Ozone Therapy for Patients with SARS-COV-2 Pneumonia: A Single-Center Prospective Cohort Study

**Hernandez A[1],
Viñals M[1], Pablos A[1],
Vilás F[1], Papadakos PJ[2],
Wijeysundera D[3] and
Vives M[4]***

1   Policlinica Ibiza Hospital, Ibiza, Spain
2   University of Rochester, New York, USA
3   St. Michael Hospital, Toronto, Canada
4   Hospital Universitario Josep Trueta,
    Girona, Spain

***Corresponding author:** Marc Vives

✉   mvives.girona.ics@gencat.cat

Department of Anesthesiology, Hospital
Universitario Josep Trueta, Girona, CP:
17007, Spain.

Tel: +34 690277088

Abstract

Background: Aside from supportive management, there remains no specific treatment for Coronavirus Disease 2019 (COVID-19).

Objective: Determine whether the use of ozonated blood is associated with shorter time to clinical improvement in patients with severe COVID-19 pneumonia.

Design: A single-center proof-of-concept prospective cohort study.

Setting: Internal Medicine ward at Policlinica Ibiza Hospital, Spain.

Participants: Eighteen consecutive patients with laboratory confirmed COVID-19 infection and severe pneumonia who were admitted to hospital between 20th March and 19th April 2020.

Intervention: Patients in the ozonated autohemotherapy arm received ozonated blood twice daily starting on the day of admission for a median of 4 days. Each treatment involved administration of 200 mL autologous whole blood enriched with 200 mL of oxygen-ozone mixture with a 40 μg/mL ozone concentration. Patients in the control arm received supportive care. Assignment to ozone group versus usual care was determined based on the admitting physician on the day of admission, with only one of the three possible physicians prescribing ozonated autohemotherapy.

Main Outcomes: The primary outcome was time from hospital admission to clinical improvement, which was defined as either hospital discharge or a two-point improvement in clinical status measured on a six-point ordinal scale. Secondary outcomes were clinical improvement measured on the 7th, 14th and 28th day after admission, as well as time to a 2-fold reduction in concentrations of C-protein reactive, ferritin, D-dimer and lactate dehydrogenase.

Results: The mean age of the cohort was 68 y and 72% (n=13) were male. Nine patients (50%) received ozonated autohemotherapy beginning on the day of admission. In unadjusted comparisons, ozonated autohemotherapy was associated with significantly shorter time to clinical improvement (median [IQR]), 7 days [6-10] vs. 28 days [8-31], P=0.04) and significantly higher proportion of patients achieving 14-day clinical improvement (88.8% vs. 33.3%, P=0.01). In risk-adjusted analyses, ozonated autohemotherapy was associated with a shorter mean time to clinical improvement (-11.3 days, P=0.04, 95% CI -22.25 to -0.42).

Conclusion: Ozonated autohemotherapy was associated with a significantly shorter time to clinical improvement in this prospective cohort study. Given the small sample size and single-center study design, these observations require evaluation in larger randomized controlled trials.

Keywords: COVID-19; Biological stimulant; Immune function

Introduction

The ongoing pandemic of Coronavirus Disease 2019 (COVID-19) infections has led to more than 16.8 million cases and 661.917 deaths globally as of July 29th, 2020 [1]. About 15% of infected adults develop severe pneumonia requiring supplemental oxygen, and an additional 5% progress to acute respiratory distress syndrome requiring mechanical ventilation (often for several weeks) [2,3]. At present, there remains no proven and efficacious treatment for this new disease. Consequently, there is a growing tendency to use novel methods. Ozone therapy is the administration of a mixture of gas of 97% oxygen and 3% ozone generated from a medical ozone generator. Ozone, is a molecule which consists of three oxygen atoms all sharing the same electrons. Because there just are not enough electrons to go around tokeep three oxygen atoms completely happy, ozone is a relatively unstable molecule. This instability is why it is such a powerful biological stimulant. Ozone therapy can be administered systemically by adding it to a sample of a patient's own blood sample and then reinfusing it, in what is termed 'ozonated autohemotherapy'. While ozone therapy is currently not available in the United States, several other countries – including Spain, Italy, Greece, Cuba, Russia, Portugal and Turkey – have incorporated ozone therapy in medical practice.

The pathogenesis of the virus is not fully understood, but it is well-known that the pathological picture varies, mainly involving the lungs where diffuse alveolar damage predominates with involvement of the microcirculation leading to marked hypoxia [4]. A dysregulation of the immune response is present in these patients and lymphocytopenia is a hallmark in the vast majority of these patients [5]. Innate immunity and coagulation pathways are intricately linked [6]. COVID-19–associated macrophage activation, hyperferritinemia, cytokine storm, release of pathogen-associated molecular patterns and damage-associated molecular proteins can result in release of tissue factor and activation of coagulation factors that create a predisposition to hypercoagulability [6].

The potential benefits of ozonated autohemotherapy include reduced tissue hypoxia, decreased hypercoagulability, renal protection, modulated immune function with inhibition of inflammatory mediators, improved phagocytic function, and impaired viral replication [7].

There are only a few publications with case reports about the efficacy and safety of ozonated autohemotherapy in patients with severe COVID-19 pneumonia, however we are aware that this therapy has been given to more COVID patients [8,9]. The Scientific Society of Oxygen-Ozone Therapy (SIOOT) describes a case series of 73 patients, of whom 32.8% were intubated and 67.1% were non-intubated [10]. Of all the intubated-patients, 62.5% were extubated after 5 sessions with ozone autohemotherapy. Of all the non-intubated patients, only 6% required intubation. Whereas, the group of patients with only usual care without ozone auothemotherapy, had only a 20% rate of recovery among the intubated patients. We therefore conducted a proof-of-concept prospective cohort study to determine if ozonated autohemotherapy was associated with a shorter time to clinical improvement in patients with severe COVID-19 pneumonia.

Materials and Methods

Study design

This prospective cohort study was performed at the Policlinica Ibiza Hospital in Spain. It was conducted in compliance with the Declaration of Helsinki and approved by a multidisciplinary human research ethics committee at the institution. Each participant gave written informed consent for administration of any interventions, collection of relevant clinical data and ascertainment of outcomes. The study cohort consisted of all consecutive adults (18 y or older) who were admitted to the hospital with a diagnosis of severe COVID-19 pneumonia between 20th March to 19th April 2020. All included patients met the following criteria: Confirmed COVID-19 infection (diagnosed by nasopharyngeal swab performed on admission); severe pneumonia with baseline chest X-ray abnormalities; PaO_2/FiO_2 ratio < 300 or oxygen saturation < 94% on room air, and tachypnea with respiratory rate exceeding 30 per minute.

Allocation to study arms

Patients admitted to the hospital during the study period were assigned to one of three possible general internal medicine physicians based on clinical convenience (i.e., new patient admission was assigned to the most immediately available physician). Each physician was responsible for the clinical care of the individual admitted patient for the duration of their hospitalization. One of the three admitting physicians prescribed ozonated autohemotherapy to all in patients with severe COVID-19 pneumonia, while the other two physicians did not. Based on the responsible admitting physician, patients were assigned to the ozonated autohemotherapy arm or usual clinical care arm.

Standard clinical care

All patients received usual clinical care for COVID-19 pneumonia, which included supplemental oxygen therapy, hydroxychloroquine, lopinavir, ritonavir, corticosteroids, and antibiotics (including azithromycin) at the discretion of the individual patient's attending physician. Decisions on endotracheal intubation, mechanical ventilation and critical care unit admission were also made at the discretion of the individual patient's attending physician.

Ozonated autohemotherapy intervention

Ozonated autohemotherapy involved intravenous infusion of ozonated autologous whole blood. Initially, 200 mL of autologous whole blood was drawn from the patient's antecubital vein into a standard plastic disposable blood collection bag (certified $SANO_3$ bag) containing 35 mL of anticoagulant citrate dextrose solution (ACD-A). The blood was then enriched with 200 mL of gas mixture oxygen-ozone with an ozone concentration at 40 μg/mL obtained by Ozonobaric P Sedecal, an ozone generator with

CE0120 certificate type IIb. The ozonized blood was then slowly re-infused into the same vein over approximately 10 minutes.

Outcomes

The primary clinical outcome was time to clinical improvement during hospital admission. This time was measured relative to the date of hospital admission. Clinical improvement was defined as a two-point reduction (relative to the patient's status on hospital admission) on a six-point ordinal scale, or discharge alive from the hospital, whichever came first. The six-point scale was as follows: death (6 points); extracorporeal membrane oxygenation or mechanical ventilation (5 points); noninvasive ventilation or high-flow oxygen therapy (4 points); oxygen therapy without need for high-flow oxygen or non-invasive ventilation (3 points); hospital admission without need for oxygen therapy (2 points); and discharged from hospital or reached discharge criteria (1 point). Discharge criteria were as evidence of clinical recovery (normalization of pyrexia, respiratory rate < 24 per minute, oxygen saturation > 94% on room air, and absence of cough) for at least 72 hours.

This six-point scale and definition of clinical improvement (i.e., two-point improvement in scale) has been used in prior research on intervention for relating to COVID-19 infection [11]. Personnel ascertaining outcomes were not blinded to whether patients received usual care versus ozonated autohemotherapy.

Secondary outcomes were clinical improvement as measured at the 7th, 14th and 28th days after hospital admission. Time to a two-fold decrease in concentrations of C-protein reactive, ferritin, D-dimer and lactate dehydrogenase were also daily measured. Patients also underwent repeat COVID-19 PCR testing at 5 and 15 days after hospital admission – provided that there was co-existing evidence of clinical improvement. Follow-up ceased at the point of hospital discharge, patient death, or 31st day after hospital admission (which ever came first).

Statistical analysis

All analyses were performed using STATA version 13.0 (StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP). Statistical significance was defined by a 2-sided P-value less than 0.05. The Shapiro-Wilk test was used to determine whether variables were normally distributed. Unadjusted differences between treatment and control arms were then calculated using the two-sample t-test (normally distributed continuous variables), Mann-Whitney U-test (continuous variables with evidence of non-normal distributions) and Fisher's exact test (categorical variables). Unadjusted times to clinical improvement were compared between the two study arms using Kaplan-Meier survival curves and the log-rank test. Patients were censored at the point of hospital discharge, death or 31st day after hospital admission (whichever came first). The adjusted association between ozonated autohemotherapy and mean time to clinical improvement was estimated using a multivariable linear regression model that adjusted for age, sex, and baseline quick SOFA score. These covariates were pre-specified on the basis of their clinical significance. Patients who

had not achieved clinical improvement within the follow-up period were assigned a time value of 31 days. There was no pre-specified sample size calculation for this proof-of-concept study. Instead, all consecutive patients admitted to the study site within a pragmatic one-month period were included in the study cohort.

Results

The cohort included 18 consecutive patients. The mean age was 68 years (SD 15) years and 72.2% (n=13) were male. The baseline characteristics of these patients are presented in **Table 1.** In total, 9 patients (50%) received ozonated autohemotherapy.

There were no statistically significant baseline differences between the study arms, but these comparisons were limited by small sample size of the present study **(Table 1)**. Nonetheless, the baseline characteristics of the two study arms were qualitatively similar, aside from age (mean age was higher in the usual care arm), weight (mean weight was higher in the usual care arm), and body mass index (mean value was higher in the usual care arm).

Primary outcome: Time to clinical improvement

Ozonated autohemotherapy was associated with a statistically and clinically significant lower time to clinical improvement (median [IQR]), 7 days [6-10] *vs.* 28 days [8-31], *P*=0.04) **(Figure 1 and Table 2)**. In unadjusted linear regression analyses, the mean time to clinical improvement was 12.4 days shorter in the ozonated autohemotherapy arm (-12.4 days; *P*=0.01; 95% CI -22.49 to -2.39). In adjusted linear regression analyses, the mean time to clinical improvement in the ozonated autohemotherapy arm was 11.3 days shorter (-11.3 days, *P*=0.04, 95% CI -22.25 to -0.42). We also conducted a post-hoc sensitivity analysis that adjusted for age, quick SOFA and weight – all of which were baseline characteristics with qualitative differences between study arms. The adjusted difference in time to clinical improvement (-11.6 days, *P*=0.05, 95% CI -23.3 to 0.41) was qualitatively similar in this sensitivity analysis. Unadjusted times to clinical improvement using Kaplan-Meier survival curves and the log-rank test showed a significant difference between groups (Log Rank (Mantel-Cox) Chi-square 4,182. *P*=0,041) **(Figure 1).**

Secondary Outcomes

Ozonated autohemotherapy was associated with a significant higher probability of clinical improvement at day 14 (88.8% *vs.* 33.3%, *P*=0.01). Ozonated autohemotherapy was also associated with a shorter time to a 2-fold decrease of C reactive protein (3.5 days [3-28] *vs.* 13 days [8-25], *P*=0.008), ferritin (8 days [5-10] *vs.* 15 days [10-25], *P*=0.016), D-dimer (4 days [1-10] *vs.* 19.5 days [10-28], *P*=0.009) and Lactate Dehydrogenase (9 days [7-9] *vs.* 25 days [12-26], *P*=0.01). There was a trend towards a lower mean time to negative PCR COVID-19 testing results [13.1 (SD 5.7) *vs.* 21.4 (SD 7.4 days), *P*=0.05]. No differences with respect to ventilator-free days at day 28 (median [IQR]), 28 days [28-28] *vs.* 28 days [0-28], *P*=0.14), in-hospital or 28-days mortality (11.1% *vs.* 22.2%; *P*=1), were observed. No adverse events were observed or unintended effects in both groups (0% *vs.* 0%).

Table 1 Baseline characteristics.

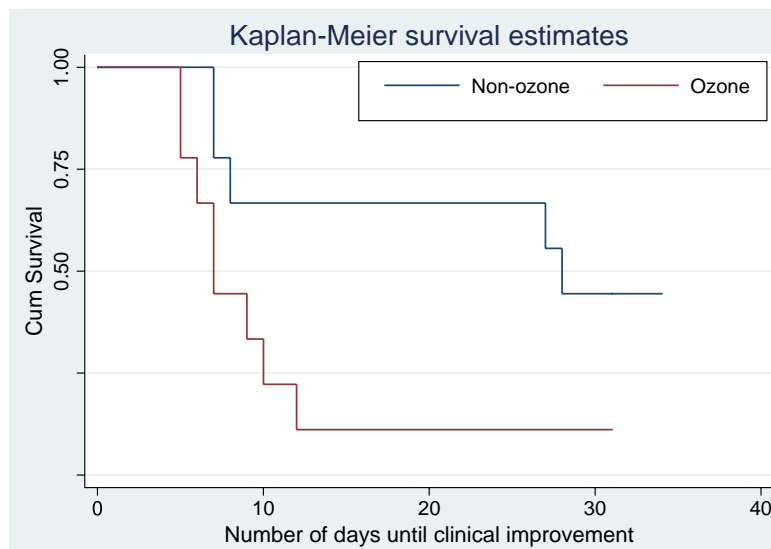| Variables	Ozonated Auto-hemotherapy (n=9)	Usual Clinical Care (n=9)	*P*-value
Age, mean (SD), years	64 (11)	71 (18)	0.35
Male sex, n (%)	7 (78%)	6 (67%)	1
Weight, Mean (SD), kg	74 (17)	85 (23)	0.25
Height, Mean (SD), cm	167 (10)	170 (7)	0.48
Body mass index, Mean (SD), kg/m^2	26.2 (4.5)	29.5 (7.1)	0.26
Hypertension, n (%)	4 (44%)	6 (67%)	0.34
Diabetes mellitus, n (%)	0 (0%)	2 (22%)	0.47
Chronic pulmonary disease, n (%)	2 (22%)	1 (11%)	1
Chronic cardiac disease, n (%)	1 (11%)	2 (22%)	1
Previous stroke, n (%)	0 (0%)	0 (0%)	1
Baseline hemoglobin, mean (SD), mg/dL	13 (2.1)	13 (3.0)	0.51
Baseline Quick SOFA score of 2 or 3, n (%)	1 (11%)	1 (11%)	1
Baseline WHO score, median [IQR]	3 [3-3]	3 [2-3]	0.60
Baseline Lactate Dehydrogenase, mean (SD), U/L	487 (168)	506 (123)	0.80
Baseline SpO$_2$/FiO$_2$ ratio, median [IQR]	350 [255-408]	339 [261-452]	0.96
Treatment			
Hydroxychloroquine, n (%)	4 (44%)	3 (33%)	0.23
Lopinavir/ritonavir, n (%)	1 (11%)	2 (22%)	1
Corticosteroids, n (%)	2 (22%)	1 (11%)	1
Ceftriaxone, n (%)	1 (11%)	1 (11%)	1
Levofloxacin, n (%)	2 (22%)	2 (22%)	1
Azithromycin, n (%)	8 (89%)	7 (78%)	1
Therapeutic anticoagulation, n (%)	0 (0%)	2 (22%)	1

Figure 1 Kaplan-Meier survival curves.

Discussion

In this proof-of-concept prospective cohort study of 18 consecutive hospitalized patients with confirmed COVID-19 severe pneumonia, twice-daily ozonated autohemotherapy was associated with a clinically and statistically significant reduction in the time to clinical improvement. Risk- adjusted analyses confirmed the results of the unadjusted analyses. No adverse events occurred. This cohort study provides novel new data pointing to the potential role of ozonated autohemotherapy for treatment of severe COVID-19 pneumonia. Our findings are consistent with recent reviews describing the potential biologically plausible benefits associated with ozonated autohemotherapy for COVID-19 infection [12-14]. As previously mentioned, there are no prior published data about the benefit of ozonated autohemotherapy for COVID-19 infection, except for case reports and an online report of the Scientific Society of Oxygen-Ozone Therapy (SIOOT) [8-10]. Medical ozone generators produce medical ozone. It is obtained from pure oxygen atoms by passing it through a high voltage gradient (5-13 KV). Single

Table 2: Outcomes.

Variables	Ozonated Autohemotherapy (n=9)	Usual Clinical Care (n=9)	*P*-value
Time to clinical improvement, median [IQR], days	7 [6-10]	28 [8-31]	0.04
Clinical improvement at day 7, n (%)	4 (44%)	2 (22%)	0.31
Clinical improvement at day 14, n (%)	8 (89%)	3 (33%)	0.01
Clinical improvement at day 28, n (%)	8 (89%)	5 (56%)	0.29
Time to Temperature <37°C, median [IQR], days	1 [1-1.5]	4 [2-5]	0.10
Time to PCR COVID-19 negative, mean (SD), days	13.1 (5.7)	21.4 (7.4)	0.05
Time to a 2-fold decreased C-reactive protein, median [IQR], days	3.5 [3-28]	13 [8-25]	0.008
Time to a 2-fold decreased D-dimer, median [IQR], days	4 [1-10]	19.5 [10-28]	0.009
Time to a 2-fold decreased ferritin, median [IQR], days	8 [5-10]	15 [10-25]	0.016
Time to a 2-fold decreased Lactate Dehydrogenase, median [IQR], days	9 [7-9]	25 [12-26]	0.01
Ventilator-free days at day 28, median [IQR], days	28 [28-28]	28 [0-28]	0.14
Intubation required, n (%)	0 (0%)	2 (22%)	0.47
ICU-length of stay, median [IQR], days	0 [0-0]	0 [0-0]	0.24
Hospital-length of stay, median [IQR], days	8 [7-12]	28 [8-31]	0.09
In-hospital mortality, n (%)	1 (11%)	2 (22%)	1
28-day hospital mortality, n (%)	1 (11%)	2 (22%)	1

oxygen atoms cannot endure alone without being regrouped into di-atomic oxygen molecules. In this recombination phase, some atoms will transform into loosely bound tri-atomic oxygen. This novel trioxygen molecule is called medical ozone. The gas mixture generated by medical ozone generators is around 97.2% oxygen and 2.8% ozone. Its molecular weight is of 48 g/mol with a solubility in water of 0.57 g/L at a temperature of 20 °C, (about ten-fold higher than oxygen). Consequently, the great solubility of ozone in water allows its immediate reaction with any soluble compounds and biomolecules present in biological fluids. Thermodynamically is unstable and spontaneously reverts back into oxygen. Concentrations ranging from 10-70 µg/ml are commonly used for medical purposes. There are multiples routes for medical ozone administration. Inhalation route may be toxic to the pulmonary system and other organs. However, ozonated autohemotherapy has been shown to be safe in multiple randomized clinical trials, observational studies and meta-analyses [15]. The incidence of side effects of ozone therapy is very low (estimated at 0.0007%), and typically manifests itself as euphoria, nausea, headaches and fatigue [16]. In general, it is a very safe therapy when administered correctly, with the recommended dose. Complications like air embolism have been described but are caused by incorrect administration practices and by using non-certified equipment.

There is a potential role for ozonated autohemotherapy for treatment of patients with severe COVID-19 pneumonia, with several biological plausible mechanisms of action. When human blood is expose to a gas mixture of oxygen and ozone, oxygen equilibrates with the extracellular and intraerythrocytic water before becoming bound to hemoglobin until it is fully oxygenated, on the contrary, ozone, more soluble than oxygen, readily dissolves in water and reacts instantaneously with biomolecules, such as amino acids, (particularly cysteine, tryptophan, methionine, phenylalanine, and tyrosine) with lipids (particularly the unsaturated fatty acids contained in membrane phospholipids) The former can yield disulfides and methionine

sulfoxide; the latter can yield hydrogen peroxide, aldehydes, and hydroxyhydroperoxides. The compounds generated during the reactions [reactive oxygen species (ROS) and lipid ozonation products (LOPs)] represent the "ozone messengers" and are responsible for its biological and therapeutic effects so ozone can be considered as a pro-drug that produces biochemical messengers [17].

Ozone might improve blood circulation and oxygen delivery to ischemic tissue as a result of the concerted effect of nitric oxide, increase intra-erythrocytic 2,3-DPG level, and increase of some prostacyclins such as PGI2 [18-24]. These effects can help to decrease the hypercoagulation that has been observed in COVID patients [25]. Another important role played by ozone in COVID-19 is its immunomodulatory effects. The immunomodulatory action of ozone is achieved through the activation of various cytoplasmic transcription factors by second messengers, specifically through the inhibition of the nuclear factor Kappa B (NF-κB) and the activation of the factor Nrf2 present in the bound cell cytoplasm to the Keap-1 protein. Ozone can achieve equilibrium between Nrf2 and NF-κB, modulating the expression of pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α counteracting the state of hyperinflammation seen in COVID patients. This immunomodulator effect and its capacity to increase the antioxidant defense system and enhance oxygen metabolism, could promote organ stress by inducing enhancement of endogenous protective parameters such as SOD, CAT, GSH-Px and GSH, in order to alleviate organ injury [26,27]. Kidney is a specific target for SARS-CoV-2 infection and acute kidney injury (AKI) can occur; indeed AKI is the most common extrapulmonary complication in intensive care so ozone has a potential protective effect which should be explore in further research [28]. In addition, ozone has potent anti-inflammatory properties through the modulation of the NLRP3 inflammasome which is recognized to play a crucial role in the initiation and continuance of inflammation in various diseases [29,30].

Regarding to the specific potential action of the ozone against coronavirus, the effectiveness of ozone against pathogens is well known. The ozone appears to be the best agent available for sterilizing water, although the *in-vivo* virucidal activity of ozone in the dosage used in this present study is unknown [31]. It has been suggested that ozone could act a signal molecule in the organism, being generated by human neutrophils and being necessary for antibody-catalyzed formation which play a role in the natural humoral response to infection [32,33]. Ozone also is capable of inducing the release and modulation of IFN-γ, TNF-α and colony stimulating factors, and is also able to modulate and stimulate phagocytic function which may have a very positive effect in COVID-19 infection [34-37].

Finally, ozone may impair viral replication, as suggested in its effects on SARS and MERS [38]. Angiotensin-converting enzyme type 2 (ACE2) cell receptors has been identified as receptor for SARS-CoV-2, which could be blocked with specific monoclonal antibodies but also through the control of the nuclear factor erythroid 2–related factor 2 (Nrf2) that regulates and blocks the activity of this receptor [39,40]. Because ozone is able to cause a rapid Nrf2 activation, it seems very likely that this may be an important physiological mechanism to block endogenous COVID-19 reduplication by preventing contact with this receptor [41,42]. Furthermore, spike proteins (S) are responsible for receptor binding and membrane fusion [43]. It contains a highly conserved transmembrane domain that consists of three parts: An N-terminal tryptophan-rich domain, a central domain, and a cysteine-rich C-terminal domain. Both, the cysteine-rich domain and tryptophan-rich domain, have been shown to be necessary for fusion [44,45]. Both cysteine and tryptophan, are sensitive to oxidation. It has been hypothesized that ozone metabolites could oxidize cysteine residues, making it difficult for the virus to enter the host cell and preventing viral replication [46]. Notably, our results, while promising, do not prove the benefit of ozonated autohemotherapy in severe COVID-19 pneumonia. This proof of concept study points to the need for further research, such as a well-designed, well-powered multicenter randomized clinical trial, to confirm our findings.

The strengths of this study include its pragmatic real-world COVID-19 population, use of objective primary clinical outcome and risk-adjustment using methods of regression modeling analyses.

Conclusion and Limitations

In conclusion, ozonated autohemotherapy was associated with a significant shorter time to clinical improvement in severe COVID-19 pneumonia patients in this small single-center cohort. Given the wide 95% CI, our study points to the need for further research in this clinical setting with larger sample size across multiples centers.

Our study has several limitations. First, the sample size of our cohort is small. Second, the 95% CIs for our adjusted estimates were wide, and do not exclude a 20-30% decrease in the coefficient for time (days) to clinical improvement. Third, as with most observational studies, we cannot exclude the possibility of residual unmeasured confounding. Fourth, it is a single-center study, limiting the external validity of our results. Finally, outcome assessors were not blinded to treatment arm assignment.

Acknowledgements

References

1 https://coronavirus.jhu.edu/map.html

2 Chen N, Zhou M, Dong X (2020) Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: A descriptive study. Lancet 395: 507-513.

3 Zhou F, Yu T, Du R (2020) Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: A retrospective cohort study. Lancet 395: 1054-1062.

4 Carsana L, Sonzogni A, Nasr A (2020) Pulmonary post-mortem findings in a large series of COVID-19 cases from Northern Italy. Br Med J 12: 1-8.

5 Guan WJ, Ni ZY, Hu Y (2020) Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med 382: 17081720.

6 Delvaeye M, Conway EM (2009) Coagulation and innate immune responses: Can we view them separately? Blood 114: 2367-2374.

7 Ricevuti G, Franzini M, Valdenassi L (2020) Oxygen-ozone immunoceutical therapy in COVID-19 outbreak: Facts and figures. Ozone Therapy 5: 9014.

8 Zheng Z, Dong M, Hu K (2020) A preliminary evaluation on the efficacy of ozone therapy in the treatment of COVID-19. J Med Virol 12: 2-4.

9 Hernández A, Viñals M, Isidoro T (2020) Potential Role of Oxygen–Ozone Therapy in Treatment of COVID-19 Pneumonia. Am J Case Rep 21: e925849.

10 http://www.ossigenoozono.it/En/Home

11 Wang Y, Zhang D, Du G (2020) Remdesivir in Adults with Severe COVID-19: A Randomised, Double-Blind, Placebo-Controlled, Multicentre Trial. Lancet 395: 1569-1578.

12 Hernandez A, Papadakos PJ, Torres A (2020) Two known therapies could be useful as adjuvant therapy in critical patients infected by COVID-19. Rev Esp Anestesiol Reanim 67: 245-252.

13 Sanchez MG, Schwartz A, Donna VD (2020) Potential cytoprotective activity of ozone therapy in SARS-CoV-2/COVID-19. Antioxidants (Basel) 9: 2-5.

14 Rowen RJ, Robins H (2020) A Plausible "Penny" Costing Effective Treatment for Corona Virus - Ozone Therapy. J Infect Dis Epidemiol 6: 113.

15 Viebahn-Hänsler R, León Fernández O, Fahmy Z (2016) Ozone in medicine: clinical evaluation and evidence classification of the systemic ozone applications, major autohemotherapy and rectal insufflation, according to the requirements for evidence-based medicine. Ozone Sci Eng 2016: 38: 322-345.

16 Jacobs MT (1982) Adverse effects and typical complications in ozone-oxygen therapy. Ozonachrichten 1: 193-201.

17 Bocci V, Aldinucci C (2006) Biochemical modifications induced in human blood by oxygenation- ozonation. J Biochem Mol Toxicol 20: 133-138.

18 Giunta R, Coppola A, Luongo C (2001) Ozonized autohemotransfusion improves hemorheological parameters and oxygen delivery to tissues in patients with peripheral occlusive arterial disease. Ann Hematol 80: 745-748.

19 Wu XN, Zhang T, Wang J (2016) Magnetic resonance diffusion tensor imaging following major ozonated autohemotherapy for treatment of acute cerebral infarction. Neural Regen Res 11: 1115-1121.

20 Borroto RV, Lima HLB, Lima GS (2013) Prevention of the stroke with the application of ozone therapy. Rev Cub de Med Fis y Rehab 5: 3-16.

21 Pandolfi S, Zammitti A, Franzini M (2017) Effects of oxygen ozone therapy on cardiac function in a patient with a prior myocardial infarction. Ozone Therapy 2: 2-4.

22 Bateman RM, Sharpe MD, Ellis CG (2003) Bench-to-bedside review: Microvascular dysfunction in sepsis--hemodynamics, oxygen transport, and nitric oxide. Crit Care 7: 359-373.

23 Bocci V (2002) Oxygen-ozone therapy: A critical evaluation. Kluwer Academic Publisher, Dordrecht, The Netherlands.

24 Schulz S, Ninke S, Watzer B (2012) Ozone induces synthesis of systemic prostacyclin by cyclooxygenase-2 dependent mechanism in vivo. Biochem Pharmacol 83: 506-513.

25 Terpos E, Stathopoulos NI, Elalamy I (2020) Hematological findings and complications of COVID-19. Am J Hematol 2: 2-4.

26 Sancak EB, Turkon H, Cukur S (2016) Major ozonated autohemotherapy preconditioning ameliorates kidney ischemia-reperfusion injury. Inflammation 39: 209-217.

27 Rodríguez ZZ, Guanche D, Alvarez RG (2009) Preconditioning with ozone/oxygen mixture induces reversion of some indicators of oxidative stress and prevents organic damage in rats with fecal peritonitis. Inflamm Res 58: 371-375.

28 Yang X, Yu Y, Xu J (2020) Clinical course and outcomes of critically ill patients with SARS- CoV-2 pneumonia in Wuhan, China: A single-centered, retrospective, observational study. Lancet Respir Med 8: 475-481.

29 Yu G, Bai Z, Chen Z (2017) The NLRP3 inflammasome is a potential target of ozone therapy aiming to ease chronic renal inflammation in chronic kidney disease. Int Immunopharmacol 43: 203-209.

30 Mangan MSJ, Olhava EJ, Roush WR (2018) Targeting the NLRP3 inflammasome in inflammatory diseases. Nat Rev Drug Discov 17: 588-606.

31 https://www.who.int/water sanitation health/dwq/S04.pdf

32 Babior BM, Takeuchi C, Ruedi J (2003) Investigating antibody-catalyzed ozone generation by human neutrophils. Proc Natl Acad Sci USA 100: 3031-3034.

33 Wentworth P, McDunn JE, Wentworth AD (2002) Evidence for antibody-catalyzed ozone formation in bacterial killing and inflammation. Science 298: 2195-2199.

34 Notarangelo L, Casanova JL, Fisher A (2004) Primary immunodeficiency diseases: An update. J Allergy Clin Immun 114: 677-687.

35 Paulesu L, Luzzi E, Bocci V (1991) Studies on the biological effects of ozone: 2. Induction of tumor necrosis factor (TNF-alpha) on human leucocytes. Lymphokine Cytokine Res 10: 409- 412.

36 https://ojs.uv.es/index.php/JO3T/article/view/10627/html

37 Frush BW, Li Z, Stiles JV (2016) Ozone primes alveolar macrophage-derived innate immunity in healthy human subjects. J Allergy Clin Immunol 138: 1213-1215.

38 http://www.triroc.com/sunnen/topics/sars.html

39 Zhang H, Penninger JM, Li Y, Zhong N, Slutsky AS (2020) Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. Intensive Care Med 46: 586-590.

40 Sagai M, Bocci V (2011) Mechanisms of action involved in ozone therapy: Is healing induced via a mild oxidative stress? Med Gas Res 1: 29.

41 Pecorelli A, Bocci V, Acquaviva A (2013) Nrf2 activation is involved in ozonated human serum upregulation of HO-1 in endothelial cells. Toxicol Appl Pharmacol 267: 30-40.

42 Re L, Sánchez MG, Bordicchia M (2014) Is ozone pre-conditioning effect linked to Nrf2/EpRE activation pathway in vivo. A preliminary result? Eur J Pharmacol 742: 158-162.

43 Chang KW, Sheng Y, Gombold JL (2000) Coronavirus-induced membrane fusion requires the cysteine-rich domain in the spike protein. Virology 269: 212-224.

44 Madu IG, Belouzard S, Whittaker GR (2009) SARS-coronavirus spike S2 domain flanked by cysteine residues C822 and C833 is important for activation of membrane fusion. Virology 393: 265-271.

45 Liao Y, Zhang SM, Neo TL (2015) Tryptophan-dependent membrane interaction and heteromerization with the internal fusion peptide by the membrane proximal external region of SARS-CoV spike protein. Biochemistry 54: 1819-1830.

46 Dussault PH, George AD, Trullinger TK (1999) Peroxides as oxidative enzyme inhibitors: Mechanism-based inhibition of a cysteine protease by an amino acid ozonide. Bioorg Med Chem Lett 9: 3255-3258.