

## Medical Ozone: The Pharmacological Mechanisms Accounting for its Effectiveness against COVID-19/SARS-COV-2

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### ABSTRACT

**Introduction:** Medical ozone has been used with safety and efficacy in different diseases of oxidative etiology, for the most part involving autoimmune diseases.

**Methods:** An analysis of the pharmacological mechanism of action of ozone was carried out to explain its clinical effectiveness and its positive response in clinical patients to COVID-19. This was done in the context of the different therapeutic targets that have been demonstrated for ozone in other diseases (autoimmune and hypoxia status).

**Results:** Based on the intestine/lung functional axis, the necessity of rectal insufflation as route of application with the aim of attaining improved results using medical ozone against COVID 19 is demonstrated. It was possible to identify at least nine adverse events/molecules which were targets of regulation through medical ozone in other diseases, including innate immune response, nuclear transcription factor NF- $\kappa$ B, "cytokine storm", inflammation, severe acute respiratory syndrome and coagulopathy. Some of them lead to multi organ failure.

Finally, a brief analysis is undertaken to show the regulatory effects of ozone versus the comorbidities contributing to virus lethality, including hyperglycemia and its vascular complications.

**Conclusions:** Medical ozone is effective against COVID-19/SARS-CoV-2: due to the multiple targets it is able to regulate and thereby achieve a positive patient response.

### Keywords

COVID 19/SARS-CoV-2, Medical ozone, Viral infection, Pharmacological mechanisms.

### Introduction

The first reports of the viral infection attracted attention in late December 2019 in Wuhan, the capital of Hubei, China. Later it was revealed that the virus responsible for causing the infections was

contagious between humans. On February 11, 2020, a taxonomic designation "severe acute respiratory syndrome coronavirus 2" (SARS-CoV-2) became the official term referring to the virus strain previously known as 2019-nCoV and Wuhan coronavirus. Within a few hours on the same day, the WHO officially renamed the disease COVID-19 [1], since then responsible for more than 2 million deaths worldwide.

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Although, in spite of an enormous scientific activity within a short time, most of the scientific research on COVID-19 still only exists in unpublished form, the reality in the field is that treatment is yet in an experimental stage. This is because its major targets have, as starting point, the knowledge available from coronavirus SARS-CoV (2002-2003) and Middle East Respiratory Syndrome coronavirus (MERS-CoV, 2012); these were largely localized to China and Saudi Arabia, respectively [2,3].

Cuba has developed and introduced different protocols in order to treat COVID 19 originating from WHO recommendations, available knowledge of the disease's pathogenesis and the recent findings from Cuban biotechnology and the pharmaceutical industry as well as from natural medicine. At present, four vaccinal candidates are in different clinical trial phases with satisfactory results.

Medical ozone is considered to be a candidate for natural medicine. It has been studied as a combined therapy in COVID-19 patients, and its satisfactory results have led to include it in Cuban treatment protocols. Briefly, rectal insufflation was used at an ozone concentration of 35-40 µg/ml. Patients received two treatments/day every 12 hours for a total of 10 days.

32 COVID-19 patients -diagnosed using nucleic acid reverse transcription polymerase chain reaction (RT-PCR)- with slight and mild symptoms were divided into two groups (n = 16 each): the first was treated with conventional protocol + medical ozone, and the second group treated using conventional procedures alone. 75% of the patients treated with medical ozone were found to be negative on day 5, coinciding with the final day of ozone treatment when compared with 43% in patients not having received medical ozone. An improvement in biochemical and hematological indicators was observed. In addition, another study involving convalescent COVID-19 patients demonstrated an improvement in general health as well as hematological and biochemical indicators. Their body weights had increased, and there was an important patient response in the form of antioxidant defense [4,5].

Medical ozone is an ozone/oxygen mixture which has showed its effectiveness in a number of diseases having an oxidative etiology [6-8]. Experimental and clinical trials have evidenced its therapeutic actions on targets that are coincident with molecules/processes recognized as pathological in SARS-CoV-2. Therefore, taking the background described above into account, the aim of this study was to establish a relevant analysis of the pharmacological mechanism of action demonstrated by ozone in previous investigations. This will explain its clinical effectiveness and its positive clinical response in patients with COVID-19 in the light of the different therapeutic targets demonstrably valid and useful for ozone found for other, relevant diseases (mainly autoimmune and hypoxia status).

The focus of this study was on the outcome in COVID-19 patients with a severe status. We have not drawn the pathological mechanisms of SARS-CoV-2 leading to a critical clinical status

into consideration -due to the generality of the findings reported as based on SARS-CoV; although they share a great similarity as to their common origin, they still display differences which have to be studied in the future.

### **What dose and which application route should be used for medical ozone in COVID-19 patients?**

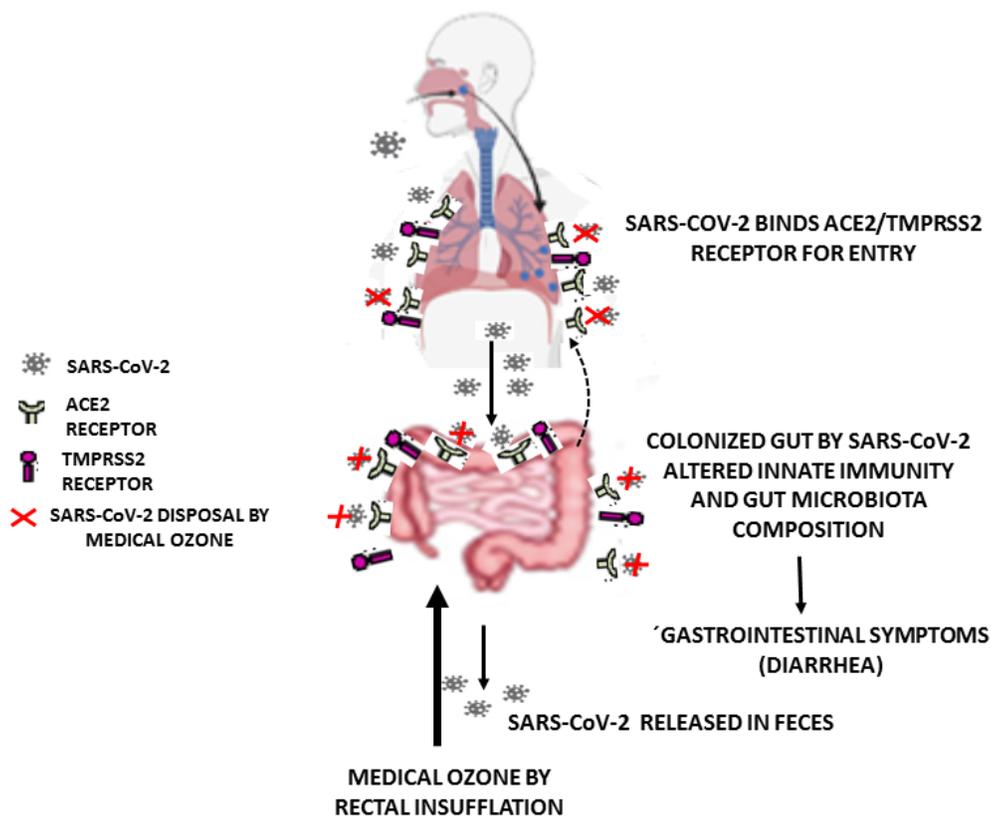
Based on experimental and clinical studies, medical ozone should be administered at a low dose (10-40 mg/L) with a controlled number of treatments as depending on the disease. In this way, medical ozone is safe and effective either via rectal insufflation or in the form of autohemotherapy [9]. Both have systemic effects. Nevertheless, with regard to COVID-19, the more effective method here is rectal insufflation, which must be considered as first choice.

The alterations in microbial community in both lungs and airways also affect the composition of intestinal microbiota [10-12]. For example, about half of the inflammatory bowel disease (IBD) patients with known alterations in their intestinal microbiota composition have reduced lung function [13]. This suggests the "gut-lung axis" as a bi-directional communication network where many respiratory infections are often accompanied by gastrointestinal (GI) symptoms or gut dysfunction or secondary gut dysfunction complications. On the other hand, respiratory viral infections can alter the intestinal microbiome, where the intestinal microbiome determines the adaptive immune responses against the respiratory pathogens and is necessary for priming the innate immune responses against the pulmonary infections. During respiratory viral infections, the level of macrophage response to the respiratory viruses depends on the presence of intestinal microbes [14,15]. This suggests that the lung and the gut are closely linked organs that affect each other's homeostasis via an immunological co-ordination between them. Some reports emerging speculate the role of targeting the gut microbiota as a new therapeutic choice or adjuvant therapeutic option in COVID-19 treatment [16,17]. In addition, diarrheas are gastrointestinal symptoms indicating for COVID-19 a gut involvement in viral infection.

The factors just described underline the importance of medical ozone rectal insufflation in COVID-19 patients as being the more suitable application. Rectal insufflation allows medical ozone to direct its antiviral effects specifically, and reduce the viral load contribution from gut colonized virus (Figure 1).

### **Pharmacological properties of medical ozone with a direct impact on COVID-19 disease**

The experimental and clinical investigations described below were carried out using rectal insufflation (dose 25-35 mg/L), for which reason they are relevant for the results obtained with COVID-19 patients treated with medical ozone. Out of all the studies carried out over the last years, those results have been selected which are close to SARS-CoV-2 symptomatology, such as cytokine storm (recognized in autoimmune diseases as rheumatoid arthritis) [18-20], diseases associated with hypoxia status (ischemic syndrome) [21,22] and other such as diabetes mellitus and its vascular complications, sometimes lethal in COVID-19.



**Figure 1:** Representation of a probable “gut-lung axis” in SARS-CoV-2 caused COVID-19 disease and medical ozone antiviral effects on infected gut contributing to reduce the virus load.

### Medical ozone and immune response in COVID-19

The uncontrolled inflammatory innate responses and impaired adaptive immune responses in severe COVID-19 patients are ubiquitous and these abnormal immune responses can lead to local and systematic tissue damage. These are consistent with clinical outcomes. According to the retrospective studies and pathological findings, many patients with COVID-19 have experienced multiple organ function damage, including acute kidney injury, cardiac injury, liver dysfunction, and cerebral damage [23-25].

A retrospective study found that 80% of critically ill patients (patients, who meet one of the three diagnostic criteria: (1) respiratory failure, (2) septic shock, (3) multiple organ failure) had lymphopenia, versus only 35% of non-critically ill patients. Moreover, the neutrophil count and neutrophil- to- lymphocyte ratio were increased in COVID-19 patients, indicating a higher disease severity and poor clinical outcome [26-28]. On the other hand, CD8+ T cells, regardless of whether they belonged to the effector, naïve, or memory subsets, declined constantly during the progression of infection [29].

Autoimmune diseases, particularly rheumatoid arthritis (RA), display a sudden cytokine release in the face of different stimuli such as autoantibodies production. Medical ozone was able to promote an increased protective response in RA patients treated

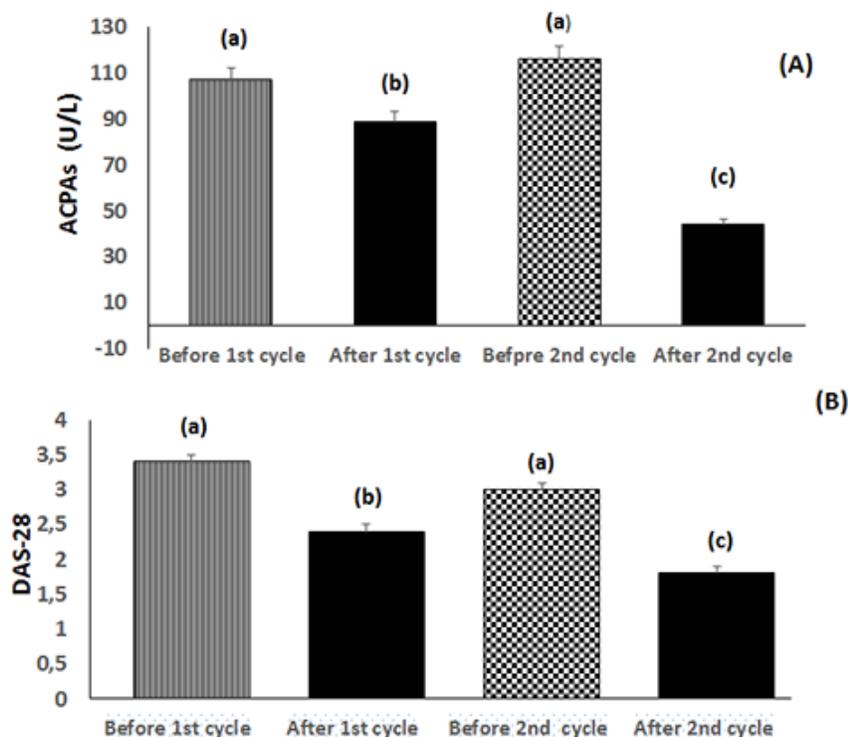
with methotrexate + medical ozone combined therapy after a second ozone exposure. The results suggest an innate memory response when they were compared with the described properties for this immune response [30-32].

Figure 2A shows how medical ozone decreased anti-citrullinated protein antibodies (ACPAs) levels at the end of second ozone exposure ( $p < 0.05$ ) with regard to the first cycle, thus indicating regulatory effects on the immune system of these patients, and suggesting an immunological innate memory deployment.

Inflammatory processes showed a similar picture in the case of ACPAs. A more favorable and robustly anti-inflammatory response was observed at the end of the second ozone exposure (Figure 2B).

On analysis, the improvement in the cellular redox balance of the patients showed a more effective pharmacological effect of ozone. A reestablishment to reference levels of protective biomarkers as well as injury indicators were observed (Table 1). An exception was the catalase activity, which showed no change. These results demonstrate that medical ozone is able to regulate an antioxidant/ pro-oxidant balance otherwise broken down by the infectious and inflammatory processes that play a role in COVID-19 patients.

Table 1.



**Figure 2:** (A) Anti-citrullinated protein antibodies (ACPAs) levels and (B) course of inflammatory events (DAS-28, 28 inflamed and painfully joints) in rheumatoid arthritis patients treated with methotrexate (MTX) + ozone before and after of receive two cycles of ozone treatment at intervals of three months between each cycle. The mean  $\pm$  standard deviation (SD) is given. Different letters mean statistical difference ( $p < 0.05$ ).

**Table 1:** Protective and pathogenic redox biomarkers in RA patients treated with MTX+ozone (n = 40) after receiving two cycles of ozone treatment at intervals of three months between each cycle.

Biomarker	Control	End first cycle	End second cycle
GSH ( $\mu\text{g/ml}$ )	800 - 1300	514	838*
SOD (U/L/min)	2 - 10	16.6	10*
CAT (U/L/min)	130 - 155	109	89
TH ( $\mu\text{M}$ )	8 - 12	38.7	9*
MDA	0.1 - 0.18	1.7	0.8*
AOPP ( $\mu\text{M}$ )	2 - 8.5	13.7	8.2*
NO ( $\mu\text{M}$ )	45 - 92	55	74*

An overproduction of reactive oxygen species (ROS) leads to oxidative stress; this is related to all the main changes observed in inflammatory and infectious diseases. Based on convincing scientific documentation, oxidative stress has been associated with changes found in patients with COVID-19, such as its participation in the amplification and perpetuation of the cytokine storm, coagulopathy, and cell hypoxia [33-38].

Oxidizing agents come from the phagocytic leukocytes such as neutrophils, monocytes, macrophages and eosinophils invading the tissue. ROS release by these cells promote cytotoxicity thus injuring cells. They degrade essential cellular components and alter the protease/antiprotease balance in the tissue interstitial. Oxidative stress mediates the activation of NF- $\kappa$ B inducing in turn the transcription of genes promoting cytokine production. Release of these cytokines, in turn, enhances the inflammatory response. An intense inflammatory response in COVID 19 seem to be

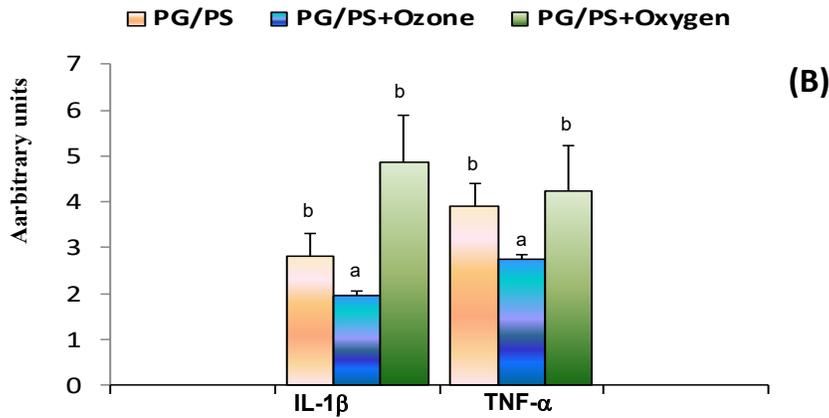
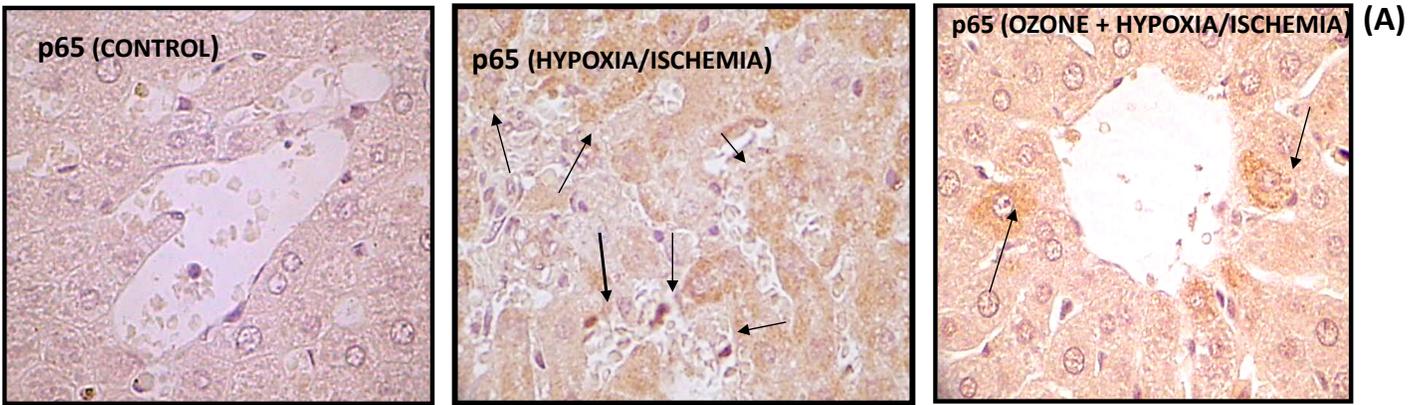
mediated by oxidative stress [39-41].

### Medical ozone and the Cytokine Storm” in COVID-19

Cytokine storm occurs when an immune system is over activated by infection, drugs, and/or some other stimuli, leading to high levels of cytokines (IFN, IL, chemokines, CSF, TNF, etc.) being released into the circulation with a widespread and detrimental impact on multiple organs. The severe inflammatory responses induced by a cytokine storm start locally and spread systemically, causing collateral damage in tissues [42-44].

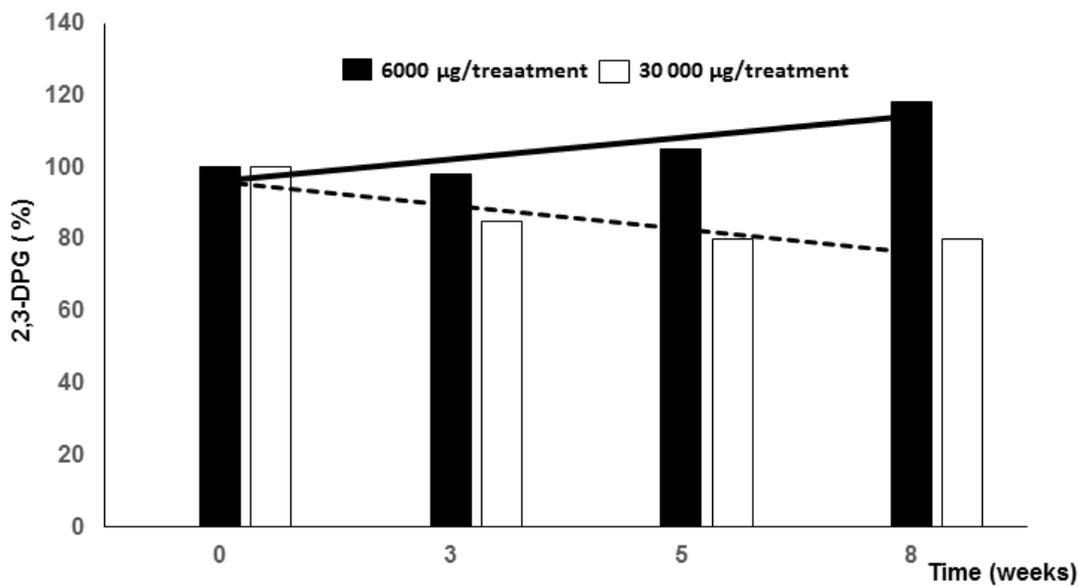
An uncontrolled, virus-induced release of cytokines leads to NF- $\kappa$ B activation; this is regulated by medical ozone in hypoxic/ischemic conditions [45]. This pathological event is associated with acute respiratory distress syndrome (ARDS) in COVID-19. Figure 3 shows medical ozone regulation of NF- $\kappa$ B immune reactivity, stronger in the hypoxia/ischemia experimental group, whereas those having received 15 previous ozone treatments displayed little and disperse reactivity. This indicates the medical ozone regulation of this important nuclear factor. In line with the above results, medical ozone reduced RNAm of IL-1 $\beta$  and TNF- $\alpha$  [46-48] produced by NF- $\kappa$ B activation and with a high pathogenicity in SARS-CoV-2 infection. Similar results were achieved with IL-6 concentrations in rheumatoid arthritis patients [49].

### The protective effects of medical ozone against SARS and coagulopathy in COVID-19 Concerning SRAS.

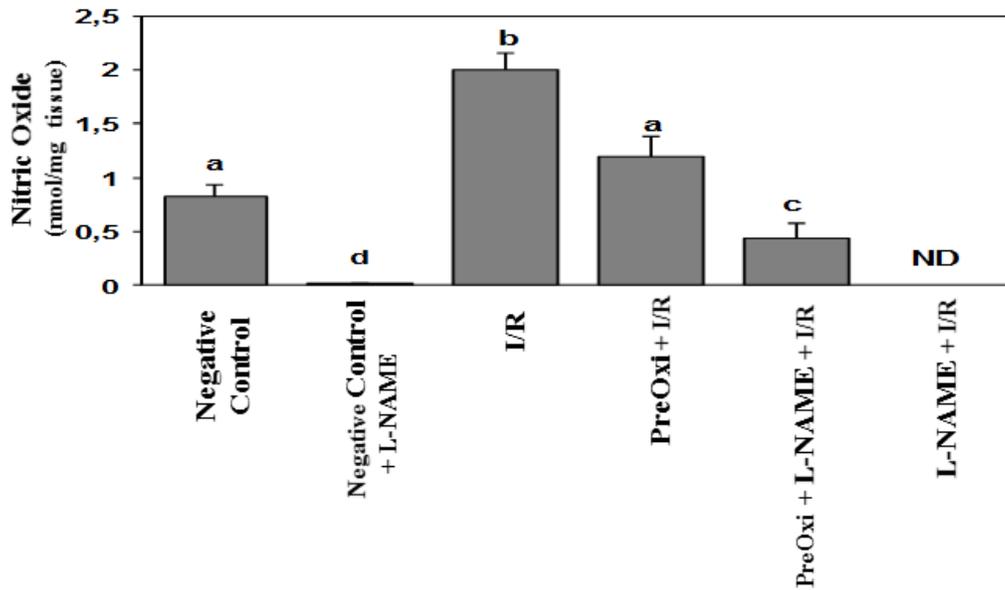


**Figure 3:** (A) Immunohistochemistry of liver slides (data shown are representative at least of seven rats). P65 subunit of NF-κB in ischemic conditions and the effects of medical ozone on the factor reactivity. Arrows indicate the antibody's reactivity areas.

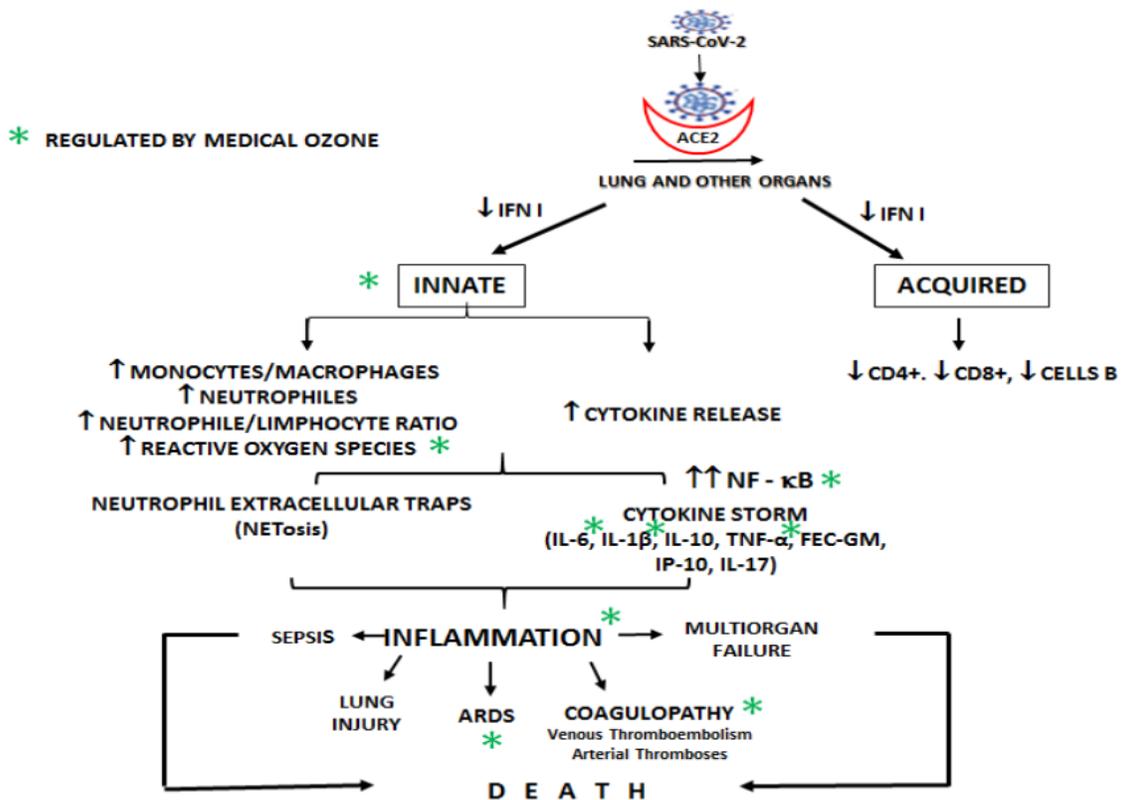
(B) mRNA levels of IL-1β and TNF-α in rat's spleen homogenates from PG/PS induced-arthritis and medical ozone effects on this model of chronic arthritis. PG/PS, bacterial cell wall peptidoglycan-polysaccharide the mean ± standard deviation (SD) is given. Different letters mean statistical difference (p < 0.05).



**Figure 4:** Pilot study in elderly, n = 11: 2,3-DPG in RBC's during and following 10 rectal ozone applications 2x per week. 6,000 µg/treatment, ozone conc. 20 µg/ml, volume V = 300 ml and 30,000 µg/treatment: 100 µg/ml, V = 300 ml.



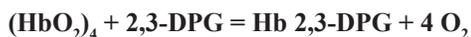
**Figure 5:** Hepatic tissue levels of nitrite/nitrate (NO<sub>2</sub>/NO<sub>3</sub>). Ischaemia–reperfusion (I/R): 90 min of ischaemia followed by 90 min of reperfusion; OzoneOP: ozone oxidative preconditioning; L-NAME: N- $\omega$ -nitro-L-arginine methyl ester. Each value is the mean  $\pm$  SEM from 10 rats. ND, not detectable levels. Different letters mean statistical difference ( $p < 0.05$ ).



**Figure 6:** Schematic representation of medical ozone effects on multiple targets associated with pathological processes described for COVID-19-SARS-CoV-2. ACE2, Angiotensin converting enzyme 2; NF- $\kappa$ B, Nuclear Factor kappa B; ARDS, acute respiratory distress syndrome.

Hypoxia in COVID-19 patients associated with SARS which, together with other symptoms, result in patient ventilation might be improved through ozone therapy [50,51].

The affinity of hemoglobin to oxygen depends a great deal on the deoxygenating substance 2,3 di-phosphoglycerate (2,3-DPG). In a healthy system, this 2,3-DPG is present in the same molar quantities as hemoglobin itself, which passes into the center of the quaternary structure of Hb to release four oxygen molecules.



As 2,3-DPG reduces oxygen affinity by a factor of 26 or, in other words, the oxygen binding curve is increasingly shifted to the right as the 2,3-DPG content rises, this results in an improved oxygen supply to the peripheries. If the release of oxygen is reduced due to a low or fluctuating 2,3-DPG content as known from diabetics, ozone application here means a re-functionalization of the red blood cells (RBCs) and an improved oxygen release [52-53].

Specific physiological or pathological conditions may result in reduced or highly fluctuating 2,3-DPG levels in diabetics, which can thus be the cause for an insufficient oxygen supply to the tissues. In a group of eleven patients with peripheral arterial circulatory disorders in Stages III and IV according to Fontaine, mostly diabetics, the influence of a series of ozone treatments on the 2,3-DPG content was determined: Only in one patient was 2,3-DPG decreased, the other ten patients showing a considerable increase to 100 % as statistic mean value. The noticeable significant increase is to be understood as a result of the extreme situation in a number of these diabetic patients. The reduction in oxygen affinity of hemoglobin and the simultaneous shift of the Hb/oxygen balance towards deoxygenated Hb found in this study constitute one of the fundamental reasons for the improved peripheral oxygen supply in diabetics after ozone therapy [52].

Figure 4 shows the results of two different ozone concentrations (low and high). A low ozone dose had a tendency to increase 2,3-DPG while a higher dose displayed a tendency to decrease it. These results demonstrate the beneficial effects at a low dose (10-40 mg/L). Patients increased ATP levels after 5 weeks (ten ozone treatments) and a remarkable additional increase 3 weeks after of the last ozone treatment [53].

### Concerning coagulopathy

A high mortality and its relationship with thromboembolic diseases in cases of COVID-19 are attracting attention to an increased extent [54,55].

Consecutive autopsy findings have revealed frequent deep vein thrombosis in 7 of 12 COVID-19 patients (58%), with complicated pulmonary embolism in 4 patients (33%).

An increased incidence of arterial thrombosis such as stroke and acute coronary syndromes has also been reported in cases with COVID-19 [56-59].

In a healthy condition, angiotensin-converting enzyme 2 (ACE2) converts angiotensin II to angiotensin 1-7, which stimulates endothelial cells to produce nitric oxide (NO). NO helps the vessels to vasodilate and suppresses platelet aggregation. In cases with COVID-19, SARS-CoV-2 occupies ACE2 and the angiotensin II level increases, which results in vasoconstriction and a decrease in blood flow. The von Willebrand factor (VWF) stored in Weibel Palade bodies is released into the circulation, promoting clot formation [60].

Nitric oxide synthesis is another target of medical ozone. In liver ischemia/reperfusion (I/R), ozone was able to regulate its concentrations (Figure 5). Through an inhibitor of nitric oxide synthase (L-NAME) and fifteen ozone applications prior to I/R, a decrease in the production of nitric oxide levels in I/R group was observed compared with negative controls, as well as the *de novo* synthesis of nitric oxide (PreOxi + L-NAME + I/R group) [61]. These results suggest that medical ozone may reduce the risk of coagulopathy in COVID-19 patients when the role of this free radical in thrombus formation is taken into account.

Finally, figure 6 gives a schematic representation of medical ozone effects on multiple targets associated with pathological processes described for SARS-CoV-2 COVID-19. Ozone produces prompt action on viral colonization in the gut when applied via rectal insufflation. Following this, it shows its systemic effects on those events of the pathological cascade which may have lethal consequences.

In addition, medical ozone has displayed beneficial effects against comorbidities in COVID-19 patients. Diabetes and its vascular complications were brought under control by medical ozone. It also reestablishes glycaemia, accelerates healing and reduces the hospitalization period in patients with diabetic foot [62-64].

In summary, with medical ozone patients can become virus negative due to its multiple therapeutic targets which work on different pathological levels (local and systemic) of SARS-CoV-2/ COVID-19 infection.

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