improved from 44.2 to 23.3 pg/mL ($p = 0.2365$) [**Table 2, Figure 3**].

Radiological variables

According to Taylor's scale, patients in Ozone-Group improved significantly from a 4.78 to a 3 grade ($p = 0.0000$) (**Table 2**). According to Taylor's scale, patients in Standard-of-care Group improved from a 4.25 to a 3.75 grade ($p = 0.3145$) (**Table 2**). The improvement was significant in favor of Ozone-Group.

A resume of the radiographic evolution between Ozone-Group and Standard-of-care Group is presented in **Figure 4**.

SECONDARY OUTCOMES

Hospitalization length-of-stay and mortality-rate

In Ozone-Group, hospitalization length-of-stay was 27.71 ± 16 days. On the contrary, in Standard-of-care Group, hospitalization length-of-stay was 37.92 ± 21.13 days. Even in the case that Ozone was administered after Standard-of-care was completed, there was a difference of 10.21 days in favor of Ozone-Group, although that difference was not statistically significant ($p = 0.0512$).

In Ozone-Group, mortality rate was 8.3% ($n = 1$), while on the Standard-of-care Group was 16.6% ($n = 2$) [**Figure 5**].

Adverse events in Ozone protocol

After a mean of 7.83 ± 2.4 sessions of rectal Ozone treatment (150mL of Ozone at 35mg / mL, total dose 5,25mg) in Ozone-Group ($n = 14$), clinical, biochemical and radiological improvement was observed. After rectal

insufflation, no side effect was observed, except slight meteorism and a feeling of bloating, which subsided spontaneously.

Discussion

To the best of our knowledge, this is the first report on the effectiveness of Rectal Ozone (for compassionate use) in a series of severe COVID-19 pneumonic patients and it was compared with a series of patients treated by Standard-of-care, in the light of this new SARS-CoV-2 pandemic. Rectal Ozone improved clinical, biochemical and radiological variables and in a significant manner ($p < 0.05$); on the contrary, the improvement observed in the Standard-of-care Group was not significant ($p > 0.05$).

To date, despite the several clinical trials performed and up-dated (more than 1661 clinical trials registered in the International Clinical Trial Registry Platform Database)¹⁴, there is no pharmacological therapy that has demonstrated effectivity in the management of SARS-CoV-2 pandemic and COVID-19 infection^{4,14}. There are 8 clinical trials (CT) that postulate Ozone as a biologically effective therapy^{4,5}. From these 8 trials, 7 CT are concerned to Ozone autohemotherapy, and one CT to rectal Ozone^{6,11}. Only one CT has published its results (Ozone autohemotherapy), while the others are still recruiting patients^{4,5}. There subsides the importance of the Study; there is no report on the literature of the effectiveness of rectal Ozone, apart from our preliminary results recently published (four cases)¹². This study presents the largest sample of COVID-19 patients treated by rectal Ozone insufflation in a case-control design.

Our study group identified up to 4 properties that could cope with the complications derived from this COVID-19 infection (anti-viral, anti-oxidant, anti-inflammatory and O₂ delivery enhancer)¹¹.

The clinical improvement observed in our preliminary results¹² and now in this case-control study confirms the promising utility of Ozone in the management of COVID-19 pandemic, as stated by some other authors^{11,15-17}.

COVID-19 has characteristics of two known syndromes: a) Macrophage Syndrome, characterized by hypercytokinemia, uncontrolled proliferation of T-cells and macrophages, where IL-6 plays a major role; and b) Antiphospholipid Syndrome, an autoimmune disease related to thrombosis, where D-Dimer is elevated (as it occurs in pneumonic patients), thrombocytopenia is observed and Ferritin is elevated (common in viral inflammatory processes)¹⁸. Therefore, we evaluated biomarkers of inflammation in our case-control study (IL-6 and Ferritin) and D-Dimer.

There are several reasons that justify Ozone use for COVID-19 management. Ozone produces antioxidant response elements (Super oxide dismutase [SOD], Catalase [CAT], Glutathione peroxidase [GPx], Hemo-oxygenase 1 [HO-1], HSP-1 [Heat Shock Protein-1]). Ozone reduces Iron overload, reducing Ferritin and oxidative stress produced by viral infection. Ozone increases 2,3DPG (diphosphoglycerate), shifting the hemoglobin curve to the right, improving blood rheology and permeability, increasing blood flow and oxygenation by the delivery of NO (nitric oxide). Ozone modulates Interferons and Cytokines; therefore, it may counteract hyperinflammation, cytokine storm and oxidative stress in COVID-19 patients. Ozone has anti platelet effect (by increasing Prostacyclin or Prostaglandin I₂ [PGI₂]), leading to vasodilation. Ozone releases NO, producing also vasodilation.

Ozone modulates Antithrombin III, reducing Fibrinogen. Therefore, Ozone could decrease hypercoagulation state in COVID-19 patients ¹⁸, as it was observed in our case-control study.

Ayanian et al have identified several biomarkers capable of predicting clinical course in COVID-19 patients, and besides, they consider these biomarkers could inform of therapeutic interventions rather than simply demonstrate a consequence of disease. Elevated levels of D-dimer, CRP, IL-6, Ferritin and LDH have been related to ICU (Intensive Care Unit) admission, intubation and death. On the contrary, lower levels of such biomarkers were related to survival and positive clinical outcomes ¹⁹. The fact that in our case-control study, Ozone was capable of decreasing such biomarkers, apart from improving O₂-saturation and radiologic amelioration, is a demonstration of the effectiveness of Ozone on COVID-19 patients, based on clinical, biochemical and radiologic variables.

Cattel has stated that Ozone properties might have direct consequences in COVID-19 patients. Ozone is antiviral and might inactivate the virus and inhibit its viral replication. Ozone could reduce inflammation and lung damage. Ozone might favor immunity and oxygenation, and decrease Oxygen support. The consequences of these effects in COVID-19 patients will be an increase in lymphocyte count, a decrease of inflammation biomarkers (CRP, IL-6, Ferritin, D-Dimer and LDH), an improvement in O₂-saturation and a decrease in O₂-supply; and finally a negativization of RT-PCR SARS-Cov-2 Test ²⁰. As Cattel stated, we have observed all these effects in our Case-control study. Ozone improved all variables and in a significant manner ($p < 0.05$); on the contrary, the change was not significant in the Standard-of-care Group ($p > 0.05$). These observations would be a confirmation of the biological properties of Ozone and this study might serve as a proof-of-concept of rectal Ozone in SARS-Cov-2 infected patients.

Regarding age and comorbidities (Charlson Index) in our study, we have treated patients older than 80 years (84.3 years in Ozone-group, 83 years in Standard-of-care Group). Their comorbidities were similar in both groups (4 in Ozone-Group and 4.42 in Standard-of-care Group). In Franzini's study (Ozone autohemotherapy in a before-and-after study), patients were 75 years and presented a Charlson Index (comorbidities Index) of 2.5 ²¹. In Tascini's study (Ozone autohemotherapy in Case-control study), age was 61 years and Charlson Index was 2. More exactly, Ozone-group was 57 years and presented 1 comorbidity, while Control-group was 65 years and 2 comorbidities ²². In Araimos' study (Ozone autohemotherapy in RCT [randomized controlled trial] design), Ozone-group was 63 years and 2.8 comorbidities, while Control-group was 60 years and 2.6 comorbidities ⁵. In Schwartz' study (Ozonized saline solution in before-and-after design), patients were 55 years and 0.84 comorbidities ²³. In Hernández' study (Ozone autohemotherapy in prospective case-control study) Ozone-Group was 64 years and 0.77 comorbidities, while Control-Group was 71 years and 1.2 comorbidities ²⁴. In the previous studies referenced, there is a clear association between age and number of comorbidities. The older the age, the higher the Charlson Index. Our study has the oldest patients and the greatest comorbidities. Even in such a case, rectal Ozone results were promising. It is expected that older patients show worst clinical outcomes, but Ozone was effective even in older COVID-19 patients ¹¹. As far as we know, our study has treated the oldest patients, if compared with other ozone studies ²¹⁻²⁴.

With regard to clinical variables, in our study Ozone-Group improved O₂-saturation from 94.3% to 94.5% and reduced O₂-supply from 7.1 to 3.5 L/min. It means that severe COVID-19 patients improved their clinical state decreasing O₂-supply. In the case of Standard-of-care Group, O₂-saturation changed slightly from 92.96% to

92.90% but more O₂-supply was needed (4.4 to 5.04 L/min). Ozone has demonstrated to be more effective than Standard-of-care in improving respiratory parameters in severe COVID-19 patients. This observation comes in line with what was reported by Franzini et al (Ozone autohemotherapy in COVID-19 patients) ²¹. In Franzini's study, O₂ saturation improved from 85% to 95% (p<0.0001) after 8.6 ± 1.4 days of treatment ²¹. In Araimo's study (Ozone autohemotherapy vs Standard-of-care for COVID-19 management in RCT design) it was observed that Ozone-group moderately reduced the need for ventilatory support (reduced use of CPAP [continuous positive air pressure], high flow nasal cannula or venturi mask) ⁵. In Schwartz' study (Ozonized Saline Solution in a prospective before-after study in COVID-19), patients that required supplemental O₂ decreased from 68% to 24% ²³. From the cited articles, it could be inferred that Ozone (by rectal, autohemotherapy or by ozonized saline solution application) is capable of improving ventilatory Indexes, mainly O₂-saturation and O₂- supply, as it was observed in our study.

Henry et al have stated that many proinflammatory biomarkers such as CRP, IL-6, Ferritin and even ESR (erythrocyte sedimentation rate) are considerably increased over the upper-limit in COVID-19 patients ²⁵. In the same line, Webb et al have considered a hyperinflammatory COVID-19 Score based on different parameters: a) Ferritin >700 ng/mL, b) LDH >400 U/L, c) D-Dimer >1500 ng/mL, d) CRP >15 mg/mL, e) IL-6 >15 pg/mL ²⁶. Ayanian et al have observed a cut-point in the levels of biomarkers with good and bad clinical outcomes in COVID-19 patients. In COVID-19 patients with no need for ICU admission, no intubation and good clinical outcomes (survivors), the range of biomarkers was: a) Ferritin 340-370 mg/L, b) LDH 863-915 U/L, c) D-Dimer 1600-1700 ng/mL, d) CRP 6.8-7.8 mg/mL, e) IL-6 50-60 pg/ml. On the contrary, in patients with need to ICU admission, intubation, mechanical ventilation and death, the range of biomarkers was greater: a) Ferritin 1320-1575 mg/L, b) LDH 1478-2050 U/L, c) D-Dimer 5800-7800 ng/mL, d) CRP 29-33.3 mg/mL, e) IL-6 188-266 pg/ml ⁵.

With regard to inflammatory biomarkers, the patients in our study presented moderate and severe pneumonia but were not critical; therefore, the levels of inflammatory markers were over upper-limit, as Ayanian, Henry and Webb have previously stated ^{5,25,26}. In Ozone-Group inflammatory biomarkers were: a) Ferritin 989 ng/mL, b) LDH 329 U/L, c) D-Dimer 3240 ng/mL, d) CRP 8.9 mg/mL, e) IL-6 85.07 pg/mL. In Standard-of-care Group the inflammatory biomarkers were: a) Ferritin 861 ng/mL, b) LDH 262 U/L, c) D-Dimer 1153 ng/mL, d) CRP 6.5 mg/mL, e) IL-6 44.2 pg/mL.

As Menendez-Cepero stated, Ozone is capable of modulating interferons and cytokines, decreasing inflammation biomarkers ¹⁸. Bocci has also stated that Ozone is capable of stimulating stem cells, improving differentiation of white cells and platelets ²⁷. This would explain why in our Study Ozone improved lymphocyte count, ameliorated inflammation biomarkers (CRP, IL-6, Ferritin, and LDH) and decreased coagulation parameters (Fibrinogen and D-Dimer). In Franzini's study, Ozone decreased inflammation Biomarkers (CRP, IL-6), thromboembolic biomarkers (D-Dimer), LDH and improved Leucocyte count ²¹. In Tascini's study, Ozone ameliorated CRP and IL-6 ²², and this comes in line with Clavo et al, who stated that Ozone effect is based on oxidative preconditioning, reducing IL-1β and IL-6 ²⁸. This would explain the decreasing of inflammation biomarkers (IL-6 and CRP) observed in Tascini's ²² and in our present study. Schwartz et al have stated that Ozonized saline solution was capable of decreasing inflammation Biomarkers from baseline (Ferritin 561 ng/mL, LDH 423 U/L, D-Dimer 905 ng/mL, CRP 33.7 mg/mL) to the end of treatment. In fact, by the 10th day of

treatment, Fibrinogen and LDH were on normal ranges in all COVID-19 patients²³. Hernandez et al have stated that Ozone autohemotherapy decreased inflammatory biomarkers (Ferritin, LDH, D-Dimer and CRP) significantly at 7 days after treatment started²⁴. Although Franzini's, Tascini's and Hernandez' Studies used Ozone autohemotherapy and Schwartz' study used Ozonized saline solution, their results were similar to our approach (rectal Ozone insufflation); that is, Ozone in its different administration techniques, was capable of decreasing such biomarkers of inflammation.

The decreasing of inflammation biomarkers observed in our study is similar to the only RCT on COVID-19-study reported and published recently (Araimo's study)⁵. In that study, all inflammation biomarkers in the Ozone-group ameliorated (Ferritin from 1337 to 1223 ng/mL, D-Dimer from 1192 to 914.8 ng/mL, CRP from 34.4 to 4.64 mg/mL, and IL-6 from 71.31 to 44.57 pg/mL). On the contrary, D-Dimer and IL-6 worsened in the Standard-of-care Group (Ferritin from 766 to 571 ng/mL, D-Dimer from 865 to 1187 ng/mL, CRP from 55.23 to 16.45 mg/mL, and IL-6 from 245.8 to 704.5 pg/ml)⁵. The variation of inflammation biomarkers in Ozone-Group was numeric but not statistical⁵. Surprisingly, it was also observed in our study, that Ferritine was the variable that worsened in the standard-of-care Group (Ferritin from 861 to 1028 ng/mL), similarly as in Araimo's study. Ferritin is a biomarker of viral inflammation and Ozone is largely recognized as an antiviral agent^{11,27}. This would explain why Ferritin decreased in Ozone-Group but increased in Standard-of-care Groups, as observed in Araimo's study and in ours⁵.

In the present study, bilateral radiographic pneumonia improved from 4.78 to 3 (on Taylor's radiologic Scale) in Ozone-group ($p=0.0000$); while in the Standard-of-care Group, improvement was just moderate (from 4.25 to 3.75) according to Taylor's Scale ($p=0.3145$). There is a clear improvement in bilateral pneumonia in favor of Ozone treatment, as evidenced by improvement in Taylor's radiologic Scale. Our findings correlate with Schwartz' study, in which radiologic signs of pneumonia changed from 60% lung affection to 24% lung affection; and the improvement was observed at 3-5 days of Ozone treatment²³. The great improvement on Taylor radiological Scale observed in Ozone-Group would explain why Ozone patients improved in O₂-saturation and decreased in O₂-supply. On the contrary, the slight increased observed in Taylor Scale in Standard-of-care Group would explain why these patients had only a slight improvement on O₂-saturation and therefore needed even more O₂-supply, as it was observed in our study (Figures 2 and 4).

In the present case-control study, although Ozone treatment (compassionate use) started after Standard-of-care was provided, hospitalization length-of-stay was inferior in Ozone-group if compared to Standard-of-care (27.71 days vs 37.92 days). In Franzini's study, Ozone reduced hospitalization with a 9 days earlier recovery, if compared with the standard-of-care (13.45 days vs 22.15 days)²¹. In Hernandez' Study, hospitalization period was inferior in the Ozone-group when compared to the Standard-of-care Group (8 days vs 28 days)²⁴. In Schwartz' study, hospitalization in ozone group was 14 days, while control group might be hospitalized from 1 to 68 days²³. Despite the fact that in our study, Ozone treatment started as compassionate when no longer improvement was observed once Standard-of-care treatment finished, all referenced studies including ours, state that hospitalization period is shorter in Ozone-groups^{21,23,24}.

In our study, mortality rate in Ozone-group was 8.3% whereas in Standard-of-care group was 16.6%. Hernandez et al have reported a mortality rate of 11% for ozone-group, and 22% for Standard-of-care Group, a rate very

similar to ours²⁴. Tascini et al have stated that poor clinical outcome was inferior in Ozone (7%) that in Standard-of-care (17%), and the mortality rate was 0% in Ozone-Group and 7% in the Standard-of-care Group²². Schwartz has reported no mortality on the Ozone-Group, but a 20.7-21.1% mortality rate in homogeneous groups treated by Standard-of-care²³. All previous studies stated a lower mortality for the Ozone-group than in the Standard-of-care Group. The expected mortality in severe COVID-19 cases is 18% and in the moderate cases is 5%²². From the previous results it can be inferred that severe cases treated by Ozone therapy reduced its mortality rate to the mortality expected in moderate COVID-19 cases²²⁻²⁴. This suggests that ozone has an impact on mortality-rate.

Finally, in our study, we have observed no severe events after rectal ozone insufflation except slight meteorism and bloating, which subside in minutes after procedure. Ozone is very safe, to the point that only 0.7 adverse events in 100.000 treatments have been reported in literature²⁹.

As a summary, the spread of COVID-19 pandemic has led to the need to determine standardized treatment for the management of SARS-Cov-2 infection. Unfortunately, no specific drug or drug regimen has been approved for COVID-19. In the pathogenesis of COVID-19, two clinical presentations are the most observed: a) respiratory failure and b) systemic coagulopathy secondary to hyper activation of complement cascade and exacerbation of cytokine cascade. As a result, hyper production of Interleukins and hypercoagulability with diffuse thrombosis in the circulation is observed. Since there is no proven efficacy of antivirals in treating COVID-19 by themselves, it is reasonable to treat COVID-19 with multimodal therapies¹⁴. Ozone is a multi-target drug with proven biological properties: a) antiviral, b) modulation of inflammatory Interleukins [IL-1, IL-6, TNF- α], c) antioxidant [via Nrf2 pathway], d) anti-inflammatory (blocking inflammasome NLRP3), e) anticoagulant (anti thrombin III effect), and f) vasodilation effect (NO release)^{11,30-33}. The multi-target profile of Ozone would explain the good clinical outcomes observed in the present study, and in the articles referenced and published in the management of COVID-19^{5,21-24,34}.

The number of sessions in autohemotherapy varied from 3 to 7 sessions^{5,21,22,24}, in ozonized saline solution was 10 sessions²³ and in Rectal Ozone the sessions were 7.8 on average.

A limitation of this case-control study is the small sample size analyzed. However, despite the number of patients evaluated, the fact that Ozone-group and Standard-of-care Group were homogeneous has made them comparable, and important conclusions can be obtained from this case-control study.

This is the first study that reports the effectiveness of rectal Ozone in the management of this new pandemic situation, so it constitutes a first proof-of-concept study. The prospective nature of this study shows the pragmatic real-world COVID-19 population. Another strength of the study is the use of objective and standardized clinical, radiological and biochemical variables to evaluate the effect of rectal Ozone in the face of this new pandemic situation.

Finally, Ozone is an anti-inflammatory therapy capable of modulating inflammation biomarkers, Ozone is cheaper and safer if compared to biological treatments (monoclonal antibodies) or antivirals (Remdesivir) and O₃ might be an alternative for low-middle income countries, where patients have to pay for their medical bills, there is scarce of economic Resources, and where the Health Systems have limited Resources (expensive drugs

and trained personnel)^{14,16}. A RCT is necessary to validate and reproduce the promising results observed in this proof-of-concept study.

Conclusion

Compassionate use of Rectal Ozone improved O₂ saturation, reduced O₂ supply, decreased inflammation biomarkers and improved Taylor's radiological scale with statistical significant difference when compared to Standard-of-care, in patients with severe COVID-19 pneumonia. Mortality and days of hospitalization was inferior in the Ozone-group, but this difference was not significant.

Rectal Ozone is a costless, safe, effective, and easy-to-perform alternative for the management of SARS-Cov-2 infection and it is presented as an adjunctive therapeutic option to consider as a compassionate use in severe bilateral COVID-19 pneumonia.

Declarations

ACKNOWLEDGMENT

The librarian Saturnino Díaz Trujillo is acknowledged for the bibliographic search for the preparation of this Study.

COMPLIANCE WITH ETHICAL STANDARDS

Funding: Authors declare no financial funding/support for the elaboration of this manuscript.

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval: The Study Protocol has been approved by the Ethical Committee of the Santa Cristina's Hospital (15th April 2020) and by Ethics Committee for Medical Investigation of La Princesa's Hospital (ACTA CEIm 12/20, 28/5/20)

Informed consent: For the treatment used in this Research Article (1) informed consent was obtained from the patient/legal representative included in the study and (2) the study protocol conforms to the ethical guidelines of the [1975 Declaration of Helsinki](#) as reflected in a priori approval by the Ethical Committee of the Hospital.

References

1. <https://www.who.int/es/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19—11-march-2020>.
2. https://www.mscbs.gob.es/profesionales/saludPublica/ccayes/alertasActual/nCov-China/documentos/Protocolo_manejo_clinico_ah_COVID-19.pdf.
3. https://www.eldiario.es/sociedad/coronavirus-sobrecarga-sanidad-Comunidad-Madrid_0_1004050083.html.
4. Coronavirus disease (COVID-2019) situation reports. 2020.

5. Araimo, F., Imperiale, C., Tordiglione, P., Ceccarelli, G., Borrazzo, C., Alessandri, F., ... & Recchia, G. E. Ozone as Adjuvant Support in the Treatment of COVID-19: A Preliminary Report of Probiozovid Trial. *Journal of medical virology* (2020), [https://doi.org/ 10.1002/jmv.26636](https://doi.org/10.1002/jmv.26636).
6. <https://rpcec.sld.cu/ensayos/RPCEC00000320-Sp>.
7. Shimabukuro-Vornhagen, A., Gödel, P., Subklewe, M., Stemmler, H. J., Schlößer, H. A., Schlaak, M., Kochanek, M., Böll, B., & von Bergwelt-Baildon, M. S.. Cytokine release syndrome. *Journal for immunotherapy of cancer*, 6(1), (2018) 56, <https://doi.org/10.1186/s40425-018-0343-9>.
8. Mehta P et al. COVID-19: consider cytokine storm syndromes and immunosuppression. *The Lancet* March 28 (2020) 1022-1034, [https://doi.org/10.1016/S0140-6736\(20\)30630-9](https://doi.org/10.1016/S0140-6736(20)30630-9).
9. Fernández-Cuadros ME, Pérez-Moro OS, Albaladejo-Florín MJ. Ozone fundamentals and effectiveness in knee pain: Chondromalacia and knee osteoarthritis. Germany: Lambert Academic Publishing; 2016.
10. Fernández-Cuadros Marcos Edgar, Pérez-Moro Olga Susana, Albaladejo-Florín María Jesús, Álava-Rabasa Sandra. El ozono intraarticular modula la inflamación, mejora el dolor, la rigidez, la función y tiene un efecto anabólico sobre la artrosis de rodilla: estudio cuasi-experimental prospectivo tipo antes-después, 115 pacientes. Intra-articular Ozone modulates inflammation, ameliorates pain and rigidity, improves function and has anabolic effect on knee osteoarthritis: a prospective quasi-experimental before-and-after study, 115 patients. *Rev. Soc. Esp. Dolor*. 2 (2020) 78-88, [https://doi.org/ 10.20986/resed.2020.3775/2019](https://doi.org/10.20986/resed.2020.3775/2019).
11. Fernández-Cuadros, Peña-Lora, Albaladejo-Florín, Álava-Rabasa, Pérez-Moro. Ozone (O₃) and SARS-CoV-2: physiological bases and their therapeutic possibilities according to COVID-19 evolutionary stage. *SN Comprehensive Clinical Medicine Insights*, 2 (2020) 1-9, <https://doi.org/1007/s42399-020-003287>.
12. Fernández-Cuadros, M. E., Albaladejo-Florín, M. J., Álava-Rabasa, S., Usandizaga-Elio, I., Jimenez, D. M. Q., Peña-Lora, D., ... & Pérez-Moro, O. S. Effect of Rectal Ozone (O₃) in Severe COVID-19 Pneumonia: Preliminary Results. *SN Comprehensive Clinical Medicine*, 2.9 (2020) 1328-1336, <https://doi.org/10.1007/s42399-020-00374-1>.
13. Taylor, E., Haven, K., Reed, P., Bissielo, A., Harvey, D., McArthur, C., & Wilson, F. A chest radiograph scoring system in patients with severe acute respiratory infection: a validation study. *BMC medical imaging*, 15.1 (2015) 61, [https://doi.org/ doi: 10.1186/s12880-015-0103-y](https://doi.org/doi:10.1186/s12880-015-0103-y).
14. Di Franco, S., Alfieri, A., Petrou, S., Damiani, G., Passavanti, M. B., Pace, M. C., ... & Fiore, M. Current status of COVID-19 treatment: An opinion review. *World journal of virology*, 9.3 (2020) 27, [https://doi.org/ 10.5501/wjv.v9.i3.27](https://doi.org/10.5501/wjv.v9.i3.27).
15. Martínez-Sánchez G, Schwartz A, Donna VD. Potential Cytoprotective Activity of Ozone Therapy in SARS-CoV-2/COVID-19. *Antioxidants (Basel)*. 5 (2020) 389, <https://doi.org/10.3390/antiox9050389>.
16. Conti P, Gallenga CE, Tetè G, Caraffa A, Ronconi G, Younes A, Toniato E, Ross R, Kritas SK. How to reduce the likelihood of coronavirus-19 (CoV-19 or SARS- CoV-2) infection and lung inflammation mediated by IL-1. *J Biol Regul Homeost Agents*. 34.2 (2020), <https://doi.org/10.23812/Editorial-Conti-2>.
17. Marini, S., Maggiorotti, M., Dardes, N., Bonetti, M., Martinelli, M., Re, L., & Tavera, C. Oxygen-ozone therapy as adjuvant in the current emergency in SARS-COV-2 infection: a clinical study. *Journal of Biological Regulators and Homeostatic Agents*, 34.3 (2020), <https://doi.org/10.23812/20-250-E-56>.
18. Menendez-Cepero, S., Marques-Magallanes-Regojo, J. A., Hernandez-Martinez, A., Tallón, F. J. H., & Baeza-Noci, J. Therapeutic effects of Ozone therapy that justifies its use for the treatment of COVID-19. *Research*

Open. J Neurol, 1 (2020) 1-6, <https://doi.org/10.31038/JNNC.2020314>.

19. Ayanian, S., Reyes, J., Lynn, L., & Teufel, K. The association between biomarkers and clinical outcomes in novel coronavirus pneumonia in a US cohort. *Biomarkers in Medicine*, 12 (2020) 1091-1097, <https://doi.org/10.2217/bmm-2020-0309>.
20. Cattell, F., Giordano, S., Bertiond, C., Lupia, T., Corcione, S., Scaldaferrì, M., ... & De Rosa, F. G. Ozone Therapy in COVID-19: a Narrative Review. *Virus Research*, 291 (2021) 198207, <https://doi.org/10.1016/j.virusres.2020.198207>.
21. Franzini, M., Valdenassi, L., Ricevuti, G., Chirumbolo, S., Depfenhart, M., Bertossi, D., & Tirelli, U. Oxygen-ozone (O₂-O₃) immunoceutical therapy for patients with COVID-19. Preliminary evidence reported. *International immunopharmacology*, 88 (2020) 106879, <https://doi.org/10.1016/j.intimp.2020.106879>.
22. Tascini, C., Sermann, G., Pagotto, A., Sozio, E., De Carlo, C., Giacinta, A., ... & Cadeo, B. (2020). Blood ozonization in patients with mild to moderate COVID-19 pneumonia: a single centre experience. *Internal and Emergency Medicine*, (2020) 1-7, <https://doi.org/10.1007/s11739-020-02542-6>.
23. Schwartz, A., Martínez-Sánchez, G., de Lucía, A. M., Viana, S. M., & Mita, C. A. Complementary application of the ozonized saline solution in mild and severe patients with pneumonia COVID-19: A non-randomized pilot study. *Journal of Pharmacy and Pharmacognosy Research*, 9.2 (2021) 126-142, <https://jppres.com/jppres>.
24. Hernández, A., Viñals, M., Pablos, A., Vilás, F., Papadakis, P. J., Wijesundera, D., ... & Vives, M. Ozone therapy for patients with COVID-19 pneumonia: preliminary report of a prospective case-control study. *International Immunopharmacology*, 90 (2021) 107261, <https://doi.org/10.1016/j.intimp.2020.107261>.
25. Henry, B. M., De Oliveira, M. H. S., Benoit, S., Plebani, M., & Lippi, G. Hematologic, biochemical and immune biomarker abnormalities associated with severe illness and mortality in coronavirus disease 2019 (COVID-19): a meta-analysis. *Clinical Chemistry and Laboratory Medicine (CCLM)*, 58.7 (2020) 1021-1028, <https://doi.org/10.1515/cclm-2020-0369>.
26. Webb, B. J., Peltan, I. D., Jensen, P., Hoda, D., Hunter, B., Silver, A., ... & Snow, G. Clinical criteria for COVID-19-associated hyperinflammatory syndrome: a cohort study. *The Lancet Rheumatology*. 2.12 (2020): e754-e763, [https://doi.org/10.1016/S2665-9913\(20\)30343-X](https://doi.org/10.1016/S2665-9913(20)30343-X).
27. Bocci, V. Is it true that ozone is always toxic? The end of a dogma. *Toxicology and applied pharmacology*. 216.3 (2006) 493-504, <https://doi.org/10.1016/j.taap.2006.06.009>.
28. Clavo B, Rodríguez-Esparragón F, Rodríguez-Abreu D, Martínez-Sánchez G, Llontop P, Aguiar-Bujanda D, Fernández-Pérez L, Santana-Rodríguez N. Modulation of Oxidative Stress by Ozone Therapy in the Prevention and Treatment of Chemotherapy-Induced Toxicity: Review and Prospects. *Antioxidants (Basel)*. 8.12 (2019): 588, <https://doi.org/10.3390/antiox8120588>.
29. Valdenassi, L., Franzini, M., Ricevuti, G., Rinaldi, L., Galoforo, A. C., & Tirelli, U. Potential mechanisms by which the oxygen-ozone (O₂-O₃) therapy could contribute to the treatment against the coronavirus COVID-19. *Eur Rev Med Pharmacol Sci*, 24.8 (2020): 4059-4061, https://doi.org/10.26355/eurrev_202004_20976.
30. Fernández-Cuadros, M. E., Perez-Moro, O. S., & Mirón-Canelo, J. A. Could ozone be used as a feasible future treatment in osteoarthritis of the knee. *Diversity Equal Health Care*, 13.3 (2016): 232-9, <https://doi.org/10.1186/s13049-016-0005-7>.

31. Fernández-Cuadros, M. E., Pérez-Moro, O. S., Albaladejo-Florín, M. J., Álava-Rabasa, S., Tobar-Izquierdo, M., & Rodríguez-de-Cía, J. A New Paradigm for the Management of Knee Osteoarthritis: The Role of Hyaluronic Acid, Platelet-Rich Plasma (PRP) and Ozone in the Modulation of Inflammation: A Review. Science Repository, (2020). In press, <https://doi.org/10.31487/j.JSR.2020.01.01>.
32. Fernandez-Cuadros, M. E., Perez-Moro, O. S., Albaladejo-Florin, M. J., & Algarra-Lopez, R. Ozone decreases biomarkers of inflammation (C-reactive protein and erythrocyte sedimentation rate) and improves pain, function and quality of life in knee osteoarthritis patients: A before-and-after study and review of the literature. Middle East J Rehabil Health Stud, 5.2 (2018) e64507, <https://doi.org/5812/mejrh.64507>.
33. Fernandez-Cuadros M E, Perez-Moro O S, Albaladejo-Florin M J, Algarra-Lopez R. Intra Articular Ozone Reduces Serum Uric Acid and Improves Pain, Function and Quality of Life in Knee Osteoarthritis Patients: A Before-and-After Study, Middle East J Rehabil Health Stud. 5.3 (2018) e68599, <https://doi.org/5812/mejrh.68599>.
34. Peña-Lora, D., Albaladejo-Florín, M. J., & Fernández-Cuadros, M. E. Uso de ozonoterapia en paciente anciana con neumonía grave por COVID-19. Revista Espanola de Geriatria y Gerontologia. 55.6 (2020)362-364, <https://doi.org/10.1016/j.regg.2020.07.005>.

Figures

Figure 1

Study design. Case-control study on 14 patients in Ozone-Group and 14 patients in Standard-of-care Group.

Figure 2

Tendency of Rectal Ozone-group (n=14) compared to Standard-of-care Group (n=14) evaluated by dispersion curve on O2-saturation and O2-supply.

Figure 3

Biochemical variables analysis (before-and-after treatment) in Ozone-Group compared to Standard-of-care Group (Control-Group). LDH, Lactate Dehydrogenase. CRP, C-reactive protein. *, $p < 0.05$

Figure 4

Radiographic evolution before and after Ozone (n=14) and Standard-of-care (n=14) is observed in both groups, based on Taylor Scale.

Figure 5

Kaplan Meier Survival curve of Ozone-Group (n=14) compared to Standard-of-care Group (n=14) in the case-control study.