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Review

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Review Article

Ozone therapy for the treatment of COVID-19 pneumonia: a scoping review.

Running head: Ozone therapy for COVID-19

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ABSTRACT

Severe forms of COVID-19 can evolve into pneumonia, featured by acute respiratory failure due to acute lung injury (ALI) and acute respiratory distress syndrome (ARDS). In viral diseases, the replication of viruses is seemingly stimulated by an imbalance between pro-oxidant and antioxidant activity as well as by the deprivation of antioxidant mechanisms. In COVID-19 pneumonia, oxidative stress also appears to be highly detrimental to lung tissues. Although, inhaling ozone (O3) gas has been shown to be toxic to the lungs, recent evidence suggests that its administration via appropriate routes and at small doses can paradoxically induce an adaptive reaction capable of decreasing the endogenous oxidative stress. Ozone therapy is recommended to counter the disruptive effects of severe COVID-19 on lung tissues, especially if administered in early stages of the disease, thereby preventing the progression to ARDS.

Keywords: COVID-19, pneumonia, ARDS, ALI, oxidative stress, ozone (O3) the

1.INTRODUCTION

Severe Acute Respiratory Syndrome Coronavirus type 2 (SARS-CoV-2), coronavirus disease 2019 (Covid-19), rapidly spread worldwide to become a pandemic on March 11, 2020 [1-3].
Seven coronavirus strains discovered thus far can cause infectious disease in humans. Whilst strains 229E, HKU1, OC43 and NL63 cause mild respiratory diseases, often presenting with common cold symptoms, the other three types can determine severe infectious diseases and include:

- The Severe Acute Respiratory Syndrome Coronavirus type 1 (SARS-CoV-1), which was associated with an outbreak in Hong Kong and elsewhere during 2002-2003 [4, 5];
- The Middle East Respiratory Syndrome Coronavirus (MERS-CoV), first appeared in 2012 and is still circulating among certain animals such as camels, mainly in the Middle-East [6]; and
- SARS-CoV-2.

There are high similarities between the latter three human coronaviruses, with SARS-CoV-2 sharing 51.8% and 79% nucleotide homology with MERS-CoV and SARS-CoV-1 [7].
The clinical pattern of COVID-19 varies extensively from mild/moderate (81%) to severe (14%) or critical (5%) [9, 10, 11]. Among 2,634 hospitalized patients with confirmed COVID-19 in New York City, Long Island and Westchester County from March 1 to April 4, 2020, 14.2% needed admission to intensive care units (ICUs), with invasive mechanical ventilation required in 12.2% of them [12]. Despite a mortality rate of approximately 2.3% - considerably lower than MERS-CoV (35%) - the base reproductive number (Ro) of SARS-CoV- 2 has been estimated to fall between 2 and 3, similar to SARS-CoV-1 (Ro=1.95) but much higher than MERS-CoV (Ro=0.5). SARS-CoV-2 is therefore more contagious as compared with MERS-CoV [12 [13,14], especially since asymptomatic/pre-symptomatic COVID-19 patients can shed high loads of virus in the surrounding environment [10]. In a recent meta-analysis on 28 high/moderate quality studies including cohorts or studies testing individuals irrespective of their COVID-19 symptoms, or case series with tracking report of asymptomatic patients, 8.7% study subjects were found to be COVID-19 positive. The percentage of asymptomatic in the latter metanalysis was 20% to 75% among COVID-19 confirmed cases [8].

In a viewpoint just published in JAMA, Kim et al., urgently called for new outpatients' therapies which, combined with an effective vaccine, could significantly contribute to end this ongoing COVID-19 pandemic [15]. Whilst some drugs (especially corticosteroids) are currently used against severe COVID-19, therapeutic remedies for initial/moderate COVID-19 pneumonia are still missing. Treatments effective in early stage COVID-19 pneumonia could have a significantly impact on patients' prognosis, reduction of hospital admissions, prevention of long-term sequelae and containment of the communicability window of COVID-19, hence reducing the respective risk of infection [15]. Leading candidates for COVID-19 treatment examined by Kim et al., included emerging antivirals, immunomodulatory drugs and antibody-based immunotherapy, with ozone (O3) being neglected [15].

Ozone is a triatomic unstable gas composed of 3 oxygen (O2) molecules featured by a 1h half time, rapidly reverting to O2 at ambient temperature [16]. Ozone has potent oxidizing activity and already proved effective cidal effect against bacteria, fungi and viruses [17-19], including SARS-CoV-1 [20], through oxidation of double bonds [16].
For its immunomodulatory and anti-inflammatory properties Ozone has also recently been suggested as potential, inexpensive and easily available adjuvant therapy also against CODVI-19, especially in mild to moderate pneumonia, to prevent the progression to critical disease [21,22].

In this study we conducted a scoping review of the evidence on the potential application of ozone (O_3) to treat/prevent the severe forms of COVID-19.

2. METHODS

Searching strategy

PubMed, Scopus, Google Scholar, Web of Science and Cochrane library were searched using the following keywords: "COVID-19 Infection AND oxidative stress"; "SARS-CoV-2 AND oxidative stress"; "Infectious disease AND oxidative stress"; "Inflammation AND oxidative stress"; "Viral disease AND oxidative stress"; "Pneumonia AND oxidative stress"; "Ozone (therapy) AND Oxidative Stress"; "Ozone (therapy) AND pneumonia"; "Ozone (therapy) AND Viral Disease"; "Ozone (therapy) AND COVID-19"; "ozone therapy AND SARS-CoV-2"; "Ozone (therapy) AND Inflammation"; "Ozone (therapy) AND acute lung injury (ALI) "; "Ozone (therapy) and Acute Respiratory Distress Syndrome (ARDS) "; "Ozone (therapy) and ARDS"; "Ozone (therapy) AND Severe Acute Respiratory Syndrome"; "Ozone (therapy) AND SARS"; "Ozone (therapy) AND cytokines"; "Angiotensin-Converting Enzyme-2 (ACE2) receptor AND Oxidative stress". Retrieved items were screen by title and abstract. Only articles in English were considered; dissertations, conference abstracts and duplicate publications were discarded.

3. DISCUSSION

3.1. Viral Diseases and Oxidative Stress

In viral diseases, the replication of viruses is seemingly influenced by an imbalance between pro-oxidant and antioxidant activity as well as by the deprivation of antioxidant mechanisms [23]. In an experimental animal model, SARS-CoV-1 infection was found to be linked to elevated reactive oxygen species (ROS) levels and disruption of antioxidant defences [24]. Hypoxia, that can be caused by viral sepsis, produces ROS such as superoxide radicals [25-28]. Increased oxidative stress is severely damaging for the lung, causing acute respiratory failure sustained by ALI and ARDS, featured by considerably high mortality and morbidity [29, 30]. ALI/ARDS also characterize patients affected by severe/critical COVID-19, especially those referred to ICUs, where multiple factors such as hypoxemia, inflammation and mechanical ventilation with high fractions of inhaled O_2 magnify oxidant generation [31, 32]. Elevated High Sensitivity C-Reactive Protein (hsCRP), an indicator of inflammation and oxidative stress, has been found in 93% of patients affected by COVID-19 pneumonia [33].

3.2. Renin-Angiotensin-Aldosterone System (RAAS) and oxidative stress

The RAAS seems to be involved in the pathogenesis of severe ALI. SARS-CoV-1 is capable of binding to the Angiotensin-Converting Enzyme-2 (ACE2) through its spike protein (**Fig 1**), downregulating its expression, which would have a physiological protective effect against ALI [34]. Likewise, SARS-CoV-2 also exploits the ACE2 receptor for cell internalization [35].

The carboxypeptidase ACE2 is a crucial element of RAAS for the control of blood pressure [36,37]. It seems that Angiotensin-Converting Enzyme (ACE) and ACE2 antagonize with each other [37]. Angiotensin I (AT1) and angiotensin II (AT2) are converted by ACE2 into the inactive molecule angiotensin 1–9 and angiotensin 1–7, respectively [38]. Angiotensin 1-7 has anti-proliferative and vasodilatory effects and reduces the oxidative stress [39]. As mentioned above, some critically ill patients with COVID-19 develop ALI and ARDS, which lead to pulmonary oedema and lung failure [40, 41]. In the pathogenesis of ALI, ACE upregulates AT2, which in turn causes severe lung injury through binding with the AT2 subtype 1a receptor [34]. AT2 has potent vasoconstrictor effects and

induces oxidative stress [42] predominantly through activation of NADPH oxidase, one of the most prominent producers of superoxide radical [43]. The serum level of AT2 is reported to be considerably elevated in COVID-19 patients and exhibits a positive linear correlation with viral load and lung injury [44]. By contrast, increasing levels of ACE2 and AT2 receptors had a protective effect in vitro against lung injury induced by SARS-CoV-1, MERS-CoV and SARS-CoV-2 [34, 44-46].

3.3. Inflammation and oxidative stress

In severe forms of COVID-19 a phenomenon known as 'cytokines storm' can be observed [40]. The increased levels of cytokines such as Monocyte Chemotactic Protein 1 (MCP1), IFN-γ-inducible protein 10, IFN-γ, IL-1β, IL-6 and IL-18, which has been found in lymphoid tissues, blood and lungs of COVID-19 patients, point toward an increased activity of the inflammasome [47-49]. The inflammasome, a protein complex of the cytosol, is one of the first components of the host innate immunity, involved in anti-viral responses by mediating the secretion of pro-inflammatory cytokines [50]. Rather than directly recognizing pathogenic elements, NLRP3 (NOD-, LRR- and pyrin domain-containing protein 3) the inflammasome appears to detect pathogenic-induced oxidative stress [51]. Nonetheless, it seems that SARS-CoV-1 directly encodes some of the known activators of NLRP3 inflammasome such as the envelope (E) protein, ORF8b, and ORF3a, which share respectively 95%, 40%, and 72% amino acid sequence with their counterpart molecules in SARS-CoV-2 [52, 53]. Significantly increased levels of NLRP3 inflammasome in leukocytes of affected lung areas have recently been reported in fatal COVID-19 pneumonia [54].

Similar to SARS-CoV-2, an excessive release of proinflammatory cytokines has been reported for SARS-CoV-1 [55,56]. A number of COVID-19 patients not presenting ARDS show signs of extrapulmonary tissue damage (e.g. elevated creatinine and liver enzymes), possibly due to pro-inflammatory cytokine storm [57].

The generation of ROS-dependent respiratory burst is one of the mechanisms used by activated phagocytic cells such as neutrophils to suppress microbes during inflammation processes [58]. However, dysregulated interactions between ROS and inflammation may be linked to the pathogenesis of cytokine storm caused by COVID-19 (**Fig 1**). While inflammation enhances ROS levels, increased levels of ROS in turn can boost inflammation, thereby creating a vicious circle [59]. The hyper-inflammatory state sustained by phagocytes likely explain the diffuse alveolar lesions with potential emphysema and even pneumothorax observed in critical COVID-19 pneumonia. On the other hand, it is hypothesized that ROS is implicated in activating the NLRP3 inflammasome [60-62].

3.4. COVID-19 risk factors and Oxidative stress

The risk of ARDS and related COVID-19 mortality increases with patients' age [63], which is associated with both cumulative damage caused by oxidative stress and reduced antioxidant activity [64, 65]. Results of a study on gene expression of type II pneumocytes revealed that the most downregulated gene in the elderly subjects is that encoding the superoxide dismutase 3 (SOD3). Genes encoding other molecules with antioxidant activity were also found to be downregulated in this population [66].

Oxidative stress and ROS are also key factors involved in pathological processes such as diabetes [67], hypertension [68], Chronic Obstructive Pulmonary Disease [69], obesity [70,71, cancer [72-74], AIDS [75] and cardiovascular disease [76, 77]. Comorbidities, which increase linearly with age, in turn, enhances the risk of severe COVID-19 [68-80].

3.5. COVID-19 and Oxidative stress

A few investigations assessed the induction of oxidative stress due to COVID-19. A recent study reported increased serum levels of sNox2-dp, a NADPH oxidase activation marker in COVID-19

patients in comparison with healthy individuals [81]. Furthermore, higher serum levels of sNox2-dp have been reported among ICU patients as compared to non-ICU patients [81].

Cellular ROS were considerably increased in human promonocyte cells expressing SARS-CoV-1 $3CL^{pro}$ (viral 3-chymotrypsin-like cysteine protease) [82]. There is 99.02 % homology between sequences of SARS-CoV-2 $3CL^{pro}$ and SARS-CoV-1 $3CL^{pro}$[83], which further strengthens the argument that SARS-CoV-2 can cause oxidative stress.

Another remarkable finding is that serum albumin, which is considered a major component of serum antioxidant defence [84], is considerably decreased in patients suffering from COVID-19 [85], pointing towards a disruption of redox balance in these patients. Therefore, oxidative stress may be implicated in the pathogenesis of COVID-19 pneumonia (**Fig1**).

3.6. Ozone Therapy and oxidative stress

Although the inhalation of O_3 gas is very toxic for the lungs [86], recent evidence on O_3 biochemical activity has shown that its administration via appropriate routes and at small doses can paradoxically be involved in induction of an adaptive reaction capable of decreasing the endogenous oxidative stress [87-90]. There is a growing consensus that an accurately adjusted oxidative stress has therefore the ability to boost the antioxidant activities.

Various experimental studies assessed the antioxidative effects of ozone therapy (Table 1), mostly in rats with ischemia-reperfusion injury (IRI) because oxidative stress largely contributes to IRI [91,92]. Hepatic [93,94], renal [95,96], intestinal [97], cochlear [98], retinal [99] and testicular [100] tissues among others have been investigated so far. According to these studies, ozone therapy has a protective role against IRI by shifting the redox balance towards the antioxidant activity.

To date, the antioxidative effects of systemic ozone therapy have been studied (**Table 2**), both on healthy volunteers [101-103] and patients with different clinical conditions such as rheumatoid arthritis (RA) [104], advanced non-small cell lung cancer [105], coronary artery disease [106], myocardial infarction [107], heart failure [108], multiple sclerosis [109], multi-drug resistance TB [110], diabetes [111, 112], knee osteoarthritis [113], cancer patients under palliative care [114], in addition to endothelial [115] and HeLa cells [116]. According to these studies, ozone therapy significantly increases the level of FRAP (Ferric Reducing Ability of Plasma), an indicator of total antioxidant capacity, as well as antioxidants (e.g., superoxide dismutase, glutathione peroxidase, glutathione, glutathione S-transferase, etc.). Furthermore, ozone therapy determines a decrease in the levels of oxidative stress markers, including peroxidation potential, total hydroperoxides, malondialdehyde, nitric oxide (NO) and advanced oxidation protein products (AOPP).

Systemic O_3 can be administered by different routes, such as major auto-hemotherapy, minor auto-hemotherapy and rectal insufflation, among others [117]. At therapeutic doses and with appropriate dose intervals, O_3 administration regulates multiple biochemical mechanisms mostly via the activation of secondary messengers [118].

O_3 therapy stimulates the expression and activity of Nuclear erythroid 2-related factor 2 (Nrf2) [119]. It is argued that low dose ozone is capable of exerting anti-inflammatory and antioxidant activities by means of activating Nrf2, which contributes substantially to the effectiveness of O_3-O_2 treatments [120-122]. In a study on multiple sclerosis patients, rectal insufflation with O_3 increased Nrf2 phosphorylation in mononuclear cells, improved the activity of antioxidant enzymes and reduced pro-inflammatory cytokines [109].

Nrf2 is defined as an important modulator of cytoprotective protein driven by the antioxidant response element, and Nrf2 pathway activation significantly prevents the oxidative stress determined by injuring cells and tissues [122]. Increasing the transcription of antioxidant enzymes (e.g. biliverdin reductase, heme oxygenase-1, peroxiredoxin 1, peroxiredoxin 6, glutathione peroxidase 2, glutathione peroxidase 4, and glutathione reductase, thioredoxin-1, etc.) is the mechanism by which Nrf2 prevents the oxidative

stress [123-126]. A study on biopsy specimens of COVID-19 patients found that the gene expression pathway of Nrf2 was suppressed [127].

Homeostatic control of ROS, accomplished by Nrf-2, can break the vicious circle of ROS and inflammation. In addition, Nrf2 reduces the generation of pro-inflammatory cytokines such as IL1β and IL-6 through prevention of RNA polymerase II transcriptional activity, which further suppresses the inflammatory response [128]. Furthermore, Nrf2 regulates gene expression in activated macrophages through two-way interactions with Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-kB) transcription factor. Nrf2 performs regulated self-transcription [129], and decreases NF-kB transcriptional activity [130].

NF- kB activation increases the generation of pro-inflammatory cytokines such as IL8, TNFα, IL6, IL1β, IFNγ, as well as proinflammatory enzymes like inducible Nitric Oxide Synthase and cyclooxygenase-2 [131]. In an animal model of ALI caused by SARS-CoV-1 infection, the generation of oxidized low-density lipoprotein (OxLDL) enhanced the innate human immune activity through Toll-like receptor 4 (TLR4)/NF-kB signalling pathway and subsequent excessive production of IL-6 by alveolar macrophages [132]. The fact that antioxidants such as vitamin E, green tea polyphenols, L-cysteine, thiols and N-acetylcysteine (NAC) can block the activating effects of almost all stimuli on NF-κB further confirms the possible role that ROS play in NF-κB activation [133, 134].

Ozone therapy decreases the level of NLRP3 inflammasome either directly or via Nrf2 activation/ROS reduction/NF-kB inhibition pathway [112]. Decreasing levels of ROS or inhibition of NF-κB prevent components of the NLRP3 inflammasome protein from being assembled, thus subsequently reducing its activity [135-138].

3.7. Ozone therapy and COVID-19

A mixture of oxygen-ozone (O_2-O_3) infusion therapy has proven beneficial for COVID-19 patients admitted to forced non-invasive ventilation, contributing to restore their O_2 saturation in a relatively short time [139]. To date, a few investigations have assessed the effects of ozone therapy in patients suffering from COVID-19. A study on 50 ICU patients with ARDS caused by COVID-19 reported clinical improvement sustained by increased O_2 saturation and PaO2/FiO2 ratio following systemic ozone therapy [139]. In addition, thromboembolic and inflammatory markers such as D-dimer, IL-6, CRP were significantly reduced in these patients. Similar findings were reported in other clinical studies [137-146]. Although O_2-O_3 autohemotherapy is regarded very safe - having a complication rate as low as 0.7/100,000 – and cost-effective, it needs to be delivered using proper devices and adapted to different phenotypes of COVID-19 patients [139,147,148].

The Italian Society of Ozone and Oxygen therapy (SIOOT) recently issued a clinical protocol, approved by the Italian National Institute of Health (ISS, Italian acronym), for the management of COVID-19 patients by O2-O3 auto-hemotherapy. The latter protocol stratifies COVID-19 patients into 5 phenotype classes, each corresponding to a different therapeutic approach, with phenotypes 1, 2, 3 (early stages COVID-19 infection) being more responsive to O2-O3 therapy (**Table 3**). Homogenous O2-O3 mixtures need to be produced with a precise and easily adjustable concentration, using devices made of ozone-resistant materials. O2 saturation of COVID-19 patients treated by O2-O3 therapy needs to be monitored on a daily basis, whereas laboratory tests (CRP, fasting glucose, ALT, creatinine, leukocytes, LDH, pro-calcitonin, L-6, among others) can be weekly checked [139,149].

The mechanisms of O2-O3 therapy against COVID-19 is still unknown, but the activation of Nrf2 induced by ozone appears to suppress the production of pro-inflammatory cytokines, hence modulating the hyper-coagulate state associated with severe forms of COVID-19 [128,139]. Furthermore, O2-O3

seems capable to directly inactivate coronaviruses spike envelope proteins - abundant of cysteine and tryptophan amino acids - thereby interfering with the binding of SARS-CoV-2 with ACE2 receptor [150]. The binding of SARS-CoV-2 with the ACE2 receptor may also be prevented by the inhibition of the palmytoilation of the spike envelope mediated by nitric oxide signalling pathways, also enhanced by O2-O3 [151,152].

4. CONCLUSIONS

Ozone therapy could be a potential resource to modulate the patient immune response against SARS-CoV-2, contributing to contain the cellular oxidative stress of COVID-19 pneumonia and breaking the vicious cycle of cytokine storm observed in severe forms of the disease. Ozone therapy may also be a useful complementary treatment to be considered in patients suffering from early stage COVID-19 pneumonia, to prevent the progression to life-threatening disease.

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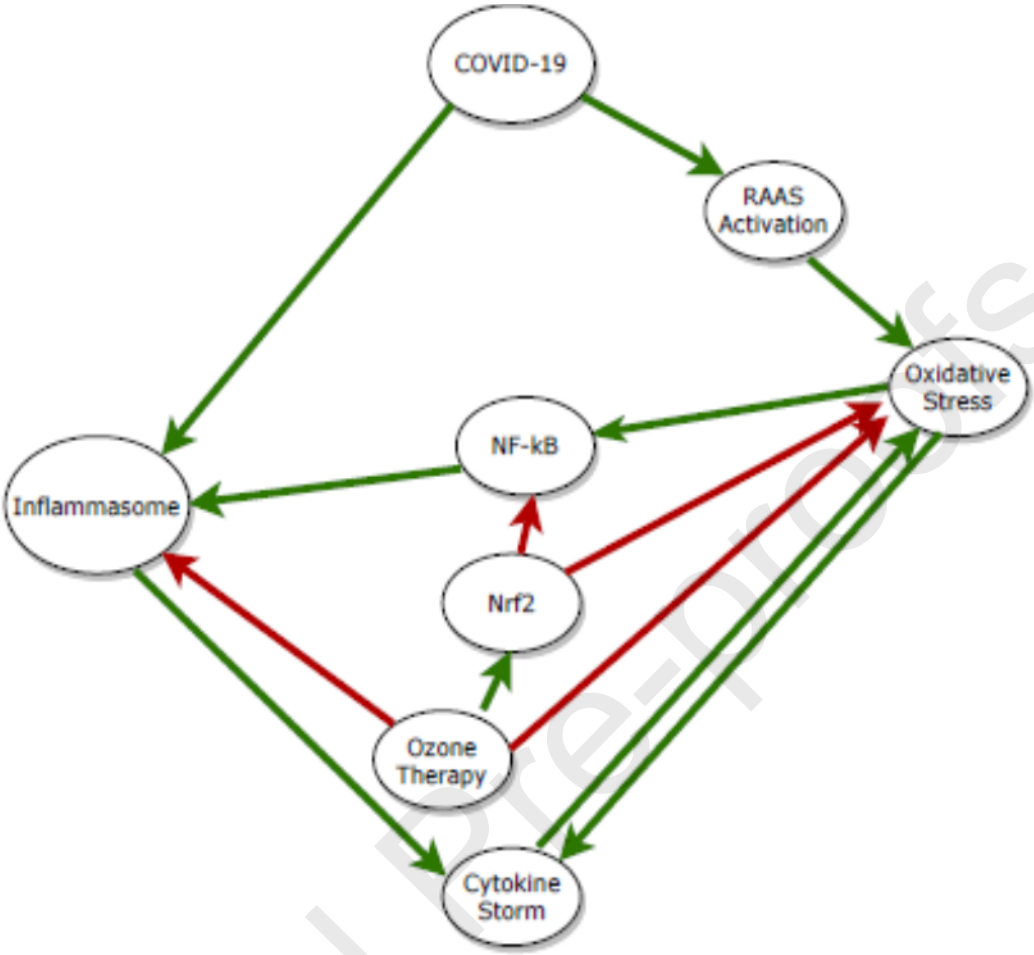


Figure 1. Possible mechanisms by which ozone therapy can reduce oxidative stress and disease severity in COVID-19 patients. Green lines denote activating effects and red lines denote inhibiting effects. NF-kB, Nuclear Factor kappa-light-chain-enhancer of activated B cells; Nrf2, Nuclear factor erythroid 2-related factor 2; RAAS, Renin Angiotensin Aldosterone System

Table 1. Experimental animal studies on antioxidative effects of ozone therapy; CAT= catalase; GSH= glutathione; GSH-Px=glutathione peroxidase; IRI= Ischemia-Reperfusion Injury; SOD= superoxide dismutase; TAC= Total Antioxidant Capacity.

AUTHORS	YEAR	SAMPLE SIZE	INVESTIGATED CONDITIONS/TISSUES	OUTCOME	REFERENCE
Peralta C et al.	1999	N=18	Hepatic IRI	Increase in SOD and preservation of GSH level	[94]
Ajamieh H et al.	2004	N=60	Hepatic IRI	Increase in SOD activity	[93]
Gonzalez R et al.	2004	N=48	Cisplatin-induced acute nephrotoxicity	Increase in GSH, SOD, CAT, and GSH-Px	[95]
Onal O et al.	2015	N=28	Intestinal IRI	Increase in SOD , GSH-Px, CAT and TAC	[97]
Kurtoglu T et al.	2015	N=32	Contrast-induced nephropathy	increase in renal antioxidant activity	[96]
Naserzadeh P et al.	2017	N=40	Brain and cochlear IRI	Increase in enzymatic and non-enzymatic antioxidants	[98]
Kal A et al.	2017	N=14	Retinal IRI	Increase in SOD, GSH-Px and TAC	[99]
Naserzadeh P et al.	2019	N=40	Testicular IRI	Increase in antioxidant capacity	[100]

Table 2. Experimental Human clinical studies on the antioxidative effects of Ozone therapy. AOPP= advanced oxidation protein products; BAP= biological antioxidant potential; CAT= catalase; CRP,C-reactive protein; FRAP= ferric reducing ability of plasma; **FiO2=Fraction of inspired oxygen;** G6PD= glucose 6 phosphate dehydrogenase; GGT= glutamyl transferase; GSH=glutathione; GSH-Px=glutathione peroxide; MDR-TB= multidrug resistance tuberculosis; MDA= malondialdehyde; NO= nitric oxide; *PaO2*=**Partial pressure of oxygen;** PP= peroxidation potential; ROM= reactive oxygen metabolites; SOD= superoxide dismutase; TH=total hydroperoxides.

AUTHORS	YEAR	SAMPLE SIZE	INVESTIGATED CONDITIONS/TISSUES	OUTCOME	REFERENCE
Hernandez F et al.	1995	N=22	Myocardial Infarction	Increase in GSH-Px and G6PD	[107]
Martinez-Sanchez G et al.	2005	N=101	Diabetic foot	Activation of SOD and normalization of organic peroxides	[112]
Inal M et al.	2011	N=11	Healthy subjects	Increase in SOD and CAT and decrease in MDA	[102]
Emma BJ et al.	2012	N=40	Non-small cell lung cancer	Decrease in dROM and increase in BAP	[105]
Martinez-Sanchez et al.	2012	N=53	Coronary Artery Disease	Increase in GSH and FRAP and decrease in PP, AOPP and MDA	[106]
Re L et al.	2014	N=6	Healthy subjects	Increased activities of SOD and CAT	[101]
Fernandez OSL	2016	N=40	Rheumatoid Arthritis	Increase in SOD, CAT, GSH and decrease in MDA, NO, AOPP	[104]
Buyuklu M et al.	2017	N=40	Heart Failure	Increase in SOD, CAT, GSH, GSH-Px and decrease in NO, MDA	[108]
Delgado-Roche L et al.	2017	N=28	Multiple Sclerosis	Increase in GSH and decrease of oxidative damage on proteins and lipids	[109]

Totolici IP et al.	2017	N=10	Cancer patients receiving palliative care	Increase in SOD and GSH-Px	[114]
Shah MA et al.	2018	N=12	Type II Diabetes	Decrease in CRP and biomarkers of lipid and protein oxidation	[111]
Loprete F et al.	2019	N=45	Healthy subjects and with various diseases	Decrease in total oxidizing capacity and increase in antioxidant response	[103]
Shah MA et al.	2019	N=7	MDR-TB	Increase in SOD	[110]
Fernandez OSL et al.	2020	N=40	Knee osteoarthritis	Increase in GGT, CAT, GSH and decrease in MDA, TH	[113]
Franzini M et al	2020	N=50	Patients undergoing ICU hospitalization for COVID-19	A notable decline of inflammatory and thromboembolic markers (CRP, IL-6, D-dimer) and improvement in the respiratory and gas exchange markers	[139]
Tascini C et al	2020	N=60	In patients affected by mild to moderate COVID-19 pneumonia	Lower PaO_2/FiO_2 and SpO_2/FiO_2 ratio and lower lymphocytes count.	[145]

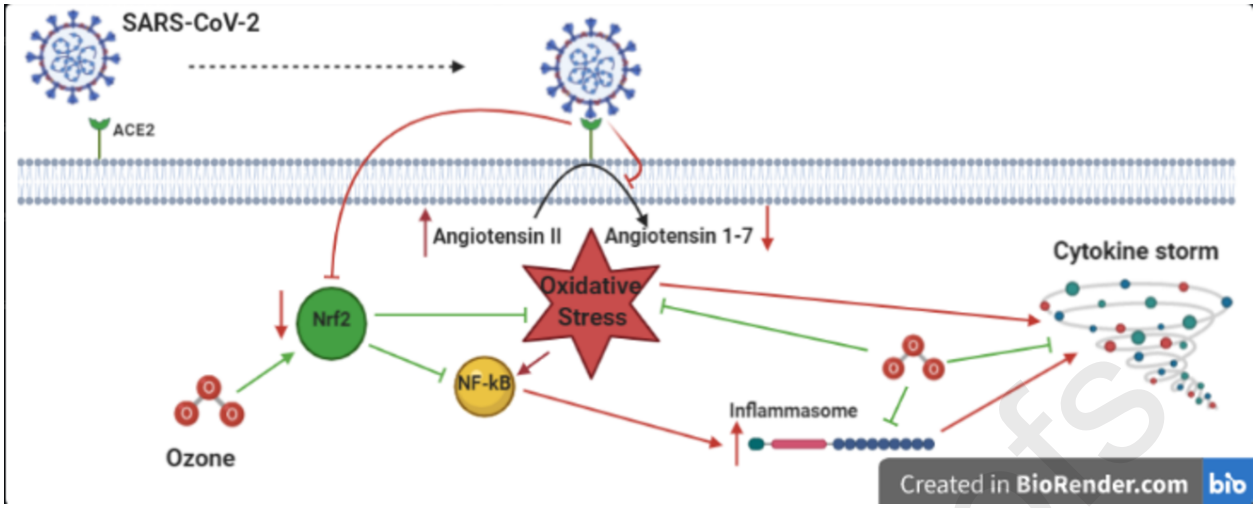
Table 3: Six different phenotypes to various therapeutic protocol

Phenotype class	Clinical Pattern	Therapeutic Management	
1	Fever With/without respiratory symptoms Negative chest X ray Normal pO2	Discharge	
		2-3 MAHT per week for 2-3 weeks (40-50 mg/150-200 cc ozone in 150/200 cc blood)	
		Ozone oil (RINOZONE) nasal spray 2/day	
		Ambient air sanitation (using AirKing)	
2	Fever GGO (at chest X ray) OR low pO_2	Admission and follow up	
		3 MAHT per week for 3 weeks (40-50 mg/200 cc ozone in 200cc blood)	
		Rinozone spray (ozonized oil) 2/3 times per day	
		Hyper-ozonized water	to drink (2 glasses/8h)
			mouth and eye rinses
		Ambient air sanitation (using AirKing)	
3	Fever Multiple GGO (at chest X ray) Low pO_2	Sub-intensive care needed	
		O_2 therapy (15 L/m)	
		4 MAHT per week for 3 weeks (40-50 mg/150-200 cc ozone in 150/200 cc blood)	
		Rectal insufflation with ozone (20-30 mg/100 cc)	
		Ozone oil (RINOZONE) nasal spray 2-3/day	
		Hyper-ozonized water	to drink (2 glasses/8h)
			mouth and eye rinses
		Ambient air sanitation (using AirKing)	
4	Pre-ARDS	CPAP	
		1st week: 1 MACHT/day for 7 days a week (40-50 mg/200 cc ozone in 200 cc blood)	
		2nd week: 4 MACHT/week (40-50 mg/200 cc ozone in 200 cc blood)	
		3rd week: 3 MACHT/week (40-50 mg/200 cc ozone in 200 cc blood)	

		Rectal insufflation with ozone (20 mg/100 cc)	
		Ozone oil (RINOZONE) nasal spray 2-3/day	
		Hyper-ozonized water	to drink (2 glasses/8h)
			mouth and eye rinses
		Ambient air sanitation (using AirKing)	
5	ARDS Very low pO2 (up to 35-40 mmHg) Pulmonary Interstitial syndrome	CPAP attempt (in case of WET interstitial syndrome)	
		Intubation (in case of DRY Interstitial syndrome)	
		1 MAHT/day for 5 days/week (40-50 mg/200 cc ozone in 200cc blood)	
		Rectal insufflation (20 mg/100 cc ozone) for 4 weeks	
		Ozone oil (RINOZONE) nasal spray 2-3/day	
		Hyper-ozonized water	to drink (2 glasses/8h)
			mouth and eye rinses
		Ambient air sanitation (using AirKing)	
6	ARDS interstitial pneumonia (at chest CT)	Oxygen-ozone (O2-O3) immunoceutical therapy	
		4 cycles of O2-O3 treatment	
		O2-O3 once a day for 5 consecutive days by 45µg/ml O2-O3mixture (Multioxygen Medical95 CPS) with 3-5 (median = 4) cycles (100–200 ml of O2-O3)	

MAHT: Major Auto-Hemo Therapy

CPAP: Continuous Positive Airway Pressure



Graphical Abstract: A scheme revealing angiotensin-converting enzyme 2 (ACE2) receptor-mediated COVID-19 following SARS-CoV-2 infection together with the mechanism of Ozone (O3)

Highlight

COVID-19 activates RAAS which induces oxidative stress leading to cytokine storm.

Ozone therapy can reduce oxidative stress.

Ozone therapy might be an excellent option as a complementary treatment for COVID-19.